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Morphological, thermal and rheological characterization of starch isolated from New Zealand Kamo Kamo (*Cucurbita pepo*) fruit – A novel source

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Abstract

New Zealand Kamo Kamo, a unique fruit, was freeze dried, ground into flour and analyzed for its proximate composition. With high levels of glucose (17.7% d.w.), fructose (22.6% d.w.) and starch (23.2% d.w.), Kamo Kamo is also a rich source of potassium, zinc and dietary fibre. The morphological, thermal and rheological characteristics of the starch isolated from Kamo Kamo fruit were measured and compared with those of a normal potato starch. The Kamo Kamo starch granules had less smooth granule surfaces and appeared as a mixture of spherical, polyhedral and dome shaped granules with sizes ranging from 3 to 23 μ*m* (medium and small) in contrast to the large size ovoid or cuboid potato starch granules, when studied by particle size analysis and scanning electron microscopy. With a low amylose content of 17.2%, the Kamo Kamo starch swelled in water to a considerable extent, and exhibited a lower solubility than potato starch in both water and DMSO. The light transmittance of the Kamo Kamo starch paste was significantly lower than that of potato starch paste, irrespective of storage period at 4 °C. The Kamo Kamo starch had significantly higher transition temperatures (T_o; T_p; and T_c), enthalpy of gelatinization (ΔH_{gel}) and range of gelatinization (R), than potato starch, which suggests differences in the strength and stability of the crystalline structures between the two starches. A range of oscillatory rheological measurements using temperature (heating, cooling) and frequency sweep testing were performed, which showed that Kamo Kamo starch dispersions had lower storage (G'), loss (G'') and complex modulus (G^*) than potato starch. Differences between Kamo Kamo and potato starches in terms of thermal and rheological properties were attributed to differences in granule structure and in the stability of the crystalline structures inside the granule.

Keywords: Kamo Kamo; Cucurbita pepo; Proximate composition; Starch; Morphological; Thermal; Rheological

1. Introduction

Maori were the first human settlers of New Zealand, arriving around 1000–2000 years ago. Cambie and Ferguson (2003) reported that early food crops cultivated by Maori include Taewa (*Solanum tuberosum*), Kumara (*Ipomoea batatas*), the taro (*Colocasia esculenta*), the “bottle gourd” or hue (*Lagenaria siceraria*), the cabbage tree or ti kouka (*Cordyline australis*), the paper mulberry or aute (*Broussonetia papyrifera*), and the yam or uhi (*Dioscorea alata* and *Dioscorea esculenta*). The Kamo Kamo (*Cucurbita pepo*) pumpkin, generally grown in spring and maturing from December to April during summer, can be added to this list. Kamo Kamo fruits are stocky in shape with heavy ribbing, and have a speckled green soft skin on white green flesh. The young immature Kamo Kamo fruits can be boiled, fried or baked. Traditionally, the young fruit after boiling is used by Maori as a baby food.

Starch is the major reserve polysaccharide of most plants and consists of amylose, a linear nearly

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unbranched α1,4-glucan, and amylopectin which possesses in addition to the linear α1,4-linkages, the branched α1,6-linkages. This polymer is used in various industries, e.g. the food, pharmaceutical, textile and chemical industries. Characterization of native starch sources is required for identifying desirable functional properties, as native starches from novel plant sources have the potential for replacing chemically modified starches (Duxbury, 1989; Singh, Singh, & Sodhi, 2002a; Kaur, Singh, & Singh, 2005). Developments in the food and pharmaceutical industries have led to more and more attention being paid to new starches with distinctive properties (Wang, Gao, Chen, & Xiao, 2005). Starch properties such as granule shape and size, amylose to amylopectin ratio and gelatinization temperature vary with genotype, environmental conditions and cultivatory practices (Barichello & Yada, 1991; Kaur et al., 2005; Singh, Singh, Kaur, Sodhi, & Gill, 2003). During processing of foods, starch undergoes variable shear rates and heating-cooling cycles, which result in variation in the texture, form, size and colour of the final products. It has been suggested that the functional behaviour of starches depends on their physico-chemical and morphological characteristics (Singh & Kaur, 2004). The thermal and rheological properties are generally interpreted in terms of the microstructure and molecular architecture of starch.

Starch is an essential component of squash fruits (Corrigan, Irving, & Potter, 2000; Irving, Hurst, & Ragg, 1997; Phillips, 1946). Structural and physico-chemical characteristics of starches from different winter squash (*Cucurbita maxima*) cultivars were studied by Stevenson, Yoo, Hurst, and Jane (2005). The studies on physico-chemical, thermal, structural and pasting properties of winter squash starches indicated their potential for food as well as non-food use on the industrial scale (Stevenson et al., 2005; Sugimoto, Yamashita, Suzuki, Morishita, & Fuwa, 1998). Many sophisticated techniques and methods for the characterization of starch have been developed which are suitable for screening starches from different sources to check their suitability for industrial use (Kim, Wiesenborn, Orr, & Grant, 1995; Singh & Singh, 2003). Only limited work has been carried out on the characterization of squash starches, and, to our knowledge, there has been no report on the characterization of Kamo Kamo fruit and its starch. In the present work, we studied some properties of Kamo Kamo fruit, isolated its starch and compared the morphological, thermal and rheological characteristics of this starch with those of a starch isolated from a modern-day potato cultivar.

2. Materials and methods

2.1. Plant materials

Kamo Kamo (*C. pepo*) fruits were obtained from the Plant Growth Unit, Massey University, Palmerston North, New Zealand (2005 harvest). The potatoes of a modern-day cultivar (Nadine (*S. tuberosum*), New Zealand, 2005 harvest) were used. Uniformly-sized fruits and potato tubers were selected before analysis and starch isolation. All the reagents used in the study were of analytical grade.

2.2. Fruit characteristics

2.2.1. Freeze-drying of Kamo Kamo fruit

Kamo Kamo dry matter was obtained by freeze-drying. Kamo Kamo fruits (with peel) were washed in warm water, cut into small pieces and deseeded. The small pieces of fruit were lyophilized and immediately ground so as to pass through a number 72 sieve (British Standard sieve). The powdered samples were then stored in a desiccator until further use.

2.2.2. Proximate analysis of freeze-dried Kamo Kamo fruit

The following standard AOAC (2000) procedures were used for analysis: dry matter (convection oven, 105 °C, AOAC 930.15, 925.10), total nitrogen (leco, total combustion method, AOAC 968.06), fat (Soxhlet extraction, AOAC 920.39), ash (furnace, AOAC 942.05), starch (α-amylase method, AOAC 996.11) and dietary fibre (enzymatic–gravimetric method, AOAC 991.43).

2.2.3. Glucose, fructose and sucrose

Glucose, fructose and sucrose were measured by the hexokinase method (with a commercially available kit from Roche Diagnostics, Basle, Switzerland) using a Cobas Fara Analyser (Roche, Basle, Switzerland).

2.2.4. Mineral composition

The mineral composition of the freeze-dried Kamo Kamo was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES).

2.3. Starch characteristics

2.3.1. Starch isolation

Potato starch was isolated as described earlier (Singh & Singh, 2001). For the isolation of Kamo Kamo starch, fruits were washed in warm water and cut into small pieces. Immediately after cutting, the fruit pieces were deseeded and peeled. The juice was extracted from Kamo Kamo fruit pieces using a laboratory scale juicer. The juice was filtered through a muslin cloth. The deposit left on the muslin cloth was washed 3–4 times with distilled water, until only a small amount of starch was passing the muslin cloth. The filtrate was collected in a glass beaker and the residue left on the muslin cloth discarded. The content of beaker containing filtrate were passed through fine sieves (200 and 100 μm mesh size, respectively) and was kept undisturbed for 6 h. A layer of starch settled down. The starch layer obtained was reslurried in distilled water, filtered using a filtration assembly and, again, starch was allowed to settle. This washing process was repeated 3–4 times until the supernatant became transparent after filtration. The starch was carefully removed from the filter and dried at 35 °C in a hot-air cabinet drier to a moisture content of ~7%.

2.3.2. Starch morphological and physico-chemical characteristics

The starch granule size distribution was determined with a laser diffraction particle size analyzer (Malvern Mastersizer, Malvern Instruments Limited, UK). Starch (0.1 g for Kamo Kamo starch and 0.1125 g for potato starch, dry weight basis) was dissolved in 150 ml distilled water and mixed at a very slow speed using a magnetic stirrer for 1 h at room temperature prior to measurement. An obscuration level of ~20% was maintained during measurements on the Mastersizer (Nayouf, Loisel, & Doublier, 2003).

Scanning electron micrographs of the starches were obtained with a scanning electron microscope (Stereoscan 250 Mk3, Cambridge Instruments Limited, Cambridge, UK) at different magnifications. Dry powdered starch was sprinkled on to double-sided sticky tape fixed on an aluminium stub, and coated with gold.

The amylose content of starches isolated from Kamo Kamo fruit and potato was estimated using iodine colorimetry by the method of McGrance, Cornell, and Rix (1998), as modified by Hoover and Ratnayake (2002). The method is based on the iodine binding capacity and spectral properties of the amylopectin- and the amylose–iodine complexes, respectively. Starch (20 mg, dry weight basis) was dissolved in 90% dimethyl sulphoxide (8 ml) in 10 ml screw-cap reaction vials. The contents of the vials were vigorously mixed for 20 min and then heated in a water bath (with intermittent shaking) at 85 °C for 15 min. The vials were then cooled to ambient temperature, and the contents diluted with water to 25 ml in a volumetric flask. The diluted solution (1.0 ml) was mixed with water (40 ml) and 5 ml of iodine (I_2)/potassium iodide (KI) solution (0.0025 M I_2 and 0.0065 M KI) and then adjusted to a final volume of 50 ml. The contents were allowed to stand for 15 min at ambient temperature before absorbance measurements at 600 nm. A standard curve was plotted for mixtures of pure amylose and pure amylopectin.

Swelling power and solubility of starches were determined by the method of Leach, McCowen, and Schoch (1959). A 2% (w/v) suspension of starch was heated at 70 and at 90 °C for 30 min, followed by rapid cooling in an ice water bath to room temperature. The suspension was then centrifuged at 3500*g* for 20 min. Supernatant was decanted carefully and residue weighed for swelling power determination. The ratio between the residue and initial starch dry matter was calculated (g/g of starch on dry weight basis) as the swelling power. The supernatant was dried to a constant weight at 110 °C. The residue obtained after drying the supernatant represented the solubility of starch (g/g of starch on dry weight basis). Light transmittance (%) of the pastes from the starches was measured using the method described by Craig, Maningat, Seib, and Hoseney (1989). A 2% (w/v) aqueous suspension of starch at near neutral pH was heated in a boiling water bath for 30 min with constant stirring. The dispersion was cooled to 25 °C. Samples were stored for five days at 4 °C and transmittance (%) was measured every 24 h at 640 nm against a water blank with a UV–Vis spectrophotometer.

The solubility (%) of the starches in anhydrous dimethyl sulphoxide (DMSO) was measured during 18 h of stirring of starch suspensions (to keep granules suspended) using the method of Yeh and Yeh (1993). Potato and Kamo Kamo starches (0.5 g, dry weight basis) were suspended in 100 ml of anhydrous DMSO in 250 ml centrifuge bottles and placed horizontally in a reciprocating shaker. After 18 h, each bottle was removed and centrifuged (10,000*g* for 15 min). In both cases, a well-defined sedimentation of undissolved substance left a clear supernatant. A 50-ml aliquot of the latter was removed by pipette and added to 150 ml of methanol with vigorous stirring. The mixture was heated in a steam bath for 20 min and then allowed to stand overnight. The heat treatment was repeated twice. The precipitated starch (A) was obtained by filtering the mixture on a medium-porosity, tared, fritted Pyrex crucible that was pre-washed with methanol and dried in a vacuum oven for 4 h at 120 °C. The solubility calculation was

$$\%\ \text{solubility} = \text{wt. of } A/0.25 \times 100.$$

The transmittance (%) of the starch suspensions (0.5%, w/v) in DMSO was measured at 640 nm against a DMSO blank with a UV–Vis Spectrophotometer after 2, 4, 6, 8, 12, 16 and 18 h of shaking.

2.3.3. Starch thermal characteristics

Thermal characteristics of starches were analyzed using a DSC (Perkin-Elmer Ltd, Norwalk, CT) equipped with a thermal analysis data station. Starch (~3.5 mg, dry weight basis) was weighed into a 40 μl capacity aluminium pan and distilled water was added with the help of a Hamilton micro-syringe to achieve a starch-water suspension containing 70% water. Pans were hermetically sealed and allowed to stand for 4 h at room temperature before heating in the DSC. The DSC analyzer was calibrated using indium, and an empty aluminium pan was used as reference. Sample pans were heated at a rate of 10 °C/ min from 20 to 100 °C. Onset temperature (T_o), peak temperature (T_p) conclusion temperature (T_c) and enthalpy of gelatinization (ΔH_{gel}) were calculated. The gelatinization temperature range (R) and peak height index (PHI) were computed as described by Vasanthan and Bhatty (1996). Enthalpies were calculated on dry starch basis.

2.3.4. Starch rheological characteristics

Small amplitude oscillatory three-step rheological measurements (temperature sweeps during heating and cooling, and a frequency sweep on the cooled sample) were made on starches from each source, with a dynamic rheometer (Physica MCR 301, Anton Paar Germany, GmbH, Germany) equipped with parallel plate system (4 cm diameter). The gap size was set at 1000 μm. Starch suspensions of 15% (w/w) concentration were stirred for 15 min (20 °C) at very slow speed using a magnetic stirrer, and loaded on to the lower plate of the rheometer (preheated to 35 °C). The sample edge was covered with a thin layer of low-density silicone oil (to minimize evaporation losses) before starting the experiments. During the temperature sweeps, strain and frequency were set at 0.5% and 1 Hz, respectively. The starch samples were heated from 35 to 80 °C and cooled from 80 to 35 °C at the rate of 3 °C/min. The cooled starch gels were held at 35 °C for 20 min and then subjected to a frequency sweep test with a frequency range of 0.1–20 Hz, with the strain set at 0.5%. Dynamic rheological parameters such as storage modulus (G'), loss modulus (G''), complex modulus (G^*), loss tangent ($\tan\delta$), complex viscosity (η^*) and dynamic viscosity (η) were determined for both types of starch as functions of temperature and frequency.

2.3.5. Starch syneresis

Starch suspensions (2%, w/v) isolated from both the sources were heated at 90 °C for 30 min in a temperature controlled water bath with constant stirring, followed by rapid cooling to room temperature (in 6 min) using an ice-water bath. The starch sample was stored for 7 days at 4 °C. Syneresis was determined at 1, 2, 3, 4 and 7 days as the amount of water released (as a percentage by mass of the sample) after centrifugation at 3000*g* for 15 min.

2.4. Statistical analysis

The data were subjected to statistical analysis using Minitab Release 14 Statistical Software (Minitab Inc., State College, PA).

3. Results and discussion

3.1. Fruit characteristics

3.1.1. Proximate analysis

The results of the chemical analyses (ash, total nitrogen, fat and fibre) performed on freeze-dried Kamo Kamo fruit are summarized in Table 1. The ash content of Kamo Kamo was 4.64% (d.w), while the nitrogen and fat contents were 0.76% (d.w) and 0.77% (d.w.), respectively. The soluble fibre content of Kamo Kamo was 3.33%. The ash, fat and fibre contents of Kamo Kamo fruit were observed to be lower than those reported for other summer squashes (USDA, 2002). The calcium and sodium levels were lower (Table 2) while the potassium levels were similar to other summer squashes (USDA, 2002). The differences in the total nitrogen and mineral composition of the Kamo Kamo compared with other squashes may be attributed to the variation in genotypic and soil characteristics (soil salinity, soil nutrients and fertilizers) (Graifenberg, Botrini, Giustiniani, & Lipucci, 1996; Chance, Somda, & Mills, 1999; Villora, Moreno, Pulgar, & Romero, 1999).

The starch and the sugar (sucrose, glucose and fructose) levels of the Kamo Kamo fruit are shown in Table 3. The starch content of Kamo Kamo fruit was 23%. The starch content of winter squashes has been reported to vary between 30% and 42% (Cumarasamy, Corrigan, Hurst, & Bendall, 2002; Phillips, 1946). The growth and formation of the starch granules has been suggested to depend on the activity of the starch synthesis enzymes (Krossmann & Lloyd, 2000). The rate of starch accumulation in fruits has also been reported to be affected by the number of days after anthesis during development (Hewitt, Dinar, & Stevens, 1982; Ho, Sjut, & Hoad, 1983; Walker & Thornley, 1977). The sucrose, glucose and fructose contents of Kamo Kamo differed considerably from those reported for other squashes (Cumarasamy et al., 2002; Rouphael et al., 2004). The sucrose level (2.74%) was lower, whereas the glucose and fructose levels were observed to be higher than those reported for both winter and summer squashes (Cumarasamy et al., 2002; Rouphael et al., 2004). The higher concentrations of glucose and fructose in Kamo Kamo may be attributed to the higher activity of sucrose

Table 1
Proximate chemical composition of freeze-dried Kamo Kamo

Parameter	Percentage[a] (% basis)
Moisture	11.47 ± 0.35
Dry matter	88.53 ± 0.35
Ash	4.64 ± 0.07
Total nitrogen	0.76 ± 0.037
Fat	0.77 ± 0.028
Insoluble fibre	11.27 ± 0.041
Soluble fibre	3.33 ± 0.060
Total dietary fibre	14.60 ± 0.15

[a] The values are the means of three determinations ± standard error.

Table 2
The mineral element composition of freeze dried Kamo Kamo

Mineral element	Composition[a]
Phosphorus (g/100 g)	0.271 ± 0.002
Potassium (g/100 g)	1.96 ± 0.004
Calcium (g/100 g)	0.154 ± 0.001
Magnesium (g/100 g)	0.211 ± 0.005
Sodium (g/100 g)	<0.005
Manganese (ppm)	7.6 ± 0.008
Zinc (ppm)	21 ± 0.007
Copper (ppm)	5.1 ± 0.002
Selenium (ppm)	<0.02

[a] The values are the means of three determinations ± standard error.

Table 3
Starch and sugar contents of freeze-dried Kamo Kamo

Minerals	Percentage[a] (% basis)
Starch	23.18 ± 0.25
Glucose	17.65 ± 0.14
Fructose	22.55 ± 0.15
Sucrose	2.74 ± 0.08

[a] The values are the means of three determinations ± standard error.

metabolizing enzymes such as acid invertase and sucrose synthase, which hydrolyze/convert sucrose into fructose and glucose (Wang, Sanz, Brenner, & Smith, 1993).

3.2. Starch characteristics

3.2.1. Morphological and physico-chemical characteristics

The full granule size distribution of the isolated Kamo Kamo and potato starches are shown in Fig. 1, and the distributions in terms of percentages of small, medium and large granules in Table 4. The granule size variation in the Kamo Kamo starch was lower than that normally observed for potato, corn and wheat starches (Singh et al., 2003). The granule size of Kamo Kamo starch ranged from 3 to 23 μm. Kamo Kamo starch contained a high percentage of medium granules and a small percentage of small granules. In contrast, the potato starch had a high percentage of large granules and a very low percentage of small granules. The mean granule volume of the Kamo Kamo starch was considerably lower than that of potato starch. The size of starch granules has been reported to vary between 1.5 and 13 μm for different cultivars of winter squashes (Stevenson et al., 2005). The granule size distributions of potato, corn, rice and wheat starches have been observed to exhibit ranges of 1–85, 1–25, 3–5 and 1–35 μm, respectively (Kaur, Singh, & Sodhi, 2002; Singh, Singh, & Saxena, 2002b; Singh, Kaur, & Singh, 2004; Singh et al., 2003; Singh & Kaur, 2004; Tester & Karkalas, 2002). The granule size distribution of starches from different botanical sources has been reported to change during the development of the storage organs of plants (Chojecki, Gale, & Bayliss, 1986).

Kamo Kamo starch appeared as a mixture of spherical, polyhedral and dome shaped granules, when viewed under SEM (Fig. 2a and b). The dome-shaped granules were

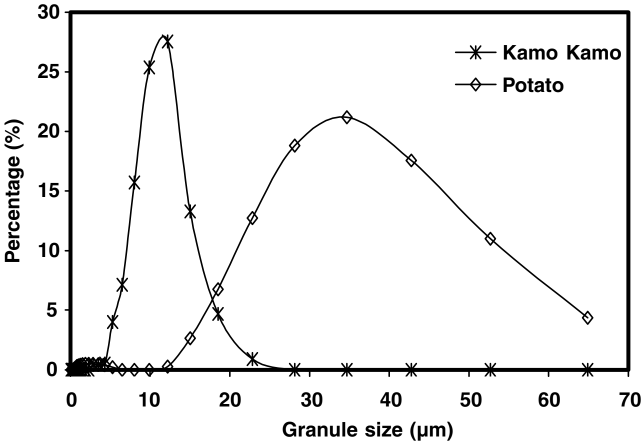

Fig. 1. Granule size distribution of Kamo Kamo and potato starches.

Table 4
Morphological parameters of Kamo Kamo and potato starches

Starch source	Small granules (%) (1–5 μm)	Medium granules (%) (6–23 μm)	Large granules (%) (>23 μm)	Mean volume (μm^3)
Kamo Kamo	5.39b	94.61b	0	432a
Potato	4.67a	22.40a	72.93	8552b

Values with the same letters in column did not differ significantly ($p < 0.05$).

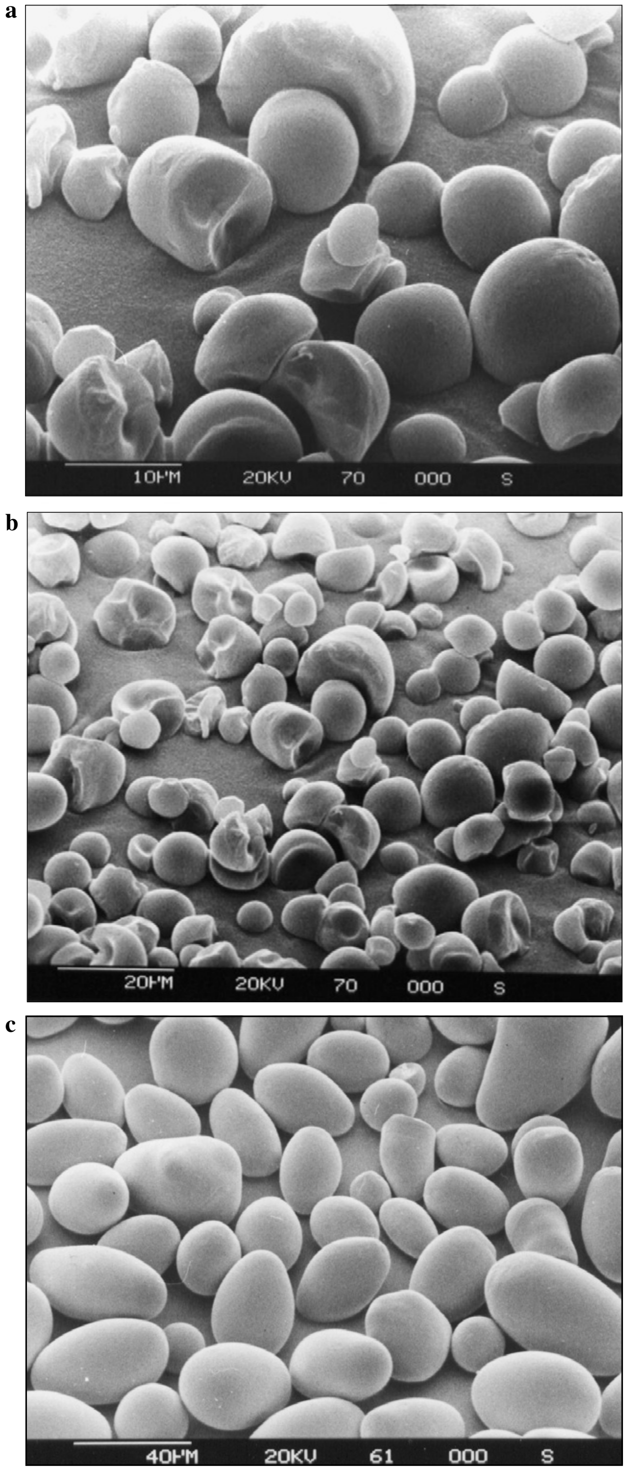

Fig. 2. Scanning electron micrographs (SEM) of Kamo Kamo and potato starches (a) Kamo Kamo (2000×, Bar = 10 μm), (b) Kamo Kamo (1000×, Bar = 20 μm), (c) Potato (500×, Bar = 40 μm).

observed to be the largest in size. The shape of the potato starch granules varied from spherical to irregular or cuboid. Many of the polyhedral and dome shaped Kamo Kamo starch granules showed indentations on their surfaces in contrast to smooth-surfaced potato starch granules (Fig. 2c). The biochemistry of the chloroplast or amyloplast, as well as the physiology of the plant, mainly dictates the morphology of starch granules (Badenhuizen, 1969). The membranes and the physical characteristics of the plastids may also be responsible for providing a particular shape or morphology to starch granules during granule development (Jane et al., 1994; Lindebooma, Chang, & Tylera, 2004).

3.2.2. Physico-chemical characteristics

The amylose contents of the Kamo Kamo and potato starches were 17.2% and 23.9%, respectively (Table 5). Starches with an amylose content ranging from 17% to 30% may be classified as normal (Thomas & Atwell, 1999). The amylose content of winter squash starches of different cultivars has been reported to vary from 13% to 18% whereas it varies between 15% and 29% for normal corn, rice, wheat and potato starches (Singh et al., 2003; Stevenson et al., 2005). The differences in amylose content between different starches may be attributed to differences in the activities of the enzymes involved in the biosynthesis of linear and branched components within starch granules (Krossmann & Lloyd, 2000). The amylose content of the starch granules has also been reported to be affected by climatic conditions and soil type during growth, and to vary with granule size (Asaoka, Okuno, & Fuwa, 1985; Cottrell, Duffus, Paterson, & George, 1995; Inatsu, Watanabe, Maida, Ito, & Osani, 1974; Juliano, Bautista, Lugay, & Reyes, 1964; Morrison & Azudin, 1987; Singh et al., 2003).

The swelling power and solubility of pastes of the starches from the two different sources varied to a greater extent than did amylose content (Table 5). The Kamo Kamo starch had lower swelling power and solubility than did potato starch at both 70 and 90 °C. The weak internal organization resulting from negatively charged phosphate ester groups within the starch granules has been reported to be responsible for the high swelling power of potato starches (Kim, Wiesenborn, Lorenzen, & Berglund, 1996). Though the swelling power of Kamo Kamo starch was lower than that of potato starch, it was found to be considerably higher than the swelling power values reported for normal corn and rice starches (Kaur et al., 2005; Sodhi & Singh, 2005). The hydration and swelling of starch during heating reflects the magnitude of interaction between the starch chains within the amorphous and crystalline domains (Liu, Ramsden, & Corke, 1999). The amylose to amylopectin ratio and the molecular weight/distribution of amylose and of amylopectin may affect the extent of this interaction, resulting in variation in the swelling power and solubility of the starch.

The light transmittance of the gelatinized starch pastes of Kamo Kamo and potato also differed considerably (Fig. 3). Potato starch paste showed a higher initial light transmittance than did Kamo Kamo starch. The differences in the granule size and swelling capabilities of both the starches may have affected the light transmittance properties of their pastes (Singh et al., 2002a, 2002b; Kaur et al., 2005; Singh et al., 2003, 2004). Starches with a higher proportion of large granules have been reported to contain fewer granule remnants in their pastes, thus allowing the light to pass through instead of being refracted and/or scattered, resulting in higher light transmittance (Singh & Singh, 2003; Singh & Kaur, 2004). The phosphorus content of starches has also been suggested to influence the light transmittance properties (Lim & Seib, 1993). The light transmittance of both the starch pastes decreased progressively during storage at 4 °C. However, this decrease was more pronounced in potato starch paste during the initial 48 h.

The solubility and light transmittance of the two different starches in DMSO varied significantly (Table 5 and Fig. 4). Potato starch was observed to be the more soluble

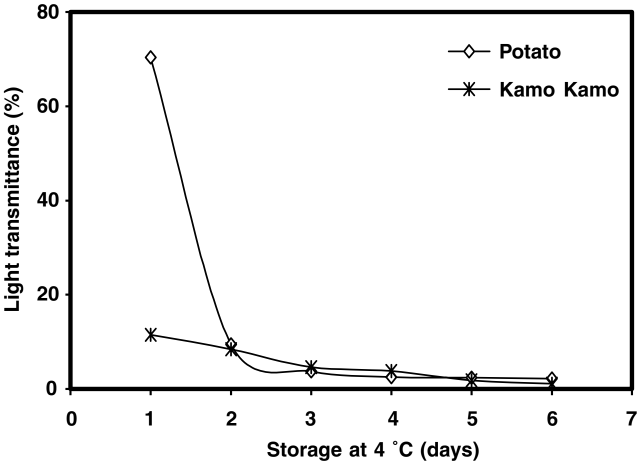

Fig. 3. Light transmittance (%) in water of Kamo Kamo and potato starch pastes as a function of storage time at 4 °C.

Table 5
Physico-chemical properties of Kamo Kamo and potato starches

Starch source	Amylose content (% basis)	Swelling power (g/g)		Solubility in water (g/g)		Solubility in DMSO (% basis)
		70 °C	90 °C	70 °C	90 °C	
Kamo Kamo	17.2a	17.5a	28.4a	0.0126a	0.0305a	30.5a
Potato	23.9b	30.8b	40.7b	0.0225b	0.0505b	71.4b

Values with the same letter in a column did not differ significantly ($p < 0.05$).

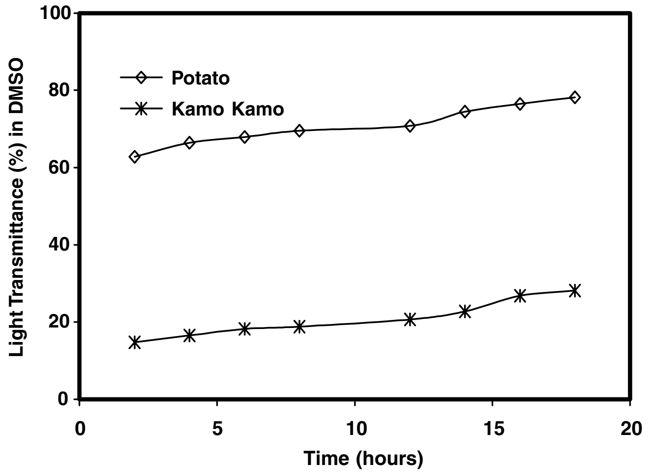

Fig. 4. Light transmittance (%) in DMSO of Kamo Kamo and potato starch pastes as a function of time.

in DMSO (71.4%). This solubility value is in accordance with those reported earlier for different potato starches (Kaur, Singh, & Singh, 2004). Kamo Kamo starch showed considerably lower solubility in DMSO (30.5%). The fairly high large granule percentage and higher mean granule volume may have resulted in the higher solubility of potato starch in DMSO. Being a hydrogen bond acceptor, DMSO breaks associative hydrogen bonding in the starch molecules (French, 1984; Cooreman, van Rensburg, & Delcour, 1995). Solubilization of the starch granules in DMSO has been reported to occur also through surface erosion (French, 1984). Therefore, Kamo Kamo starch, owing to its relatively small granules and lower mean granule volume, dissolved in DMSO to a lower extent. Sahai and Jackson (1996) reported that the extent of starch solubilization in DMSO varied significantly within a population of starch granules of different sizes, presumably reflecting inherent structural heterogeneity among the granules. The light transmittance (%) in DMSO of the starches increased steadily with time (Fig. 4). After 12 h, potato starch showed more than 68% transmittance in DMSO, while the Kamo Kamo starch showed less than 25% even after 18 h.

3.2.3. Thermal characteristics

Thermal properties of Kamo Kamo and potato starch are presented in Table 6. Both starches showed thermal profiles typical of normal starches from different sources. For both the Kamo Kamo and potato starches, a single endothermic transition was observed, which corresponds to the dissociation of the amylose and amylopectin molecules within the starch granules, and leaching out of amylose to the continuous phase (Fujita, Lida, & Fujiyama, 1992; Liu, Lelie'vre, & Ayoung, 1991). The thermal profile values of potato starch are in accordance with the reported values for potato starches in the literature (Kim et al., 1995; Singh & Singh, 2001). Kamo Kamo starch showed values of T_o, T_p and T_c of 64.1, 68.7 and 77.5 °C, which were considerably higher than those of potato starch (Table 6). T_o, T_p and T_c values ranging between 61 and 70 °C have been reported by Stevenson et al. (2005) for starches separated from different cultivars of winter squashes. The T_c for most normal starches from different sources has been observed to vary between 66 and 80 °C, depending on their T_os (Singh et al., 2003). It has been suggested that the amylopectin branch chain length of the starches may influence the transition temperatures (Asaoka et al., 1985; Jane, Shen, Lim, Kasemsuwan, & Nip, 1992; Yuan, Thompson, & Boyer, 1993; Kuakpetoon & Wang, 2006). The higher transition temperatures of Kamo Kamo starch may also be due to its small granule population; starches containing smaller granules have been reported to exhibit higher transition temperatures (Singh & Kaur, 2004; Yeh & Yeh, 1993). The enthalpy of gelatinization (ΔH_{gel}) was observed to be higher for Kamo Kamo starch (16.0 J/g) than for potato starch (14.25 J/g). Enthalpy of gelatinization gives an overall measure of crystallinity (quality and quantity) and is an indicator of the loss of molecular order within the granule that occurs on gelatinisation (Cooke & Gidley, 1992; Hoover & Vasanthan, 1994; Tester & Morrison, 1990). The ΔH_{gel} has been reported to be influenced by the degree of crystallinity of the starches (Eliasson & Gudmunsson, 1996; Thirathumthavorn & Charoenrein, 2006). It has been reported in earlier studies that starch granule size, phosphorus content, granule shape, amylopectin chain length, and crystalline regions of different stability and/or size mainly influence the thermal properties of starches (Noda, Takahata, Sato, Ikoma, & Mochinda, 1996; Singh & Kaur, 2004; Stevens & Elton, 1971; Singh & Singh, 2001; Wang et al., 2005; Yuan et al., 1993).

Table 6
Thermal properties of Kamo Kamo and potato starches

Starch source	T_o	T_p	T_c	ΔH_{gel}	*PHI*	R
Kamo Kamo	64.1b	68.7b	77.5b	16.0b	3.5a	13.4b
Potato	61.7a	65.0a	70.3a	14.3b	4.3b	8.5a

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH_{gel}, enthalpy of gelatinization (dwb); *PHI*, peak height index ($\Delta H_{gel}/(T_p - T_o)$) and *R*, gelatinization range ($T_c - T_o$).

Values with the same letter in a column did not differ significantly ($p < 0.05$).

The *PHI* and *R* of the starches also differed to a considerable extent (Table 6). A higher *PHI* (4.31) was calculated for potato starch, while it was lower for Kamo Kamo starch (3.48). An exceptionally higher (13.4) *R* value was calculated for Kamo Kamo starch. The differences in the *R* values between the two starches may be due to the presence of small crystallites of slightly different crystal strengths within the crystalline regions of their starch granules that delay the completion of the gelatinization process to different extents (Banks & Greenwood, 1975). Further investigations on the molecular structure of Kamo Kamo starch is required to explain these differences.

3.2.4. Rheological characteristics

The structural transitions associated with phase change in the starch systems are reflected by changes in the rheological profiles and are described by rheological properties such as G', G'', G^*, $\tan\delta$, η^* and η'. The G', G'' and G^* of starch dispersions showed a tendency to increase to maxima during initial heating ($G^* > G' > G''$) (Fig. 5) and then dropped with further heating, which agrees with previous findings (Gonzalez-Reyes, Mendez-Montealvo, Solorza-Feria, Toro-Vazquez, & Bello-Perez, 2003; Hsu, Lu, & Huang, 2000). At early stages of heating, the amylose molecules would have dissolved from the starch granules and the suspension becomes a sol; thus increases in G' and G'' were relatively small. With further increase in temperature, G' and G'' increased and reached maxima, which may be attributed to the formation of a network of swollen starch granules (Hsu et al., 2000). The decrease in G' and G'' with further increase in temperature indicates the destruction of this gel structure with prolonged heating (Gonzalez-Reyes et al., 2003; Hsu et al., 2000).

The temperature at which G' was maximal (TG') showed a significant variation between the two starches. Potato starch showed a lower TG' (63.5 °C) than did Kamo Kamo starch (69.4 °C). The higher G' and G'' of 4390 and 1190 Pa, respectively, were observed for potato starch compared with the lower values of 1880 and 315 Pa, respectively, for Kamo Kamo starch, at their respective TG's (Fig. 5a and b). The other rheological parameters such as G^*, η^* and η' followed the same trends (Figs. 5c and d). The large granules of potato starch may have been responsible for its higher G' and G'' values during the heating cycle, while the presence of small granules with lower swelling capability in the Kamo Kamo starch may have been responsible for its lower G' and G'' values. Disintegration of starch granules occurred as the heating continued and this extent of disintegration was quantified by calculating the difference between the maximal G' at TG' and the minimal G' (that occurred at 85 and 75 °C for Kamo Kamo starch and potato starch, respectively). The drop in G' value also differed significantly between the two starches (Fig. 5a). A higher drop of 935 Pa in G' was observed for potato starch whereas it was lower, at 730 Pa, for Kamo Kamo starch. The $\tan\delta$ values during heating at and above the gelatinization temperature of both the starches were considerably less than unity, indicating a high degree of solid-like behaviour (Table 7).

The values of all the rheological properties except $\tan\delta$ increased during cooling of the heated starch pastes from 85–35 °C (Fig. 6a–d and Table 7). Potato starch exhibited higher G' and G'' values compared with Kamo Kamo starch. A decrease in $\tan\delta$ values during cooling of starches has been suggested to be evidence for gel formation (Reddy

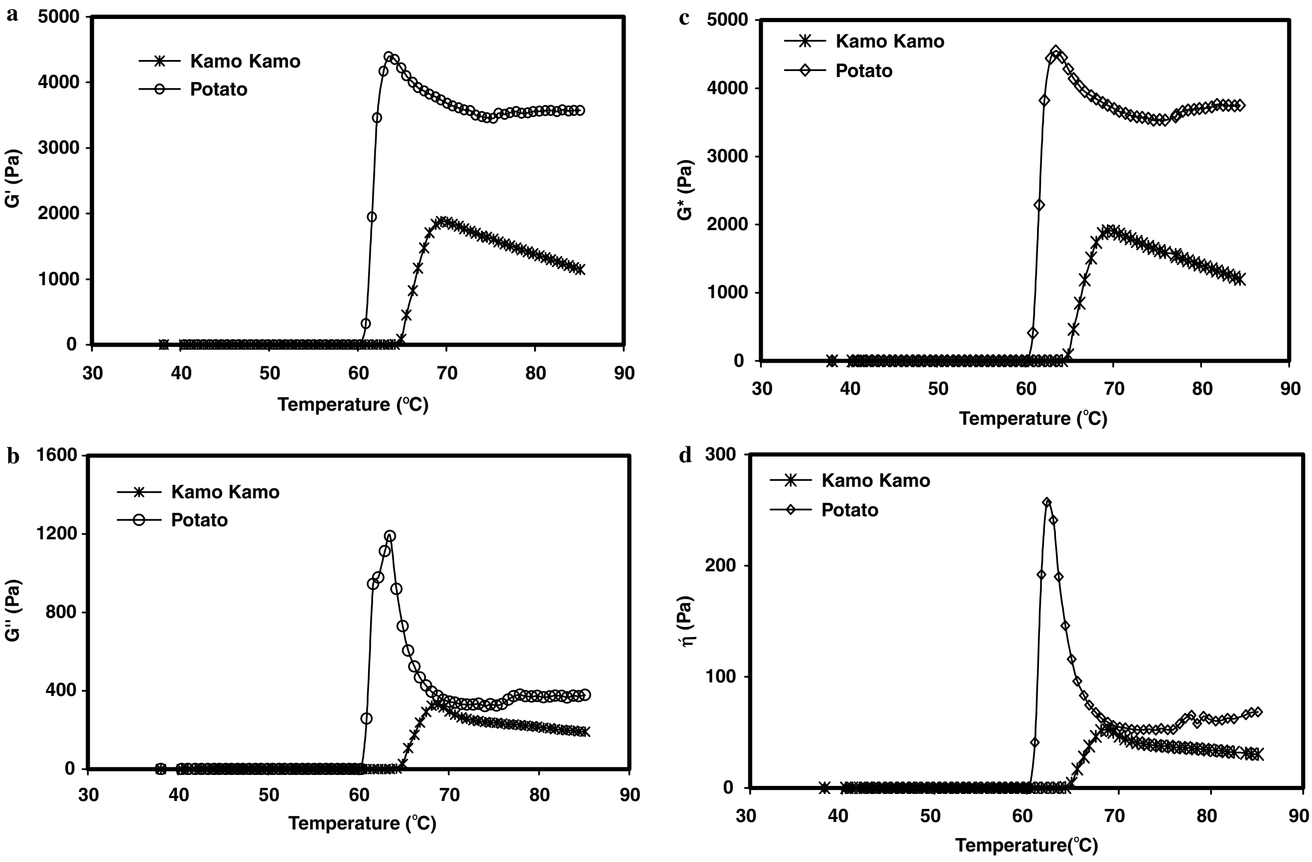

Fig. 5. Rheological properties of Kamo Kamo and potato starches during temperature sweep – heating (a) storage modulus (G'), (b) loss modulus (G''), (c) complex modulus (G^*), (d) dynamic viscosity (η').

Table 7
Rheological properties of Kamo Kamo and potato starches

Starch source	Test	G'	G''	Tan δ	G^*	η^*	η'
Kamo Kamo	Heating	1880a	315b	0.167c	1910a	303b	50c
Potato	Heating	4390d	1190d	0.272d	4550c	725d	190d
Kamo Kamo	Cooling	1790a	195a	0.109b	1800a	287b	31b
Potato	Cooling	4190c	297b	0.071a	4200d	668c	47b
Kamo Kamo	Frequency	2900b	315b	0.109b	2910b	23.2a	2.5a
Potato	Frequency	6690e	514c	0.076a	6710e	53.4a	4.1a

Values with the same superscripts in a column did not differ significantly ($p < 0.05$).

(G'), storage modulus; (G''), loss modulus; (G^*), complex modulus; (tan δ), loss tangent; (η^*), complex viscosity and (η'), dynamic viscosity. Heating, rheological parameter values at 69.4 °C for Kamo Kamo starch and 63.5 °C for potato starch. Cooling, rheological parameter values at 35 °C. Frequency, rheological parameter values at 35 °C and 20 Hz.

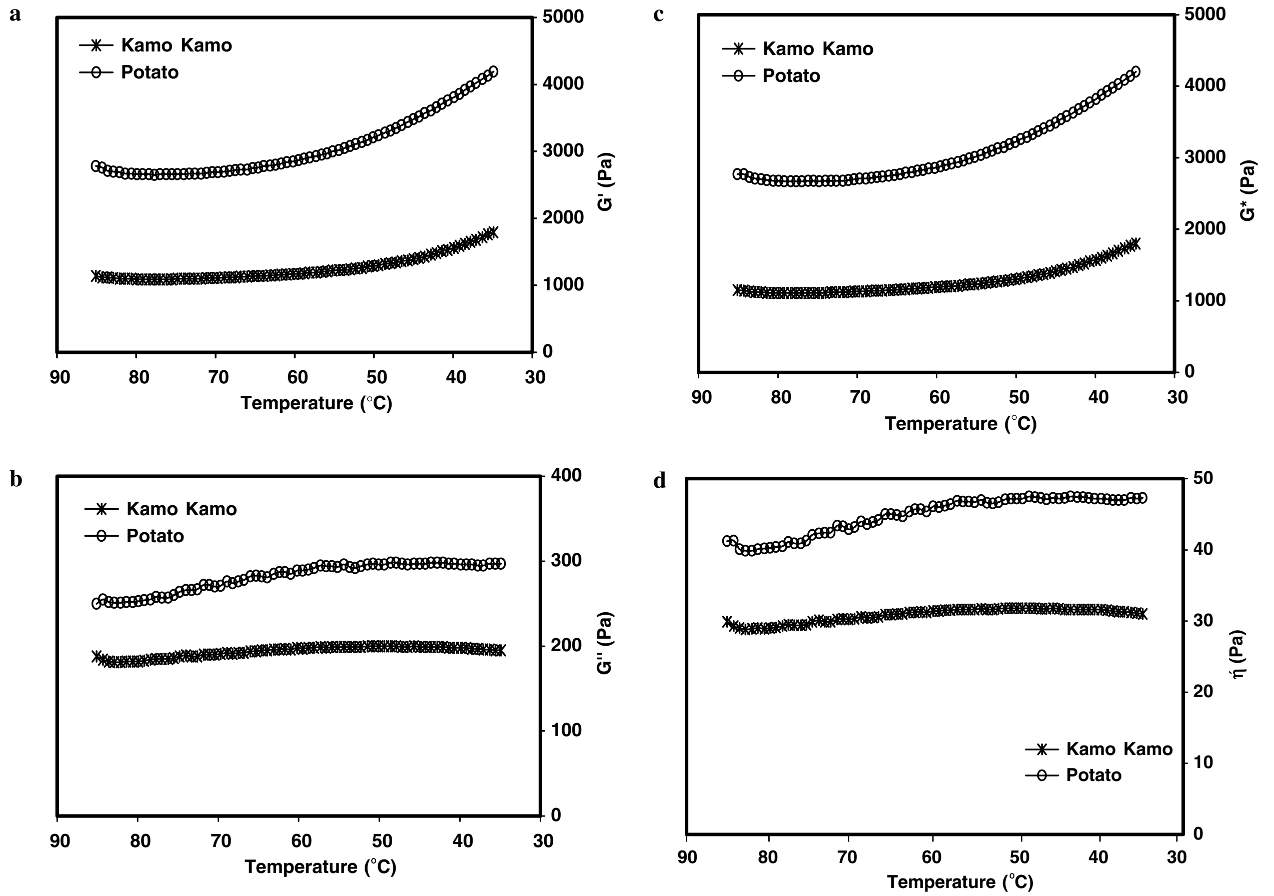

Fig. 6. Rheological properties of Kamo Kamo and potato starches during temperature sweep – cooling (a) storage modulus (G'), (b) loss modulus (G''), (c) complex modulus (G^*), (d) dynamic viscosity (η').

& Seib, 2000). The decrease in tan δ might be due to retrogradation of leached components and interaction between molecules remaining inside the granule, reinforcing the gel structure during cooling (Hsu et al., 2000). The frequency dependence of the G' and G'' may also give valuable information about the structure. A material that is frequency independent over a large time scale range is solid-like; a true gel system is such a material. In contrast, strong frequency dependence suggests a material structure with molecular entanglements that behaves more like a solid at higher frequencies and more like a liquid at lower frequencies (Ross-Murphy, 1984). All of the rheological properties showed significant differences between Kamo Kamo and potato starches with respect to frequency dependence (Fig. 7a–d). G', G'' and G^* increased while η^* and η' decreased with increasing frequency. Kamo Kamo starch showed lower values for rheological properties compared with potato starch (Table 7 and Fig. 6a–d). Differences in rheological properties between the two starches can be attributed to differences in granule size and shape, the presence of phosphate esters and the amylose to amylopectin ratio (Wiesenborn, Orr, Casper, & Tacke, 1994).

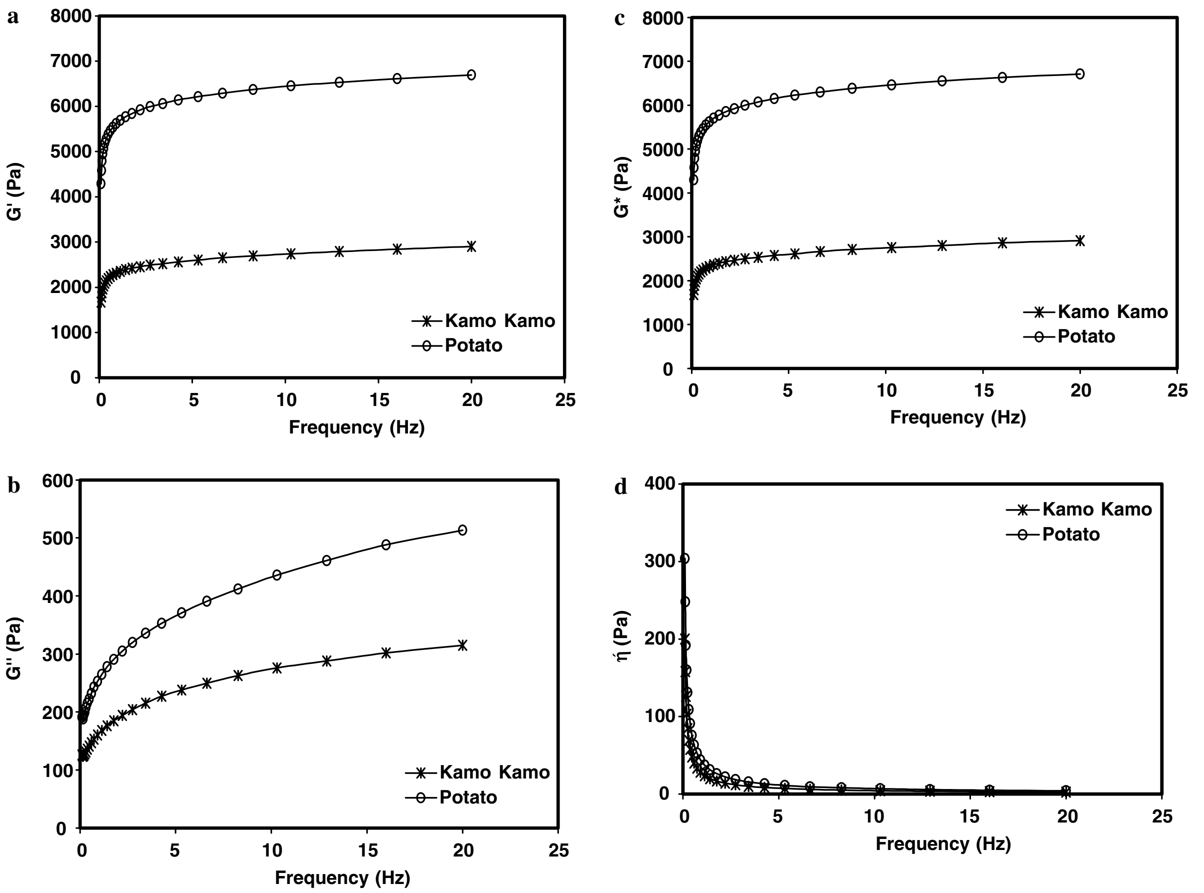

Fig. 7. Rheological properties of Kamo Kamo and potato starches during frequency sweep (a) storage modulus, (b) loss modulus, (c) complex modulus, (d) dynamic viscosity.

3.2.5. Syneresis

The syneresis % of the gelatinized starch pastes are shown in Fig. 8. Kamo Kamo starch paste showed lower syneresis than did the potato starch paste after 24 h, the difference increasing during 7 days of storage at 4 °C (Fig. 8). Both the starch pastes tended to synerese at a higher rate during the first four days than later. Thereafter, the syneresis (%) increased steadily in both starch pastes up to the 7th day of storage at 4 °C. The syneresis (%) of Kamo Kamo starch was lower than that of potato starch at all times. Amylose aggregation and crystallization have been reported to be complete within the first few hours of storage while amylopectin aggregation and crystallization occur at later stages (Miles, Morris, Orford, & Ring, 1985). The syneresis of starches are indirectly influenced by the structural arrangement of starch chains within the amorphous and crystalline regions of the ungelatinized granule, because this structural arrangement influences the extent of granule breakdown during gelatinisation and also influences the interactions that occur between starch chains during gel storage (Perera & Hoover, 1999).

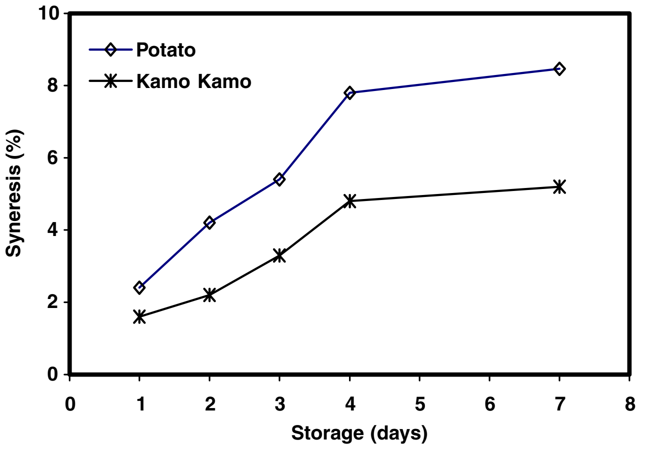

Fig. 8. Syneresis (%) of Kamo Kamo and potato starch pastes as a function of time at 4 °C.

4. Conclusions

Kamo Kamo differs from other winter and summer squashes mainly owing to differences in the reducing and non-reducing sugar levels. Starch, the predominant component of Kamo Kamo fruit could be classified as normal type (based on amylose content) small or medium size granule starches. Kamo Kamo starch exhibited behaviour similar to that of potato starch. However, it presented considerable differences in physico-chemical and functional properties. Based on the observed composition and functionality, the Kamo Kamo fruit and its starch may find suitable applications in the food processing industry. Further research on Kamo Kamo fruit and its starch is needed to utilize their unique characteristics for novel product development.

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