The effect of concentration on rheological properties of sago (*Metroxylon sagu*) starch

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Abstract

The concentration of starch is one of the main factors influencing the rheological characteristics of starch-based foods. The rheological behavior of sago starch at varied concentrations of 3, 5, 10, 20 and 30% (w/w) were investigated using small oscillatory temperature and frequency sweep measurements. All concentrations of sago starch expressed less solid-like behavior, with tan δ values of approximately 0.2. A flowable viscous paste of sago starch was generated at low concentrations (3 and 5% (w/w)). The gel network formation of sago starch initially occurred at a critical concentration of 10% (w/w). Further increases in the concentration to 20 and 30% (w/w), stronger gel network formation was occurred. In addition, the tan δ values for concentrations of 20 and 30% (w/w) were approximately 0.2 with elastic and soft gel characteristics. A positive power law correlation between G' and sago starch concentration was found, with a coefficient of determination (R^2) value of 0.99. The gelatinized sago starch was unstable as a frequency sweep was applied, indicating that it may be less stable during storage or processing.

1. Introduction

The stems of the sago palm are a source of sago starch, which is commonly consumed as a staple food, especially in the equatorial region. Sago starch is a source of carbohydrates (as energy) and has a low price (Cui and Oates, 1997). Sago starch has been utilized to produce various traditional desserts and foods. The sago starch provides clear, sticky, and soft textural characteristics which are unique features and are notably different from other sources such as tubers, cereals, and legumes. Nowadays, native flours, such as sago flour, are increasingly attractive and important ingredients for food industries because of customer preferences for healthy foods containing natural ingredients. In addition, the glycemic Index (GI) value of sago starch was around 28, categorized as low GI food (GI < 55) (Syartiwidya *et al*., 2019). Sago starch also contains resistant starch (Syartiwidya *et al*., 2019) and phenolic compounds (such as flavonoids and tannin), resulting in antioxidant activity (Momuat *et al*., 2016). Hence, sago starch is an interesting alternative ingredient for use in the design and development of functional food products. The starch

concentration is the important driving factor in rheological properties (Mohd Nurul *et al*., 1999, Ahmed *et al*., 2008, Udomrati *et al*., 2022). The rheological properties of sago starch with various concentrations must be investigated so that it can be suitably utilized in product development to produce the desired attributes in various foods.

The purpose of the current research was to study the starch rheological behavior of sago starch with various concentrations. This information should provide information on relevant textural characteristics that should aid in the design of new food products. Furthermore, rheological data are useful for predicting the stability of products and in the process design (such as in pumping, filtration, and extrusion) (Mandala *et al*., 2004; Liu *et al*., 2015).

2. Materials and methods

2.1. Materials

Sago starch was obtained from southern Thailand. The dried sago starch particles were ground using a

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pestle and mortar and then passed through a 100-mesh sifter. The sieved starch samples were kept in sealed polypropylene bags and stored at 4°C before further analysis.

2.2 Amylose content

An amylose/amylopectin assay kit (MegaZyme International Ireland Ltd., Wicklow, Ireland) was used to determine the amylose content.

Starch was heated and fully dispersed in dimethyl sulfoxide. Lipid and amylopectin in dispersed starch were precipitated and eliminated using ethanol and lectin concanavalin A, respectively. Amylose in supernatant was enzymatic hydrolyzed to D-glucose by amyloglucosidase. The total starch also hydrolyzed to D-glucose with amyloglucosidase. D-glucose was measured using spectrophotometric method at 510 nm. The concentration of amylose in starch sample was estimated with ratio of absorbance at 510 nm of supernatant (contained only amylose) to that of total starch sample.

2.3 Proximate analysis of sago starch

The moisture, protein, fat and ash contents of the sago starch were determined following the methods of AACC International (2000).

2.4 Gelatinization and retrogradation determination

The gelatinization transition enthalpy (ΔH_{gel} expressed in joules per gram, J/g) and the melting temperatures of gelatinization (onset temperature (To_{gel}), peak temperature (Tp_{gel}), and conclusion temperature (Tc_{gel})) were determined using differential scanning calorimetry (DSC) equipment (Diamond DSC, Perkin-Elmer, USA). Sago starch samples (2–2.5 mg) were weighed into the DSC equipment pan, then distilled water was added (ratio of starch-to-water of 30:70 on a dry basis). The DSC pan containing the sample was sealed and heated from 25 to 95°C at 10°C/min. Then, the DSC pan containing the gelatinized sample was stored at 4–5°C for 20 days for retrogradation determination. The sample pan was heated from 25 to 95°C at 10°C/min. The retrogradation transition enthalpy (ΔH_{retro} expressed as J/g) and the melting temperatures of retrogradation (To_{retro}, Tp_{retro}, and Tc_{retro}) were determined. An empty pan was used as the reference. The percentage of retrogradation was calculated using Equation (1):

$$Degree\ of\ retrogradation\ (\%) = \left[\frac{Enthalpy\ of\ retrogradation\ transition}{Enthalpy\ of\ gelatinization\ transition}\right] \times 100 \quad (1)$$

2.5 Pasting properties

The pasting characterization of sago starch (9.5% concentration) were determined using a Rapid Visco Analyzer (RVA 4, Newport, Australia).

2.6 Rheological properties

All determinations of rheological properties of the sago starch samples were performed using an MCR 305 rheometer (Anton-Paar, Graz, Austria) with parallel plate geometry and a diameter of 50 mm, with the distance of gap set at 1 mm. Each sample of approximately 2 mL was transferred to the bottom plate of the rheometer and the edge of the sample was covered with paraffin oil to prevent sample dehydration during the experiments. The sample temperature was controlled using a Peltier system with an accuracy of ±0.1°C. The sago starch concentration was 5% (w/w) for steady shear measurement. The oscillatory temperature sweep and frequency sweep measurement was performed with sago starch concentrations of 3, 5, 10, 20 and 30% (w/w).

2.6.1 Steady shear properties

Sago starch suspension (5% (w/w) on a dry basis) was capped and heated until fully gelatinized by boiling in water for 35 mins. The sample was gently shaken to prevent rice granule precipitation. Then, the gelatinized rice paste was placed in a water bath at room temperature for 30 mins. Three rheological tests were performed.

The viscosity of gelatinized sago paste sample as a function of the shear rate was determined at 25°C. The shear rate range was 0.01–100 s^{-1}. Experimental data were fitted to the Herschel-Bulkley model represented by Equation (2):

$$\tau = \tau_0 + k \cdot \gamma^n \quad (2)$$

where τ is the shear stress (Pa), τ_0 is the yield stress (Pa), γ is the shear rate (s^{-1}), n is the dimensionless flow behavior index, and k is the consistency index (Pa.s^n).

2.6.2 Oscillatory temperature sweep test and oscillatory frequency sweep test

The sago starch suspensions were prepared using starch concentrations of 3, 5, 10, 20 and 30% (w/w on a dry basis) with stirring using a vortex mixer at room temperature for 5 mins before loading the starch suspension of approximately 2 mL onto the bottom plate of the rheometer.

Each sago starch suspension sample was initially equilibrated at 25°C for 5 mins, and then was heated from 25 to 95°C at a rate of 5°C/min at a frequency of 1 Hz and a strain of 0.1%. The storage or elastic modulus (G', Pa), loss or viscous modulus (G'', Pa), loss tangent (tan $\delta = G''/G'$), and complex viscosity (η^*, Pa.s) were collected automatically as a function of temperature. Then, the gelatinized sample was cooled to 25°C for further frequency sweep testing. The frequency sweep of

0.1–10 Hz was performed with a constant strain of 0.1% at 25°C. The G', G'', tan δ, and η^* values were collected as a function of frequency. The η^* value was calculated based on the correlation expressed using Equation (3):

$$\eta^* = \left[\left(\frac{G'}{\omega}\right)^2 + \left(\frac{G''}{\omega}\right)^2 \right]^{1/2} \qquad (3)$$

where ω is the angular frequency (rad/s), G' is the storage or elastic modulus (Pa), and G'' is the loss or viscous modulus (Pa).

2.7 Statistical analysis

Data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test at the 95% confidence level ($p < 0.05$) using the SPSS statistical software (IBM Corp., New York, USA).

3. Results and discussion

3.1 Composition of sago starch

The amylose content of the sago starch was 20.36 (% dry basis) as shown in Table 1. The amylose content of sago starch has been variously reported in the ranges 24–31% (Ahmad *et al.*, 1999), and 18–21% (Piyachomkwan *et al.*, 1999) because the amylose content depends on many factors such as the variety, the stem part of the sago palm, and the cultivation area and method (Ahmad *et al.*, 1999). The average amylose molecular size of sago starch was reported by Piyachomkwan *et al.* (1999) in the range of 2,500–2,800 glucose units. Sago starch contains fat, protein, and ash contents of 0.12%, 0.23% and 0.60%, respectively, on a dry basis (Table 1). Sago starch had low fat and protein content. This was in agreement with Kumkanokrat (2001), who reported that the protein and lipid contents of sago starch were 0.13 and 0.12%, respectively, on a dry basis. The moisture content of sago starch (9.62%) was a suitable moisture content for storage because it was less than 13% and so inhibited microbial growth (Thai Industrial Standards, 2020).

3.2 Gelatinization and retrogradation of sago starch

The enthalpy (ΔH) and temperatures (To, Tp, Tc) of gelatinization and retrogradation of sago starch are shown in Table 2. ΔH$_{gel}$ is the total energy required to destroy the crystalline structure of starch, including breaking hydrogen bonding and some Van der Waals force (Tester, 1997). The ΔH$_{gel}$ value of sago starch (30% (w/w)) was 4.37 J/g of starch and the gelatinization temperature was in the range 71–83°C which was close

to result of Cui and Oates (1997), who reported sago starch gelatinized in the temperature range 67–88°C. The gelatinization of sago starch needed more energy and a higher temperature than non-glutinous rice flours (ΔH$_{gel}$~2.3-3.6 J/g of flour and a gelatinization temperature range of ~62–81°C) at the same flour concentration (30% (w/w)) (Udomrati *et al.*, 2020). These results indicated that sago starch was hardly gelatinized compared to non-glutinous rice flour because of its stronger and denser starch crystalline structure.

After storing the gelatinized sago starch gel at refrigeration temperature for 20 days, the gel sample was determined for retrogradation using DSC. The enthalpy of retrogradation (ΔH$_{retro}$) related to the quantity of re-crystallized structure of starch molecules after storage. The ΔH$_{retro}$ value of stored sago gel was 2.03 J/g of starch and the degree of retrogradation was 46.5%, as shown in Table 2. These results were in agreement with Noor Fadzlina *et al.* (2005), who reported gelatinized sago (~33% starch) had a retrogradation ratio (ΔH$_{retro}$/ΔH$_{gel}$) of 0.41 when stored at 4°C for 7 days. This indicated that almost all the amylopectin retrogradation of sago starch occurred within one week at refrigeration temperature. The sago starch tended to have higher amylopectin retrogradation compared to some flours and starches with the same concentration (30% of flour/starch) and storage temperature (4–5°C), such as tapioca starch with an expressed degree of retrogradation of 19% after storage for 20 days (Tongta *et al.*, 2007) and rice flours (14–30% amylose content) with degrees of retrogradation in the range 4–30% after storage for 14 days (Udomrati *et al.*, 2020). The retrogradation enhanced resistant starch (RS III), which is digested more slowly by human digestive enzymes than fresh gelatinized flour or starch (Cui and Oates, 1997). Sago starch may be attractive ingredient for developing healthy food products in term of digestibility and released-sugar or energy.

The melting temperatures of retrograded crystals for refrigerated sago gel were in the range 44–66°C (Table 2). This range of melting temperature indicated retrograded amylopectin, which melts at about 45–60°C (Biliaderis, 1992; Alonso and Escrig, 1999). Almost-retrograded amylopectin crystals were formed by helical intramolecular bonding of the amylopectin molecule and intermolecular interaction among close amylopectin molecules. The melting temperature of retrograded crystals shifted much lower than those for gelatinization

Table 1. Composition of sago starch

Amylose (%)	Moisture (%)	Fat (% dry basis)	Protein (% dry basis)	Ash (% dry basis)
20.36±0.58	9.62±0.49	0.12±0.01	0.23±0.02	0.60±0.03

Values are presented as mean±SD.

Table 2. Thermal properties for gelatinization and retrogradation of sago starch.

Gelatinization	ΔH_{gel} (J/g of starch)	4.37±0.64
	To_{gel} (°C)	71.50±0.01
	Tp_{gel} (°C)	76.41±0.25
	Tc_{gel} (°C)	82.61±0.35
Retrogradation	ΔH_{retro} (J/g of starch)	2.03±0.17
	Degree of retrogradation (%)	46.53±2.84
	To_{retro} (°C)	43.72±0.39
	Tp_{retro} (°C)	53.56±0.09
	Tc_{retro} (°C)	66.04±0.14

Values are presented as mean±SD.

(Table 2) due to the weaker crystalline structure.

3.3 Pasting properties of sago starch

The pasting properties and profiles of sago starch were measured using an RVA and the results are shown in Table 3 and Figure 1, respectively. The pasting temperature, peak viscosity, final viscosity, and setback of sago starch were higher than those of KTH17 rice flour (pasting temperature ~63°C, peak viscosity~245 rapid visco units (RVU), final viscosity~290 RVU, setback ~63 RVU (Udomrati *et al.*, 2020), whereas they had similar amylose contents (~20%). Although the amylose content plays an important role in determining the pasting and rheological properties of starch (Ye *et al.*, 2016), the pasting behavior also depends on various factors such as a granular crystalline structure, the starch granule size and size distribution, chemical composition (proteins and lipids), and the starch molecular structure (Kraithong and Rawdkuen, 2019). The average starch granule size of sago starch (25 μm) was larger than that of rice starch (5 μm) (Pomeranz, 1991) and sago starch had a higher swell capacity than rice starch (Wattanachant *et al.*, 2002), providing a higher peak viscosity for the sago starch. The protein and lipid contents of sago starch (Table 1) were lower than that of KHT17 rice flour, with 10.46% protein and 0.41% lipid contents, respectively (Udomrati *et al.*, 2020). The protein decreased the swelling of starch granules, and leached-amylose molecules (Sun *et al.*, 2008), resulting in low viscosity. The lipid also inhibited water penetration into starch granules due to the reduced water binding capacity of the starch molecules (Chinma *et al.*, 2015; Ali *et al.*, 2016) and the lipid was able to form complexes with the starch as amylose-lipid complexes, which reduced the hydrophilic parts of the starch molecules (BeMiller and Whisler, 2009), resulting in less swelling of starch granules (Tester and Morrison, 1990).
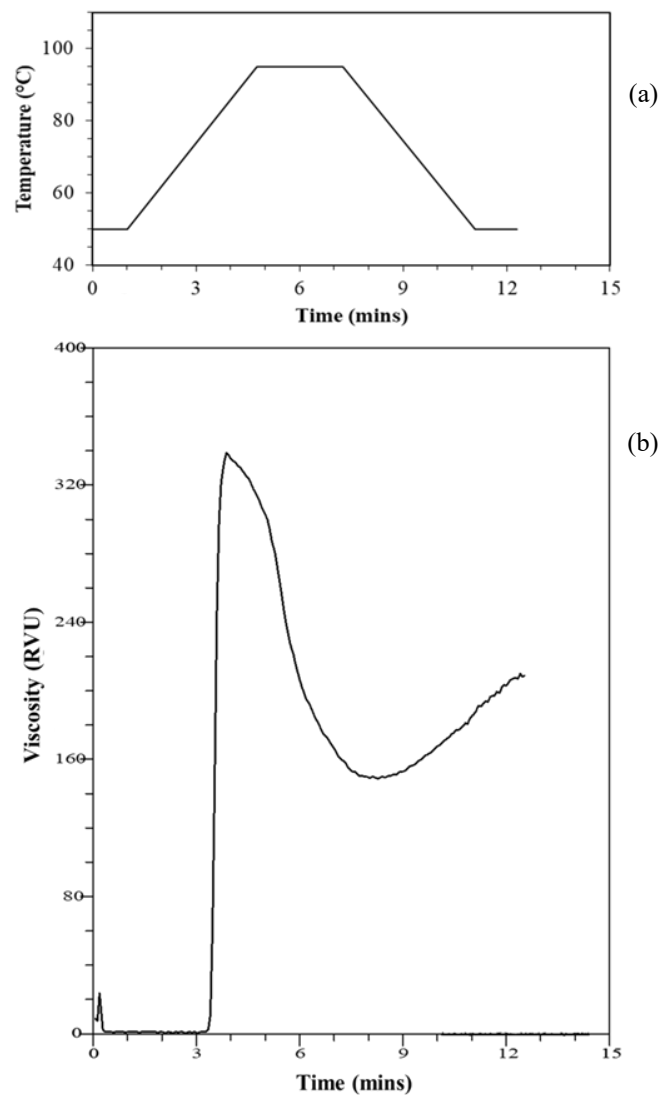

Figure 1 Pasting characteristics of sago starch; (a) RVA temperature profile, and (b) RVA pasting profiles.

Table 3. Pasting properties of sago starch.

Peak viscosity (RVU)	Trough (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Setback from Trough (RVU)	Pasting temperature (°C)
340.29±1.82	152.21±4.42	188.09±2.60	212.79±5.36	127.50±3.45	77.63±0.11

Values are presented as mean±SD.

3.4 Rheological properties of sago starch

3.4.1 Steady shear properties of sago starch with concentration of 5% (w/w)

The gelatinized sago paste (5% (w/w) of starch) had a rather low viscosity (1.13Pa.s at a shear rate of 50 s^{-1}) (Figure 2) and a flowable paste (no gel formation) because the concentration of 5% (w/w) was too low and there were insufficient starch molecules to form the gel network. The consistency index (k) value, shown in Table 4, is related to the viscosity of natural fluids. The starch crystalline structure was disrupted, the granules became swollen, and starch molecules leached from the gelatinized starch granules during gelatinization. These phenomena were the main driving factors resulting in an increase in the viscosity and k values of the system. In Figure 2, the viscosity of the sago paste decreased when the shear rate increased, expressed as shear thinning behavior with a flow behavior index (n) of less than 1 (Table 4). The shear thinning behavior may be due to the deformation entanglement of starch molecules and a reduction in the volume fraction of agglomerated and swollen granules as a high shear force was applied.
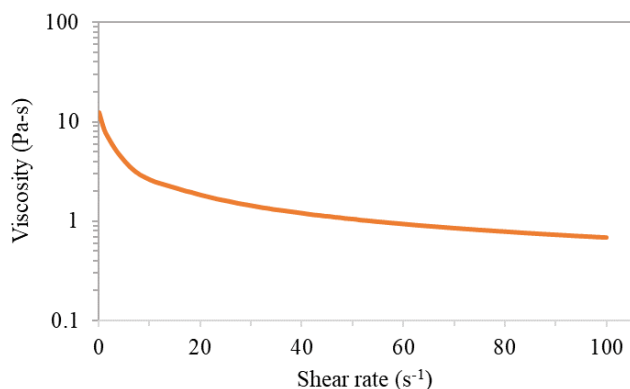

Figure 2. Flow curves of gelatinized sago paste (5% (w/w)).

Table 4. Rheological characteristics of gelatinized sago starch (5% (w/w)).

Flow behavior index (n)	Consistency index (k) (Pa.s^{n})
0.402±0.004	10.984±0.523

Values are presented as mean±SD.

3.4.2 Oscillatory temperature sweep determination of sago starch with concentration range of 3–30% (w/w)

The rheological investigation of sago starch (3–30% (w/w)) was performed based on a small sample deformation temperature sweep test during heating from 25 to 95°C. The G' value indicates gel rigidity or gel strength. When the temperature was below 70°C, the G' value of all samples was low (almost 0.1), as shown in Figure 3a, indicating a system without rigidity due to the lack of gelatinization. The gelatinization temperature of sago starch (71–83°C) was analyzed using DSC, as

shown in Table 2. The sample at all concentrations still exhibited liquid-like-behavior (flowable), as explained by their high values (≥1) of tan δ (Figure 3b). The values of G' and $\eta*$ for all concentrations initially increased as the temperature was about 70°C because the gelatinization of the sago starch started to occur (Figure 3a, 3c). The DSC thermogram indicated the gelatinization of sago starch and $\eta*$ during heating (25–90°C), as shown in Figure 3d. The relationship between the gelatinization temperature and an increase in the $\eta*$ profile was observed. As the temperature increased (> 70°C), the values of G' and $\eta*$ for all concentrations rose steeply to the expressed peak G' and $\eta*$ (Figure 3a, 3c) because the starch granules were swollen and much larger than their original size and were completely gelatinized, resulting in increased viscoelastic behavior. The tan δ value decreased noticeably because of the system transition from sol (suspension) to solid (gel) due to the cross-linking of polymers (Isuka and Winter, 1994). The three-dimensional gel network of the starch molecules was formed by leached-out starch molecules interaction and the swollen starch particles, resulting in increased solid-like behavior (Eliasson, 1986; Hsu et al., 2000). Gelatinized sago starch at all concentrations was a soft gel with a tan δ value of approximately 0.2 (Figure 3b), indicating less semi-solid behavior. At high temperatures (>85°C), the tan δ value plateaued due to complete gelatinization. However, the G' and $\eta*$ values at all concentrations clearly decreased when the applied temperature exceeded the gelatinization temperature (Figure 3a, 3c). This might be due to the fact that the disentanglement of amylopectin molecules progressed, resulting in weakened and softened starch granules (Tsai et al., 1997), and the swollen starch granules were disrupted (Anandha Rao, 1999). In addition, this result might have been due to the dynamic acceleration of amylose gel matrix formation (Ahmed, 2010) and gel network collapse, resulting from the loss of interaction between particles (Ahmed et al., 2008). Furthermore, proteins and lipids were able to induce resistance to amylose leaching, leading to a constant G' value over a broader temperature range (Biliaderis et al., 1986). Sago starch contains low contents of protein (0.23% on a dry basis) and fat (0.12% on a dry basis) (Table 1); hence, a distinct decrease in the G' value was observed.

The G' value increased as the concentration increased, as shown in Figure 4. The positive power law correlation was evident between the concentration and G', with an R^2 value of 0.99 (Figure 4). This result was in agreement with Biliaderis and Juliano (1993) who studied rice starch in the concentration range 8–40% and found a power law correlation between concentration and G'. At low concentration (≤10% (w/w)), the G' value slightly increased as the concentration increased from 3
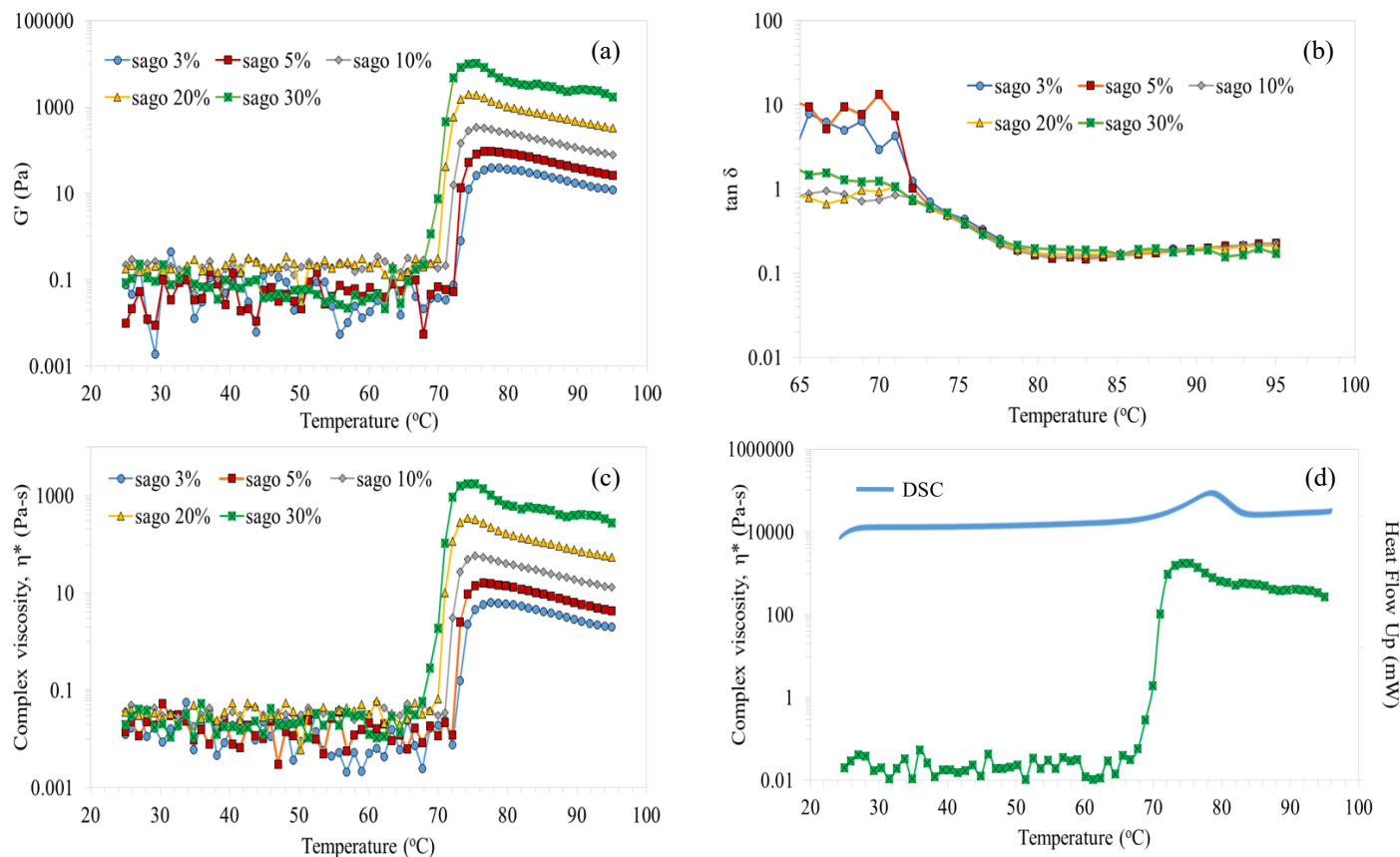
Figure 3. Temperature-dependent oscillation profile of different concentrations (3–30% (w/w)) of sago starch: (a) storage modulus (G'), (b) loss tangent (tan δ), (c) complex viscosity (η^*), and (d) DSC thermogram compared with dynamic rheological data.

to 10% perhaps because that amount of starch molecules was not enough to form a strong gel, as expressed by the low G' value (Figure 4). There was no gel formation, confirmed by the appearance of gelatinized sago starch at difference concentrations (Figure 5). In Figure 5, the sample did not form a gel network and was flowable at a concentration of 3% (w/w). Fragments of gelatinized starch film and free water were observed at a concentration of 5% (w/w) and gelatinized starch film was found at a concentration of 10% (w/w), which could be interpreted as indicating that a concentration of 10% (w/w) was the critical concentration (c^*) for sago starch network formation. However, the G' value substantially increased at high concentrations ($\geq$ 20% (w/w). This may be caused by the increased rigidity of the gel network since the swollen granules and amylose network formed a firmer gel and solid behavior with limited availability of water (Ahmed, 2010). The firm gel formation was confirmed by gel appearance, as shown in Figure 5. Fakharian *et al*. (2015) studied rheological properties of modified sago starch (combined modification of acid hydrolyzation and hydroxypropylation) and found that the modified sago starch (concentration of 25%) was not able to form gel and expressed low G' (2 Pa) value, which was similar to that of native sago starch at concentration of 3% (w/w). This result indicated that the starch modification obviously changes the rheological properties of sago starch.
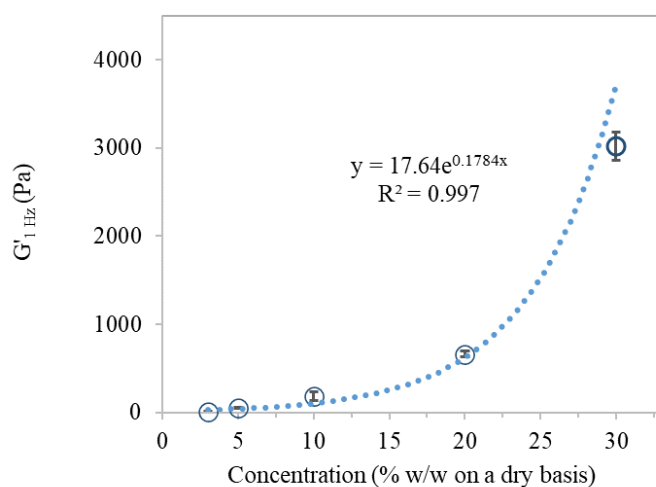

Figure 4. G'_{1Hz} of gelatinized sago starch as a function of starch concentrations (3–30% (w/w)).

3.4.3 Oscillatory frequency sweep determination of gelatinized sago starch with concentration range of 3–30% (w/w)

The G' value was higher than the G'' value throughout the applied frequency range of 0.1–10 Hz for all concentrations (Figure 6a), indicating that all systems (3–30% (w/w)) were in the semi-solid state. The difference between the G' and G'' values at a lower concentration was less than at a high concentration (Figure 6a) because the increase in the starch concentration induced a solid-like system (gel
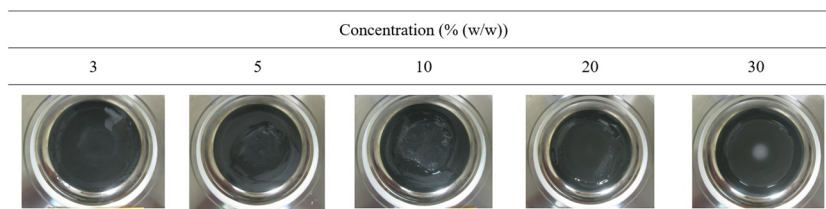
Concentration (% (w/w))				
3	5	10	20	30

Figure 5. Appearance of sago starch at various concentrations (3–30% (w/w)) after heating at 95°C using a rheometer.

formation). In addition, G' and G'' were dependent on the frequency for all samples, indicating a less semi-solid-like system. The dependency of G' and G'' on the frequency may have been due to relaxation processes that occurred even for short time scales (Lopes da Silva and Anandha Rao, 1999). The less semi-solid-like behavior of all sample systems was also indicated by tan δ values above 0.1 (Figure 6b). The tan δ value tended to increase as the frequency increased, as shown in Figure 6b. This may have been due to the gel structure of sago starch being quite unstable as a higher frequency or shear force was applied because the interconnection of starch molecules and junction zones in the less semi-solid-like system may have been easily destroyed (Ahmed *et al.*, 2008). The η^* of all concentrations decreased as the frequency increased (Figure 6c) because the deformation of the inner structure may have decomposed with the extension of the applied frequency (Ahmed, 2010).

4. Conclusion

Understanding the rheological behavior of sago starch is very important in the control and development of food products to satisfy customers' needs. Various rheological properties at different concentrations of sago starch are used to design and produce food products with various characteristics. Low sago starch concentrations (≤ 5% (w/w)) were suitable for viscous and flowable foods. A very weak gel was produced using a concentration of 10% (w/w). Concentrations of 20 and 30% (w/w) were able to form a soft gel with completely elastic behavior. The rheological data estimated using the oscillatory frequency sweep test indicated that the stability of sago paste or gel may be quite unstable during storage or transportation. Hence, improvement in the stability of the sago starch system should be considered as well such as adding food additives and processing modification.

Conflict of interest

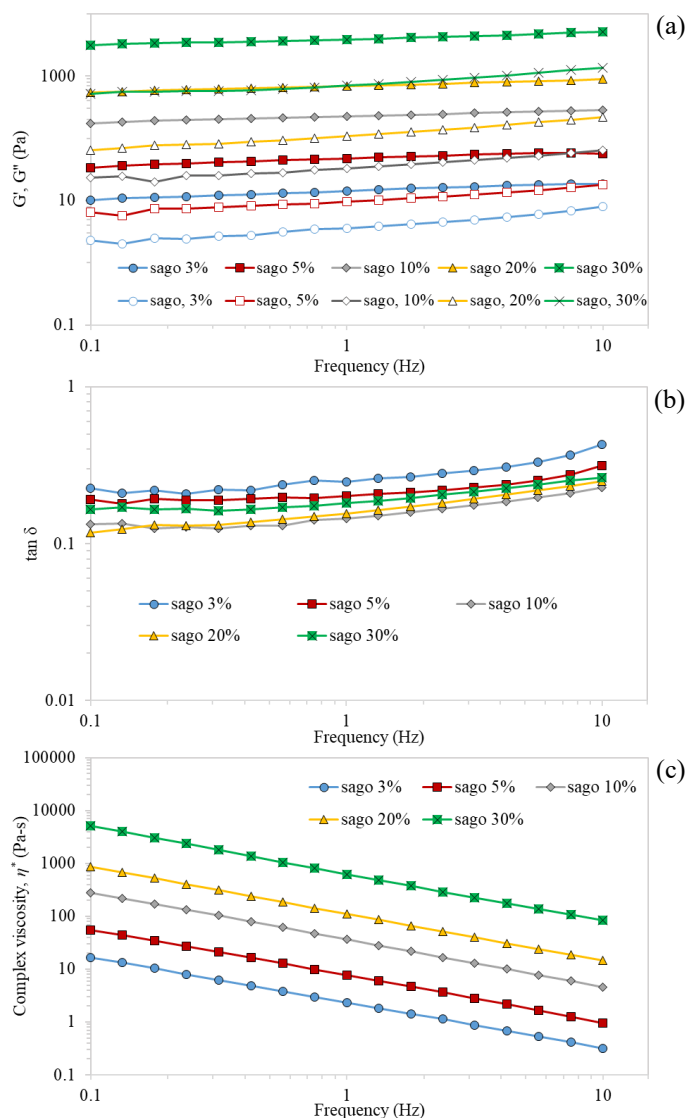The authors declare no conflict of interest.

Acknowledgments

Figure 6. Frequency-dependent oscillation profile of gelatinized sago gel (3–30% (w/w): (a) storage modulus (G') and loss modulus (G''), (b) loss tangent (tan δ), and (c) complex viscosity (η^*).

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