Synergistic interaction of *Auricularia auricula-judae* polysaccharide with yam starch: effects on physicochemical properties and in vitro starch digestibility

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Abstract Thermal stable polysaccharides from *Auricularia auricula-judae* (AP) have unique molecular structures and multiple bioactivities. The effects of AP on the physicochemical properties and in vitro starch digestibility of yam starch (YS) were studied. The addition of AP induced a significant increase in the swelling power, solubility, mean volume diameter and adhesiveness as well as a dramatic decrease in the hardness and gumminess ($p < 0.05$). AP showed a strong suppressive effect on in vitro starch digestibility. Higher modulus (G' , G'') and stiffness parameters (A_α), and lower order of relaxation function (α), were observed in oscillatory rheological measurements, indicating that the gels were more elastic-like and had higher pseudoplasticity in the presence of AP. Furthermore, AP remarkably decreased the syneresis and storage modulus (G'), and also retarded the retrogradation process of YS gel at 4°C, revealing a synergistic interaction between AP and YS, which could also be demonstrated by scanning electron microscopy.

Keywords Polysaccharide · *Auricularia auricula-judae* · Yam starch · Physicochemical property · In vitro starch digestibility

Introduction

Auricularia auricula-judae has been used globally as food and medicine for hundreds of years (Yan et al., 2004). *A. auricula-judae* polysaccharides (AP), an important bioactive component, have been shown to possess multiple pharmacological activities, such as inhibiting acinar cell carcinoma proliferation and inducing S-180 tumor cell apoptosis (Ma et al., 2010), decreasing the levels of total cholesterol, triglycerides, and low-density lipoprotein cholesterol in the blood of hyperlipidemic rats (Zeng et al., 2013), and promoting antioxidant, anticoagulant and hypoglycemic effects (Khaskheli et al., 2015; Yoon et al., 2003). A water-soluble, neutral polysaccharide extracted from *A. auricula-judae* was characterized as a β -(1 → 3)-D-glucan with two β -(1 → 6)-D-glucosyl residues for every three main glucose residues, displaying a comb-branched structure, a rigid chain conformation, as well as parallel self-orientation behavior. Additionally, its chain shape and stiffness was stable below 140°C (Xu et al., 2013). Furthermore, our previous reports on AP have also demonstrated that the AP gel network structure is formed by hydrogen bonds between the carboxyl groups of the uronic acid on AP chains, and the AP gels exhibited high viscosity, pronounced shear-thinning and excellent thermal stability (5 → 80°C) at the concentration range of 0.5–2% (w/w) (Bao et al., 2016; Bao et al., 2018). These particular physicochemical properties and biological functionalities have attracted increasing interest from food and pharmaceutical producers due to the potential of incorporating AP into product formulations as a healthy ingredient or a fibrous carrier.

Yams (*Dioscorea* spp.), which are extremely widespread in tropical and subtropical countries including China, Korea, Japan and Nigeria, are the fourth most important

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root and tuber crop after cassavas, potatoes and sweet potatoes, and are widely consumed as staple foods in various forms, mainly boiled, fried or roasted (Akinoso and Olatoye, 2013). As a major and important component amounting to 75–84% of the total dry weight of a yam tuber, yam starch (YS) is closely associated with the processing characteristics, product quality and functional applications of yam tubers (Wanasundera and Ravindran, 1994). Compared with commercial starches from maize, wheat, and sweet potato starch, raw starches from yam tubers have better anti-digestion, and potential anti-constipation and hypolipidemic effects, mainly due to the higher resistant starch content (Huang et al., 2016). Tischer et al. also reported that YS amylopectin has fewer branch points, shorter average chain lengths and more linear structure than that of maize starch, promoting intermolecular binding (Tischer et al., 2006). Additionally, YS gel exhibits higher gel strength and viscosity as well as better heat tolerance than that of cassava starch, but lower storage stability (Mali et al., 2003).

Customarily, hydrocolloids, particularly non-starch polysaccharides, are frequently used to modify gelatinization and rheology, improve stability and texture, and control the overall quality of starch-based foodstuffs without chemical modification (BeMiller, 2011). More importantly, hydrocolloids can influence starch hydrolysis by digestive enzyme, which is one of the key metabolic processes in human digestion to maintain proper levels of postprandial blood glucose (Sasaki and Kohyama, 2011). According to the rate of digestion, starch is classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst et al., 1992). RDS is rapidly and thoroughly hydrolyzed in the small intestine and causes a rapid increase in the blood glucose level, while SDS is digested slowly but completely in the small intestine and is generally considered a desirable form of dietary starch. RS is not hydrolyzed in the gastrointestinal tract, but is partially fermented in the large intestine, which can improve blood sugar and insulin sensitivity, and also prevent and control the occurrence of obesity and diabetes (Englyst et al., 1992). Several research groups have reported on the processing and storage properties of yam starch in the presence of hydrocolloids. Some hydrocolloids, including xanthan and guar gum, iota-carrageenan, locust bean gum and xyloglucan improve some YS functionalities, such as reducing exudate production under refrigerated storage, enhancing thermal stability and gel strength, as well as decreasing the gelatinization enthalpy and increasing final viscosity (Freitas et al., 2015; Huang, 2009; Mali et al., 2003; Tischer et al., 2006). Although we have confirmed that synergistic interaction between AP and YS with a lower amylose content (31.23%), occurring under different processing conditions, that improved the

rheological properties and thermal resistance of the mixtures (Zhou et al., 2017), it is of great importance to explore the interaction of AP with YS containing higher amylose contents, which greatly influences the hydrocolloid-starch synergism (BeMiller, 2011). However, to the best of our knowledge, the effects of synergistic interactions between AP and YS with higher amylose content on the swelling of granules, rheological properties, texture, and retrogradation, as well as digestive characteristics of YS have not been systematically evaluated during processing and cold storage.

Therefore, the objective of this study is to investigate the effects of AP on the physicochemical properties and in vitro digestibility of YS with high amylose content (34.79%). Rotational rheometry and a texture analyzer were used to characterize the viscoelasticity and textural properties, while field-emission scanning electron microscope (FE-SEM) was used to examine the microstructure. The swelling power, solubility index, leached amylose content, particle size distribution, and syneresis were also determined to further elucidate the synergism with AP. The present work is applicable to regulating gelatinization and rheological properties, and for predicting the final textural attributes of starch-based formulated foods, as well as developing hypoglycemic foods. Additionally, it is critical to gain a deeper insight into the YS/AP mixture system to explore the potential applications of AP as a new-type of functional hydrocolloid in the food industry.

Materials and methods

Materials

Starch from commercial yams (*Dioscorea batatas*) originating from Andong, Korea, was prepared by water extraction (Zhou et al., 2017). The extracted yam starch (YS) had moisture, ash, crude fat, and protein contents of 9.17 ± 0.31 , 0.09 ± 0.01 , 0.11 ± 0.01 , and $0.16 \pm 0.02\%$, respectively, as determined by a previously described method (AACC, 2010); the amylose content ($34.79 \pm 0.28\%$) was determined using the iodine colorimetric method (Williams et al., 1970). Water-soluble polysaccharide (AP) from the fruiting bodies of *A. auricula-judae* was prepared as previously reported (Bao et al., 2016), with the chemical composition mostly consisting of carbohydrate (72.1%), protein (8.6%), water (9.6%), uronic acid (5.3%), and ash (4.4%). α -Amylase (A3176, from porcine pancreas, Type VI-B, 16 U/mg) and amyloglucosidase (A7095, from *Aspergillus niger*, 300 U/mL, aqueous solution) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). A D-Glucose assay kit (glucose oxidase/peroxidase, GOPOD Format K-GLUK)

was purchased from Megazyme International Ireland Ltd. (Bray, Ireland). All chemicals and reagents used were of analytical grade.

Preparation of gels

Regarding the gels preparation for determination of particle size, and rheological, textural, syneresis, and microstructural properties, AP dispersions were first prepared at concentrations of 0.2, 0.4 and 0.8% by dispersing AP in distilled water with constant stirring at 600 rpm for 20 min using a magnetic stirrer (SP131320-33Q, Thermo Fisher Scientific, Massachusetts, USA), followed by heating in a water bath at 85°C for 20 min with continuous stirring at 600 rpm and then quickly cooling to room temperature. Then, the required amount of YS was added to yield 6% total polysaccharide with the YS/AP mixing ratio of 6.0/0.0, 5.8/0.2, 5.6/0.4 and 5.2/0.8 (w/w). The mixture was allowed to evenly disperse and thoroughly hydrate by vortexing for 1 min before constantly stirring (600 rpm) for 1 h at 25°C. The YA suspensions were then heated in a boiling water bath for 30 min with stirring (600 rpm) for full gelatinization. After heating, the hot pastes were quickly transferred to an ice-water bath and cooled for 5 min. Freshly prepared gels were used for further analysis. To prevent water evaporation from influencing the concentrations of blended materials, all samples were prepared in a closed system using a vial with a deep-skirted screw cap.

Swelling power, solubility index and leached amylose

The swelling power (SP) and solubility index (SI) of YS alone and YA mixtures were determined by slightly modifying the previous method of Rosell et al. (2011) (Rosell et al., 2011). Briefly, YS or YA dispersions (20 g) were prepared by suspending the yam starch in the AP solutions (0, 0.05, 0.1 and 0.2%, w/w) to obtain a final dispersion concentration of 1.5% (w/w) with the YS/AP mixing ratio of 6.0/0.0, 5.8/0.2, 5.6/0.4 and 5.2/0.8 (w/w) in 50 mL centrifuge tubes. The tubes were sealed with screw caps followed by vortexing for 1 min before heating in a boiling water bath for 30 min with a shaking rate of 90 rpm. After immediately cooling in an ice bath for 5 min, the centrifuge tubes were centrifuged at $7000 \times g$ for 15 min at 25°C. The supernatant was carefully removed into previously weighed aluminum dishes and dried to a constant weight (W_1) in a hot oven at 105°C. The wet precipitate was also weighed (W_2) for determination of the swelling power. Meanwhile, the leached amylose (LA) content of the soluble supernatant was determined by iodine binding (Williams et al., 1970) and calculated by dividing the

amylose content in the supernatant by the original weight of YS. The SI and SP were calculated as follows:

$$SI (\%) = (W_1/W_0) \times 100 \quad (1)$$

$$SP (g/g) = W_2/[W_0(1 - SI)] \quad (2)$$

where W_0 is the sample weight (g, db).

Particle size distribution

The particle size distributions of the YA gels were determined at room temperature using a particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd., UK) according to the method given by Li et al. (2016) with slight modifications. Approximately 5 g of freshly prepared gel was diluted to 10 mL with distilled water and gently stirred for 20 min before measurements. The sample particle refractive index (RI) was set to 1.530, the absorption was 0.1, and the obscuration was about 8%. The dispersant was water and the dispersant RI was 1.330. The mean volume diameter ($D_{[4,3]}$) is presented in μm .

Textural measurements

The freshly prepared pastes were poured into cylindrical glass containers (diameter: 50 mm, height: 20 mm), capped with a glass cover, covered with parafilm, and then packed in plastic bags followed by aging at 4°C for 24 h. The textural properties of stored YS and YA gels were determined using a Texture Analyzer (TA.XT.plus, Stable Micro Systems, UK) equipped with a P/36R cylinder probe and a 25 kg load cell by a standard two-cycle compression test (Wang et al., 2013). After cold storage, the gels were placed at room temperature for 1 h prior to analysis. The parameters were set as follows: pretest speed 2.0 mm/s, test speed 1.0 mm/s, post-test speed 2.0 mm/s, trigger force 5 g, compression deformation rate 50%, and interval time 5 s for two compressions. Textural parameters of hardness, adhesiveness, springiness, cohesiveness, and gumminess of gels were calculated from the instrument software.

In vitro starch digestibility

Yam starch (0.5 g, db) was replaced with AP at 0, 2, 4 and 8% by weight, and the effects on in vitro starch digestibility were evaluated according to the method described by Englyst et al. (1992) with minor modifications. Porcine pancreatic α -amylase (3.0 g) was dispersed in distilled water (20 mL), centrifuged for 10 min at $2500 \times g$, and 13.5 mL of supernatant was collected. Next, 3.15 mL of amyloglucosidase was diluted to 6.75 mL with distilled water, and then 1.5 mL of amyloglucosidase

solution was added to the supernatant. The enzyme solution was freshly prepared beforehand for the digestion analysis. Subsequently, the YA gels were prepared according to the section of “preparation of gels”. After heating, the mixture was cooled to 37°C, and then 10 mL of sodium acetate buffer (0.2 mol/L, pH 5.2) and four glass balls (10 mm in diameter) were added to each tube. After equilibration in a 37°C water bath with 100 rpm shaking for 5 min, the enzyme solution (2.5 mL) was added to each tube and incubated at 37°C with shaking (100 rpm). Aliquots (0.1 mL) were removed at different time intervals (0, 20, 40, 60, 90, 120 and 180 min) and added to 0.9 mL of absolute ethanol in a 1.5 mL microcentrifuge tube to stop the reaction. After centrifugation at 9000×g for 2 min, the hydrolyzed glucose content of the supernatant was measured using a glucose oxidase/peroxidase (GOPOD) kit. Values for the digested starch fractions are expressed as milligrams of glucose multiplying 0.9. Values for rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) were calculated from the G_{20} and G_{120} values, using the follow equations:

$$C(\%) = (G_t - G_0) \times 0.9/TS \times 100 \quad (3)$$

$$RDS(\%) = (G_{20} - G_0) \times 0.9/TS \times 100 \quad (4)$$

$$SDS(\%) = (G_{120} - G_{20}) \times 0.9/TS \times 100 \quad (5)$$

$$RS(\%) = (TS - RDS - SDS)/TS \times 100 \quad (6)$$

where C is rate of starch hydrolysis, t is hydrolysis time, G_t is the released glucose content at hydrolysis time t, G_0 is the free glucose at 0 min, G_{20} is the released glucose content after 20 min, G_{120} is the released glucose content after 120 min, and TS is total dry mass of yam starch.

Dynamic viscoelasticity measurement

The dynamic viscoelasticity of the YA gels was measured with a rheometer (AR 2000ex, TA Instruments Inc., New Castle, DE, USA) using a parallel plate system (40-mm diameter with a 1 mm gap). Frequency sweep tests over the range of 0.1–10 Hz were performed at a constant strain of 2% and 25°C. The 2% strain was within the linear viscoelastic region for all samples determined through a strain-sweep measurement at 1 Hz and 25°C beforehand. The storage modulus (G'), loss modulus (G''), complex-modulus ($G^* = [(G')^2 + (G'')^2]^{1/2}$), and loss-tangent ($\tan \delta = G''/G'$) values as a function of frequency were obtained to characterize the mechanical spectra. The power-law correlation constants between G' and G'' versus ω for the YA gels were determined using the formulas (Razavi et al., 2016):

$$G' = k' \times \omega^{n'} \quad (7)$$

$$G'' = k'' \times \omega^{n''} \quad (8)$$

where, k' (Pa s^{n'}) and k'' (Pa s^{n''}) are intercepts, n' and n'' are slopes of the frequency dependence of G' and G'' , respectively, and ω is the angular frequency (rad s⁻¹). The material stiffness parameter (A_α , Pa rad^{-α} s^α) and the order of relaxation function (α) were calculated from the Friedrich and Heymann model according to the equation (Friedrich and Heymann, 1988):

$$G^* = A_\alpha \times \omega^\alpha \quad (9)$$

Additionally, the interaction coefficients were calculated by comparing the value of each parameter for the corresponding YS/AP blends with that of the single component system (Razavi et al., 2016). Using a ternary blending system (polymer a—polymer b—water), we can calculate the synergism percentage (σ) by the equation:

$$\sigma(\%) = (\text{Experimental data}_{a+b} - \text{Experimental data}_a - \text{Experimental data}_b) / \text{Experimental data}_{a+b} \times 100 \quad (10)$$

To evaluate the effect of cold storage on the viscoelasticity of the YA gels, the fresh YA pastes prepared in vials with a deep-skirted screw cap were placed directly into a refrigerator and stored at 4°C for 168 h before small oscillatory rheological test, in which the YS paste was more prone to retrogradation with firm structure under refrigerated storage. Thus, the YS gel sample was prepared according to the method described by Sasaki and Kohyama (Sasaki and Kohyama, 2011). Briefly, the fresh YS paste was placed between two glass plates with a 1 mm spacer, covered with plastic film, and then sealed in an airtight bag followed by storage at 4°C. Immediately before dynamic rheological measurements, YS gels were cut from the center of the gel into disks 40 mm in diameter (1 mm thick) using a mold to fit the plate geometry. All samples were held on the plate for 10 min at the initial temperature for equilibration and relaxation of the pastes prior to measurements. The exposed edge of the gap was covered with a thin layer of light paraffin oil to minimize evaporation.

Syneresis measurements

The syneresis of sample gels was measured by the method described by Shaikh et al. (2017) with slight modifications. Accurately weighted fresh pastes were transferred into 2.0 mL of microcentrifuge tubes, sealed with caps, covered with plastic film, and then placed in a refrigerator at 4°C for 0–168 h. After cold storage, the tubes were taken out

and held at room temperature for 1.5 h followed by centrifugation at $9000\times g$ for 5 min. The watery top layer was decanted and the residue was weighed. Syneresis (%) was expressed as the ratio of separated water to total sample gel before centrifugation by weight.

Scanningelectron microscope (SEM)

Field-emission scanningelectron microscope (FE-SEM) (JSM-7500F; JEOL, Ltd., Tokyo, Japan) was employed to evaluate the microstructure of YS and YA gels formed at 4°C for 24 h. The YA gels were immediately freeze-dried and cut with a razor blade to expose cut-surfaces, which were attached on a SEM stub with double-sided sticky tape. Stubs and samples were sputter-coated with a thin layer of gold–palladium alloy in a vacuum, and the cross-sectioned area was examined and photographed. The accelerating voltage and magnification were 5 kV and 500, respectively.

Statistical analysis

All tests were carried out at least in triplicate, and the results were expressed as means $\pm$ standard deviation. All statistical computations and one-way analysis of variance (ANOVA) were performed using Statistical Analysis System software for Windows (version 9.2, SAS Institute, Cary, NC, USA). Duncan's multiple-range test was also applied to establish the significance of differences among the mean values, using a significance test level at 5%.

Results and discussion

Swelling power (SP), solubility index (SI), leached amylose (LA), and particle size distribution

As shown in Table 1, with increased AP concentrations, the SP of YA mixtures rapidly increased initially and then steadily rose as the mixing ratio increased from 5.8/0.2 to 5.2/0.8, while the SI and the mean volume diameters

($D_{[4,3]}$) dramatically increased for all replacement levels ($p < 0.05$). Similar studies were reported for tapioca starch/hydrocolloid (guar or xanthan gum) mixtures (Chaisawang and Supphantharika, 2006). These observations could be attributed to the shear forces or osmotic pressure exerted by AP onto the surface of the starch granules, which are responsible for the enlargement of granular submicropores, the increase of water uptake, the expansion and breakdown of granules as well as the subsequent exudation into the continuous phase during gelatinization (Rosell et al., 2011; Song et al., 2006). Compared with SP and SI, LA increased at a lower YS/AP mixing ratio (5.8/0.2) and then continuously decreased with increasing AP fraction due to higher viscosity by adding AP, which might impede the settlement of the swollen granules during centrifugation, leading to the decreased LA value of the unit volume in the supernatant (Song et al., 2006), as well as the enhanced intermolecular interaction between YS (mainly leached amylose) and AP via hydrogen bonding, reducing the free amylose content in mixtures (Freitas et al., 2015). A similar phenomenon was previously reported in mixtures of rice starch/hydrocolloid (xanthan or hydroxypropylmethylcellulose) (Rosell et al., 2011).

Textural properties

Texture analysis was used for YA gels with different mixing ratios after 24 h incubation at 4°C. As shown in Table 2, AP addition has a significant influence on the textural parameters of YA gels under cold storage ($p < 0.05$). With increasing AP fraction, YA gel exhibited a drastic decrease in the magnitudes of hardness and gumminess, while an opposite trend was observed in adhesiveness ($p < 0.05$). In contrast, the springiness and cohesiveness significantly ($p < 0.05$) decreased at lower AP replacement levels (5.8/0.2 w/w), but gradually increased with the higher proportion of AP. In particular, the 5.2/0.8 YS/AP mixture with the lowest values of hardness and gumminess also has the least syneresis [1.09%, Fig. 2(B)], which has potential applications in

Table 1 Swelling power (SP), solubility index (SI), leached amylose (LA), and mean volume diameter ($D_{[4,3]}$) of YS and YS/AP mixtures

YS/AP ¹ mixing ratio	SP (g/g)	SI (%)	LA (%)	$D_{[4,3]}$ (μm)
6.0/0.0	26.02 $\pm$ 0.91 ^{b2}	12.75 $\pm$ 0.26 ^c	8.56 $\pm$ 0.27 ^b	56.50 $\pm$ 0.28 ^c
5.8/0.2	29.58 $\pm$ 1.10 ^a	16.44 $\pm$ 0.55 ^b	12.44 $\pm$ 0.45 ^a	75.17 $\pm$ 1.52 ^b
5.6/0.4	30.20 $\pm$ 1.14 ^a	17.49 $\pm$ 0.60 ^b	11.52 $\pm$ 0.34 ^a	79.69 $\pm$ 3.25 ^b
5.2/0.8	31.59 $\pm$ 0.61 ^a	19.70 $\pm$ 0.28 ^a	9.31 $\pm$ 0.35 ^b	90.50 $\pm$ 3.62 ^a

¹AP, *A.auricula-judae* polysaccharide; YS, yam starch

²The values showed are the means $\pm$ standard deviation. For the same characteristic test, values for different YS/AP mixing ratios in the same column followed by the different upper-case letter (a–c) are significantly different at $p < 0.05$ by Duncan's multiple range test

Table 2 Textural properties of YS and YS/AP mixture gels stored at 4°C for 24 h

YS/AP ¹ mixing ratio	Hardness (N)	Adhesiveness (mJ)	Springiness	Cohesiveness	Gumminess (N)
6.0/0.0	11.64 ± 0.28 ^{a2}	0.22 ± 0.01 ^d	0.80 ± 0.04 ^a	0.54 ± 0.02 ^a	6.35 ± 0.17 ^a
5.8/0.2	4.70 ± 0.08 ^b	0.43 ± 0.02 ^c	0.67 ± 0.02 ^c	0.32 ± 0.01 ^d	1.51 ± 0.04 ^b
5.6/0.4	3.09 ± 0.10 ^c	0.70 ± 0.05 ^b	0.70 ± 0.03 ^{bc}	0.40 ± 0.02 ^c	1.22 ± 0.06 ^c
5.2/0.8	2.57 ± 0.08 ^d	0.79 ± 0.03 ^a	0.73 ± 0.02 ^b	0.45 ± 0.02 ^b	1.16 ± 0.07 ^c

¹AP, *A.auricula-judae* polysaccharide; YS, yam starch

²The values showed are the means ± standard deviation. For the same characteristic test, values for different YS/AP mixing ratios in the same column followed by the different upper-case letter (a–d) are significantly different at $p < 0.05$ by Duncan's multiple range test

jelly, confectionery, desserts, yogurt and other sticky or frozen foods. The main reason might be the reinforcement of a three-dimensional network structure induced by the synergistic interactions between AP and YS (mainly amylose) chains, leading to the reduction of hydrogen-bonding reassociation or recrystallization between starch molecular chains, and the subsequent lower retrogradation and syneresis of the blended system in the refrigerated period (Liu et al., 2006).

In vitro starch digestion

The effects of AP on the in vitro digestibilities of yam starch gels were investigated by determining the hydrolysis rate (data not shown) during starch digestion. The contents of RDS, SDS and RS in the gel samples are shown in Table 3. Compared to the control gel (YS), the gels with AP exhibited lower levels of RDS and higher total amounts of SDS and RS. Moreover, the inhibitory effect of starch digestion was enhanced with increasing AP ($p < 0.05$). This result agrees with previous reports indicating that using β -glucans from *Lentinus edodes* and oat as a source of soluble dietary fiber can remarkably reduce the glycemic index (GI) response (Regand et al., 2011; Zhuang et al., 2017). The most pronounced suppressive effect of AP on yam starch digestibility might be attributed to the inter-molecular associations or entanglements between AP and

starch molecules reducing the contact between amylase and starch (Zhuang et al., 2017), the increased viscosity influencing glucose release and diffusion, as well as the encapsulation (coating) of starch granules decreasing enzyme accessibility (Sasaki and Kohyama, 2011).

Dynamic viscoelasticity

Figure 1 shows the frequency dependence of G' and G'' for fresh YA gels with AP blending ratios of 0–0.8% (w/w). For all samples, both G' and G'' increased with increasing ω , and G' values were always greater than the values of G'' throughout the frequency range, demonstrating a typical gel-like system with associated network. The correlation coefficients ($R^2 > 0.99$) between G' or G'' and ω were high for all samples. The magnitudes of n' and n'' are indices of the power-law's viscoelastic functions (G' and G''). YS gel showed the largest frequency dependence of the modules than other samples, and this dependence rapidly declined with increasing AP fraction in mixtures, as achieved by n' and n'' reduction (data not shown). On the basis of the report of Hesarinejad et al., high n' value is a characteristic of viscous gel; whereas, for n' value close to 0, the polymer behaves as an elastic gel (Hesarinejad et al., 2014). As for YA gels, increasing AP concentration lead to lower n' values than the control (0.196), while an increase in the magnitudes of k' and k'' was observed, indicating that the

Table 3 Starch digestion fractions of yam starch gels with AP at different replacement levels (0–8%) under in vitro starch digestion

AP ¹ replacement (%)	RDS (%)	SDS (%)	RS (%)
0	75.81 ± 1.72 ^{a2}	7.28 ± 0.17 ^a	16.91 ± 0.59 ^d
2	73.30 ± 2.04 ^{ab}	6.25 ± 0.18 ^b	20.45 ± 0.56 ^c
4	69.28 ± 1.90 ^b	5.94 ± 0.13 ^b	24.78 ± 1.71 ^b
8	60.37 ± 2.04 ^c	5.28 ± 0.19 ^c	34.35 ± 1.33 ^a

¹AP, *A.auricula-judae* polysaccharide. RDS, SDS, and RS mean rapidly digestible starch, slowly digestible starch, and resistant starch, respectively

²The values showed are the means ± standard deviation. For the same type of starch fraction, values for different AP replacements in the same column followed by the different upper-case letter (a–d) are significantly different at $p < 0.05$ by Duncan's multiple range test

Fig. 1 G' and G'' as a function of frequency for fresh YA gels with different blending ratios. YA, yam starch and *A. auricula-judae* polysaccharide mixture

elastic or solid-like behavior was strengthened by AP. This synergism effect could be explained that the phase separation and thermodynamic incompatibility probably contribute to the co-polymer network formation (BeMiller, 2011). In addition, the values of stiffness parameter (A_α) and the order of relaxation function (α) were calculated by the Friedrich and Heymann model with high correlation ($R^2 > 0.99$) (Friedrich and Heymann, 1988). Higher value of A_α corresponds to a more strength of network, while lower α value presents a higher number of interactions. Accordingly, the largest value of A_α was found for 5.2/0.8 YS/AP (178.61 Pa rad $^{-\alpha}$ s $^\alpha$) with the 75.94% synergism percentage, confirming in the blending system the largest molecular interaction occurred between YS and AP. The α value reduced with an increase in the AP fraction with the least value of α for 5.2/0.8 YS/AP gel (0.143), which suggests the shear thinning in small deformation is strengthened with increasing AP and the highest pseudo-plasticity was found for 5.2/0.8 YS/AP gel.

Cold storage stability of YA gels

The variances in the storage modulus (G') at 1 Hz and 2% strain for YA gels with different mixing ratios as a function of cold storage time (168 h at 4°C) are presented in Fig. 2(B). Among fresh gels the 5.2/0.8 YS/AP sample had the highest G' value (227.1 Pa), while the lowest value was observed for the control (104 Pa). In contrast, in the initial storage process (0 → 12 h), the values of G' significantly increased and the highest and the lowest values were observed for YS and 5.2/0.8 YS/AP, respectively. During the time between 12 and 24 h, the YS gel still showed increased G' and exhibited a tight appearance with pronounced syneresis (18.19%) indicating a strong

Fig. 2 Changes in G' for YA gels (6%, w/w) at 1 Hz and 2% strain during aging at 4°C for 168 h as a function of different blending ratios (A). Syneresis rate (%) as a function of storage time at 4°C for YA gels (6%, w/w) with different blending ratios (B). For the same storage time, bars followed by the different lower case superscript letters (a–d) are significantly different at $p < 0.05$ by Duncan's multiple range test. For the sample with same YS/AP mixing ratio, bars followed by the different lower case superscript letters (x–z) are significantly different at $p < 0.05$ by Duncan's multiple range test

retrogradation trend, and could not be used in the next oscillatory test. However, the G' values of YA gels dramatically decreased with increasing the AP fraction. As the storage time increased from 24 to 168 h, G' values of YA gels slowly increased and eventually reached a plateau, with the exception of the 5.8/0.2 YS/AP sample stored for 72 h showed a brittle structure with more water (12.55%) expelled from the polymer network. Additionally, a significant difference ($p < 0.05$) in syneresis for YA gels with different blending ratios under refrigerate storage (4°C) could also be observed in Fig. 2(B). The syneresis for all samples obviously increased during the storage period of 24–168 h, while it decreased remarkably with increasing AP fraction ($p < 0.05$). Accordingly, the largest syneresis reduction was found for 5.2/0.8 YS/AP gels during different storage times (94.01% for 24 h, 91.22% for 72 h, and 85.30% for 168 h). These results indicate that the

addition of AP increased the short-term structural development rate, but generally retarded the starch retrogradation process over longer cold storage. A possible explanation is the entanglement between chain segments and/or the molecular interactions between AP and amylose competing with amylose–amylose interactions, as well as the hydrophilic character of AP preventing water separation (Sudhakar et al., 1996). This may be useful for application in chilled YS-based food. Similar observations were made in studies on the yam starch gel stability in xanthan or guar gum added systems (Mali et al., 2003); and the retrogradation of the tapioca starch gels mixed with xanthan gum under refrigerated storage (Pongsawatmanit et al., 2013).

Scanning electron microscopy

The microstructures of YS gels with and without AP stored at 4°C for 24 h were examined by SEM in Fig. 3. Distinct differences between the gels were observed in the presence of AP. As seen in Fig. 3(A), the YS gel after aging exhibited a fragile and honeycomb-like structure with a thinner matrix, larger sized pores and more propagated cracks due to starch polymer (amylose) network formation resulting from chain aggregation and recrystallization (BeMiller, 2011). In contrast, smaller pore size with no cracks and more flexible segments throughout the heterogeneous system were found in Fig. 3(B), while Fig. 3(C) showed more interactive entanglements and larger junction zones with local–regional lamellar membrane structures. Surprisingly, compared with other samples, Fig. 3(D) exhibited a more firmer cross-linked network structure with a thicker and more elastic matrix

Fig. 3 Scanning electron microscopy images of YS and YS/AP mixture gels stored at 4°C for 24 h. (A–D) depict 6.0/0.0, 5.8/0.2, 5.6/0.4 and 5.2/0.8 (w/w) YS/AP mixtures respectively, with $\times 500$ magnification. YS, yam starch; AP, *A.auricula-judae* polysaccharide

surrounding pores as the mixing ratio increased to 5.2/0.8, confirming the largest synergistic interaction between AP and YS. This might be attributed to the neutral AP, with a comb-branched structure and a rigid chain conformation in aqueous solution, shows unique characteristics, such as excellent water solubility, high intrinsic viscosity, and strong thermal stability over a wide temperature range (5–80°C) (Bao et al., 2016; Xu et al., 2013). This favoured intermolecular entanglements and/or hydrogen-bond interactions between AP and leached starch molecules, and helped to inhibit syneresis, as well as stabilized the YS gel structure during refrigerated storage.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

References

- AACC. Approved Method of the AACC. 11th ed. Method 76-13.01. American Association of Cereal Chemist, St. Paul, MN, USA (2010)
- Akinoso R, Olatoye KK. Energy utilization and conservation in instant-pounded yam flour production. *Int. Food Res. J.* 20: 575–579 (2013)
- Bao H, You SG, Cao L, Zhou R, Wang Q, Cui SW. Chemical and rheological properties of polysaccharides from fruit body of *Auricularia auricula-judae*. *Food Hydrocoll.* 57: 30–37 (2016)
- Bao H, Zhou R, You SG, Wu SF, Wang Q, Cui SW. Gelation mechanism of polysaccharide from *Auricularia auricula-judae*. *Food Hydrocoll.* 76: 35–41 (2018)
- BeMiller JN. Pasting, paste, and gel properties of starch–hydrocolloid combinations. *Carbohydr. Polym.* 86: 386–423 (2011)
- Chaisawang M, Supphantharika M. Pasting and rheological properties of native and anionic tapioca starches as modified by guar gum and xanthan gum. *Food Hydrocoll.* 20: 641–649 (2006)
- Englyst HN, Kingman SM, Cummings JH. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* 46: S33–S50 (1992)
- Freitas RA, Woehl MA, Kaminski GAT, Silva R, Sierakowski MR. Rheological description of the interaction of xyloglucan and starches: effect of the amylose content in starches. *CYTA-J. Food.* 13: 235–242 (2015)
- Friedrich CHR, Heymann L. Extension of a model for crosslinking-polymer at the gel point. *J. Rheol.* 32: 235–241 (1988)
- Hesarinejad MA, Koocheki A, Razavi SMA. Dynamic rheological properties of *Lepidium perfoliatum* seed gum: Effect of concentration, temperature and heating/cooling rate. *Food Hydrocoll.* 35: 583–589 (2014)
- Huang CC. Physicochemical, pasting and thermal properties of tuber starches as modified by guar gum and locust bean gum. *Int. J. Food Sci. Tech.* 44: 50–57 (2009)
- Huang H, Jiang Q, Chen Y, Li X, Mao X, Chen X, Huang L, Gao W. Preparation, physico-chemical characterization and biological activities of two modified starches from yam (*Dioscorea opposita* Thunb.). *Food Hydrocoll.* 55: 244–253 (2016)
- Khaskheli SG, Zheng W, Sheikh SA, Khaskheli AA, Liu Y, Soomro AH, Feng X, Sauer MB, Wang YF, Huang W. Characterization of *Auricularia auricula* polysaccharides and its antioxidant properties in fresh and pickled product. *Int. J. Biol. Macromol.* 81: 387–395 (2015)
- Li Y, Zhu K, Guo X, Peng W, Zhou H. Interaction of barley β -D-glucan with wheat starch: Effect on the pasting and rheological properties. *Int. J. Biol. Macromol.* 92: 70–76 (2016)
- Liu H, Eskin NAM, Cui SW. Effects of yellow mustard mucilage on functional and rheological properties of buckwheat and pea starches. *Food Chem.* 95: 83–93 (2006)
- Ma Z, Wang J, Zhang L, Zhang Y, Ding K. Evaluation of water soluble β -D-glucan from *Auricularia auricula-judae* as potential anti-tumor agent. *Carbohydr. Polym.* 80: 977–983 (2010)
- Mali S, Ferrero C, Redigonda V, Beleia AP, Grossmann MVE, Zaritzky NE. Influence of pH and hydrocolloids addition on yam (*Dioscorea alata*) starch pastes stability. *Lebensm. Wiss. Technol.* 36: 475–481 (2003)
- Pongsawatmanit R, Chantaro P, Nishinari K. Thermal and rheological properties of tapioca starch gels with and without xanthan gum under cold storage. *J. Food Eng.* 117: 333–341 (2013)
- Razavi SMA, Alghooneh A, Behrouzian F, Cui SW. Investigation of the interaction between sage seed gum and guar gum: steady and dynamic shear rheology. *Food Hydrocoll.* 60: 67–76 (2016)
- Regand A, Chowdhury Z, Tosh SM, Wolever TMS, Wood P. The molecular weight, solubility and viscosity of oat beta-glucan affect human glycemic response by modifying starch digestibility. *Food Chem.* 129: 297–304 (2011)
- Rosell CM, Yokoyama W, Shoemaker C. Rheology of different hydrocolloids-rice starch blends. Effect of successive heating-cooling cycles. *Carbohydr. Polym.* 84: 373–382 (2011)
- Sasaki T, Kohyama K. Effect of non-starch polysaccharides on the in vitro digestibility and rheological properties of rice starch gel. *Food Chem.* 127: 541–546 (2011)
- Shaikh M, Ali TM, Hasnain A. Utilization of chemically modified pearl millet starches in preparation of custards with improved cold storage stability. *Int. J. Biol. Macromol.* 104: 360–366 (2017)
- Song JY, Kwon JY, Choi J, Kim YC, Shin M. Pasting properties of non-waxy rice starch-hydrocolloid mixtures. *Starch/Stärke* 58: 223–230 (2006)
- Sudhakar V, Singhal RS, Kulkarni PR. Starch-galactomannan interaction: functionality and rheological aspects. *Food Chem.* 55: 259–264 (1996)
- Tischer PCSF, Nosedá MD, Freitas RA, Sierakowski MRS, Duarte MER. Effects of iota-carrageenan on the rheological properties of starches. *Carbohydr. Polym.* 65: 49–57 (2006)
- Wanasundera JPD, Ravindran G. Nutritional assessment of yam (*Dioscorea alata*) tubers. *Plant Food. Hum. Nutr.* 46: 33–39 (1994)
- Wang L, Xie B, Xiong G, Wu W, Wang J, Qiao Y, Liao L. The effect of freeze-thaw cycles on microstructure and physicochemical properties of four starch gels. *Food Hydrocoll.* 31: 61–67 (2013)
- Williams PC, Kuzina FD, Hlynka I. A rapid colorimetric procedure for estimating the amylose content of starches and flours. *Cereal Chem.* 47: 411–421 (1970)
- Xu S, Lin Y, Huang J, Li Z, Xu X, Zhang L. Construction of high strength hollow fibers by self-assembly of a stiff polysaccharide with short branches in water. *J. Mater. Chem. A.* 1: 4198–4206 (2013)
- Yan PS, Luo XC, Zhou Q. RAPD molecular differentiation of the cultivated strains of the jelly mushrooms, *Auricularia auricula* and *A. polytricha*. *World J. Microbiol. Biotechnol.* 20: 795–799 (2004)

- Yoon SJ, Yu MA, Pyun YR, Hwang JK, Chu DC, Juneja LR, Mourao, PAS. The nontoxic mushroom *Auricularia auricula* contains a polysaccharide with anticoagulant activity mediated by antithrombin. *Thromb. Res.* 112: 151–158 (2003)
- Zeng F, Zhao C, Pang J, Lin Z, Huang Y, Liu B. Chemical properties of a polysaccharide purified from solid-state fermentation of *Auricularia auricula* and its biological activity as a hypolipidemic agent. *J. Food Sci.* 78: 1470–1475 (2013)
- Zhou R, Bao H, Kang YH. Synergistic rheological behavior and morphology of yam starch and *Auricularia auricula-judae* polysaccharide-composite gels under processing conditions. *Food Sci. Biotechnol.* 26: 883–891 (2017)
- Zhuang H, Chen Z, Feng T, Yang Y, Zhang J, Liu G, Li Z, Ye R. Characterization of *Lentinus edodes* β -glucan influencing the in vitro starch digestibility of wheat starch gel. *Food Chem.* 224: 294–301 (2017)