Carbohydrate Polymers 78 (2009) 462–468

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

New starches: Physicochemical properties of sweetsop (*Annona squamosa*) and soursop (*Anonna muricata*) starches

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ARTICLE INFO

Article history:
Received 23 February 2009
Received in revised form 30 April 2009
Accepted 6 May 2009
Available online 13 May 2009

Keywords:
Sop starches
Composition
Physicochemical properties
Rheological properties

ABSTRACT

Starch from the fruits of sweetsop (*Anonna squamosa*) and soursop (*Anonna muricata*) were isolated and purified and the fat, ash, phosphorus and protein contents measured. The amount of amylose present was determined spectrophotometrically and found to be very similar (~19%) for both starches. Scanning electron microscopy showed very small indented and spherical granules from both with an average granule size of 4.84 μm and 4.72 μm, respectively. The physicochemical properties, namely the swelling power, solubility, pasting characteristics, paste clarity and freeze–thaw stability were studied to assess the functionality of the starch pastes as hydrocolloids. The sweetsop starch showed higher swelling power and solubility compared to soursop starch and had a lower gelatinization temperature indicating a weaker granular structure. Sweetsop starch exhibited a lower pasting temperature, higher viscosity peak, higher viscosity breakdown and lower setback, higher paste clarity and freeze–thaw stability compared to soursop starch. The low gelatinization temperature and high freeze thaw stability of sweetsop starch are comparable to that of waxy corn. The properties of sweetsop indicate that it has potential for application as a thickener in frozen foods.

1. Introduction

Starch remains a major source of calories in the human diet and can be found in high concentration in the main storage organs of plants including roots/tubers, stems, seeds/grains and fruits. Starches from roots/tubers (Nwokocha, Aviara, Senan, & Williams, 2009; O'Hair, 1990; Peroni & Rocha, 2006; Tetchi, Rolland-Sabate, N'guessan Amani, & Colonna, 2007) and seeds/grains (Baik & Jackowski, 2004; Lorenz, 1976) have been extensively studied and have application in processed food products. The search for novel starches has resulted in recent focus on fruit starches (Adewusi, Udio, & Osuntogun, 1995; Fuke & Matsuoka, 1984; Goni, Escribano, & Meridio, 2008; Harshe & Bhagwat, 2006; Kayisu, Hood, & van Soest, 1981; Ketiku, 1973; Kwok et al., 2006; Loos, Hood, & Graham, 1981).

Annona squamosa L. (sweetsop or sugar apple) and *Annona muricata* L. (soursop) are small tropical trees or shrubs which belong to the genus *Annona* (family: *Annonaceae*). They are presently cultivated on small scale for their fruits. Fifteen plants have been listed as belonging to this genus in PlantFiles (Dave's garden plant database). Starch from *Annona reticulata* (Harshe & Bhagwat, 2006) and *Annona cherimoya* (Goni et al., 2008) fruits have been isolated and characterized. Starches from sweetsop and soursop fruits have not been reported. The fruits of sweetsop and soursop are oval or heart-shaped with tender soft pliable spines which breakup easily when the fruit is ripe. Soursop fruits are bigger and larger; about 10–30 cm long and could weigh as much as 4.5 kg (Morton, 1987) while sweetsop fruits weigh about 0.1–0.15 kg. Sweetsop and soursop fruits have been used as food for a long time and for making exotic drinks (Morton, 1987). The carbohydrate content has been reported to be 14.63–15.1% in soursop pulp (Morton, 1987) and 19–25% in sweetsop (Duke & DuCellier, 1993). We have isolated starch from sweetsop and soursop fruits and examined the granule structure, pasting and rheological properties, as a way of finding its suitability for food application.

2. Materials and methods

2.1. Isolation of starch

The fruits were washed, peeled and seeds extracted. The pulp was cut into pieces 5–6 cm cubes and immediately rinsed in sodium sulphite solution and sun dried. The dried chips were converted to flour using a Philips blender mill. Known weight of the flour was dispersed in five times its weight of distilled water for 2 h. This was sieved with a muslin cloth. The residue was washed with water until the wash water was clean. The dirty starch milk was then centrifuged at 5000 rpm for 30 min and the

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doi:10.1016/j.carbpol.2009.05.003

supernatant was decanted. The resulting starch sediment, which contained a thin brown mucous layer, was dispersed in a solution of 0.3% sodium hydroxide (w/v) and washed repeatedly with the same until a clean white starch resulted on centrifuging. The clean starch was dispersed in distilled water and washed repeatedly until the washing water was neutral to litmus. The recovered starch was sun-dried and stored in an airtight container.

2.2. Proximate analysis

Moisture was determined by oven-drying the starch sample at 105 °C overnight. The oven dried samples were used in further analysis. The ash, fat, crude protein ($N \times 6.25$), phosphorus, free sugar and starch were determined by standard methods (International Institute of Tropical Agriculture, 1995).

2.3. Blue value and amylose content

To 0.1 g starch in a test tube was added 1 ml of ethanol (95%) to disperse the starch followed by 9 ml of 1 M NaOH solution and heated in a water bath to gelatinize the starch. This was transferred quantitatively into a 100 ml standard volumetric flask and made up to the mark with distilled water. About 5 ml of the solution was taken into a 100 ml volumetric flask and 1 ml of 1 M acetic acid added followed by 2 ml stock iodine (0.2 g I_2/2 g KI) solution and made up to mark with distilled water. This was left for 20 min for the colour to fully develop. The solution was put in a 1 cm cuvette and scanned in a Lambda 25 UV/visible spectrophotometer (wavelength 350–950 nm, scan speed 480 nm/min) using iodine solution at the same concentration but without starch in the reference cell. A calibration curve was prepared with pure potato amylose (Sigma–Aldrich, UK) from which the amylose content of the starch was obtained by extrapolation from the absorbance–amylose concentration curve. The absorbance readings were measured at 620 nm (Juliano, 1971).

The blue value was calculated as:

$$\frac{\text{Maximum absorbance} \times 4}{\text{Starch concentration (g/dL)}}.$$

2.4. Starch microscopy

Granule micrographs were obtained with a JSM 35 Genie Scanning Electron Microscope (SEM). The starch was sprinkled onto a double-backed adhesive carbon tab stuck to a circular aluminum stub. The aluminum stub with the starch sample on it was placed in the vacuum chamber of a Polaron PS 3 sputter coater, after attaining a vacuum of 0.1–0.2 torr and plasma current of 42 mA, gold coating process was carried out for 140 s. The stub with gold coated starch was then placed in the SEM chamber which was evacuated before the electron beam was turned on. A 10 kV/2.05 A setting was used for the subsequent imaging work on starch, the aperture size being fixed at 3. Granule size analysis was obtained on a BT1600 Image Analyzer at a magnification of 40×.

2.5. Physicochemical characterization

2.5.1. Determination of swelling power and solubility

Swelling power and solubility was determined using an aqueous dispersion of 0.5 g starch (d,b) in a pre-weighed centrifuge tube and 25 ml distilled water. The tubes were immersed in a thermostatic Clifton water bath maintained at 80 °C for 30 min and thoroughly stirred with a glass rod all through the heating period. The tubes were removed, cooled to room temperature and centrifuged at 5000 rpm for 15 min. The supernatant was carefully transferred into a pre-weighed crucible, evaporated over a steam bath and dried in the oven at 120 °C to constant weight. The weight of the paste was determined and used to calculate the swelling power as weight of sedimented paste per gram of dry starch. The difference in weight after drying the supernatant gave the weight of the soluble material and was used to calculate the percentage solubility as weight of soluble material per dry weight of starch.

2.5.2. Determination of pasting characteristics

The pasting characteristics were determined with a Rapid Visco-Analyzer ((RVA series 4, Newport Scientific, NSW, Australia) on a 12% starch slurry. Sample (3 g, dry basis) was weighed and dispensed into a canister together with 25 ml of water. The paddle was placed centrally inside the canister and then inserted into the RVA machine. The measurement cycle was initiated and the profile determined over 12 min. The time-temperature regime used was: Idle temperature 50 °C for 1 min, heated from 50 °C to 95 °C in 3 min 45 s, then held at 95 °C for 2 min 30 s, the sample was subsequently cooled to 50 °C over a 3 min 45 s period followed by a period of 2 min where the temperature was controlled at 50 °C.

2.5.3. Determination of paste clarity

Paste clarity was determined by the method of Singhal and Kulkarni (1990) by measuring the percentage light transmitted by different concentrations of starch (0.5–4.0%) at 660 nm on a UV/visible spectrophotometer. Distilled water was used in the reference cell.

2.5.4. Determination of freeze–thaw stability

The freeze–thaw stability was determined according to the method of Singhal and Kulkarni (1990). The determination was carried out by heating 5% (w/v) starch (d,b) in distilled water at 95 °C for 30 min with constant stirring. This was then removed and stirring continued during cooling to avoid formation of skin. About 10 ml of paste was transferred to weighed centrifuge tubes. The weight of the paste was then determined. This was subjected to alternate freezing and thawing cycles (18 h, 3 h, respectively) for 9 days, centrifuged at 5000 rpm for 10 min after each cycle and the percentage water separated determined as weight of exudates to the weight of paste.

2.5.5. Rheological properties

The rheological properties of the starch pastes were measured on 8% starch pastes. The pastes were prepared by heating starch in aqueous dispersion (8%, w/v) in a water bath maintained at 99 °C for 30 min. The starch dispersion was stirred within the first minute of immersion during which pasting occurred. The stirring was stopped and the sample left to cook for 30 min. The paste was removed and left at 25 °C and the rheological properties examined after for 1 h. The flow properties were measured on a controlled stress Rheometer (AR 2000, TA Instruments Ltd.) with cone and plate geometry (40 mm, 2°Cone and 52 μm gap). Measurements were carried out at 25 °C at shear rates of 10^{-1} to 120 s^{-1}. The TA Data Analysis software was used to fit the Power law model (Eq. (1)) into the viscosity-shear rate profiles.

$$\sigma = \eta(\gamma)^{N}, \tag{1}$$

where σ = shear stress (Pa), η = viscosity (Pa s), γ = shear rate (1/s) and N = rate index.

The viscoelastic properties of the starch pastes were determined by carrying out a frequency sweep in the range of 10^{-1} to 120 $rads^{-1}$ within the viscoelastic region (strain, 0.05%). The linear viscoelastic region was obtained by performing a stress sweep within the range of 0.01–50 Pa at an angular frequency of 2.683 $rads^{-1}$. The variation of storage modulus (G′) and loss modulus

(G″) with angular frequency was analyzed by the TA Data Analysis software.

2.5.6. Statistical analysis

Analysis of variance was done with MATLAB 7.6.0. One-way ANOVA was used to compare sample means at 95% confidence level.

3. Results and discussion

3.1. Starch composition

Table 1 shows the yield and composition of sweetsop (*A. squamosa*) and soursop (*A. muricata*) starches. Starch composition of sweetsop was 25.6% and soursop 27.3%. Harshe and Bhagwat (2006) reported starch yield of 19% for *A. reticulata*. The moisture content was ~9% for both starch samples. Both starches showed a statistical difference ($p < .05$) in the content of fat, ash, amylose and starch purity. Starch phosphorus of 25.5 mg/100 g has been reported for Kiwi fruit starch (Fuke & Matsuoka, 1984), this value is similar to that found for soursop starch. Starch phosphates have been reported to greatly affect starch physicochemical properties. The presence of phosphate weakens intermolecular association of the starch molecules and lowers the energy requirements for the gelatinisation process (Smith, 1982). Fig. 1 shows the starch–iodine absorption spectra for sweetsop and soursop starches. The two starches had similar absorption characteristics (Table 2). The blue value of sweetsop was 0.4295 and soursop 0.4226. The amylose content of sweetsop (19.35%) and soursop (19.31%) was in the range (18–30%) found in normal starches. Lower amylose composition has been reported for kiwi fruit starch (10.8%) (Fuke & Matsuoka, 1984) and passion fruit starch (yellow variety, 8.7%; purple variety, 5.8%) (Kwok et al., 2006). Amylose composition in starch has been reported to affect starch physicochemical properties and reactivity (Kitahara et al., 2007; Kuakpetoon & Wang, 2006; Thomas & Atwell, 1999).

3.2. Granule size distribution, morphology and gelatinization properties

The scanning electron micrographs of sweetsop and soursop starches are presented in Fig. 2a and b, respectively. The shape of the granules is spherical, truncated and irregular. The granule size distributions of sweetsop and soursop obtained using an image particle size analyzer are presented in Fig. 3a and b, respectively, while the particle size analysis is in Table 3. Sweetsop starch has a granule size range of 2.92–6.42 μm, granule average of 4.84 μm, L/D of 1.20 and roundness of 0.73. For soursop, starch granule size ranged from 2.49 to 7.68 μm, granule average 4.72 μm, L/D 1.23 and roundness 0.70. The granules of sweetsop and soursop can be classified as very small to small according to the classification of Lindeboom, Chang, and Tyler (2004). Granules of passion fruit are of the order of 6.4–7.8 μm (Kwok et al., 2006) while kiwi fruit starch granules averaged 5.5 μm. This might indicate the *Annona* fruit starches consist of very small granules. The

Table 1
Chemical composition of sweetsop and soursop starches.

Starch property	Sweetsop	Soursop
*Yield (%)	25.6	27.30
Moisture (%)	9.11 ± 0.1[a]	9.07 ± 0.1[a]
Fat (%)	0.2305 ± 0.1[a]	0.0107 ± 0.0[b]
Ash (%)	1.12 ± 0.1[a]	2.30 ± 0.2[b]
Protein (%)	0.2142 ± 0.02[a]	0.2324 ± 0.03[a]
Phosphorus (%)	0.031 ± 0.01[a]	0.025 ± 0.01[a]
Sugar (%)	3.17 ± 0.3[a]	2.965 ± 0.2[a]
Starch (%)	68.92 ± 0.5[a]	70.44 ± 0.7[b]
Amylose (%)	19.35 ± 0.0[a]	19.31 ± 0.0[b]

Averages are means of three determinations ± SD.
[a,b] Values in the same row with different superscripts are statistically different ($p < .05$).
* Based on dry weight of flour.

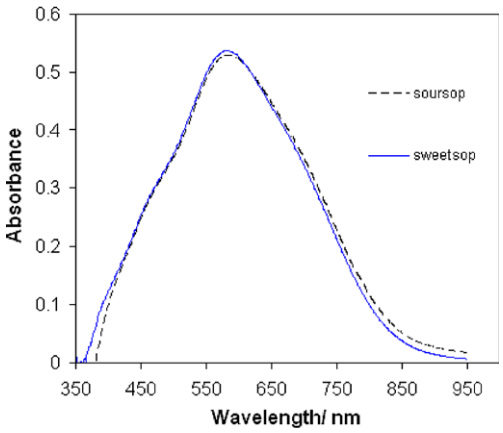

Fig. 1. Absorption spectra of starch–iodine complexes of sweetsop and soursop starches.

Table 2
Starch–iodine absorption characteristics of sweetsop and soursop starches.

Starch source	Sweetsop	Soursop
Wavelength of maximum absorbance (nm)	581–583	585
Absorbance	0.5369	0.5283
Blue value	0.4295	0.4226

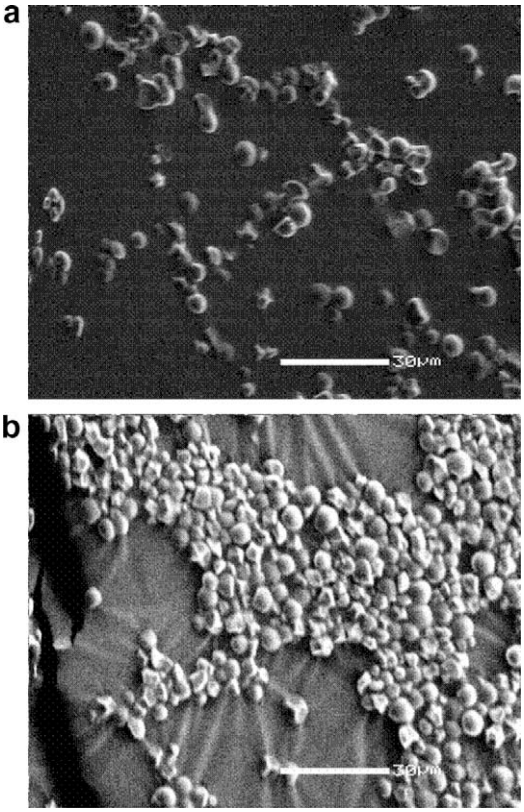

Fig. 2. (a) Scanning electron micrographs of soursop starch granules (X1600). (b) Scanning electron micrographs of sweetsop starch granules (X1600).

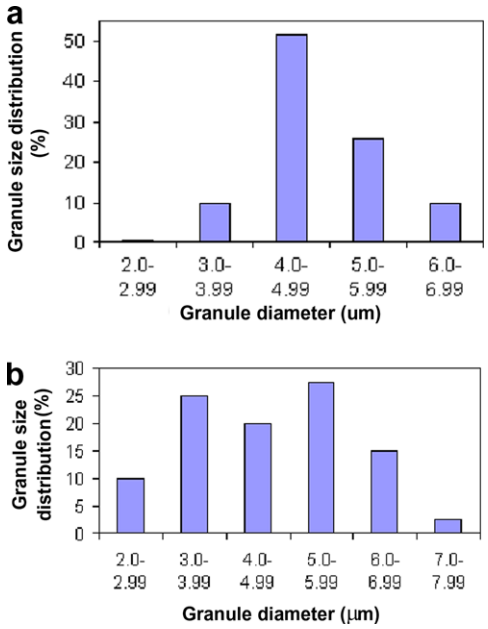

Fig. 3. (a) Granule size distribution of sweetsop starch. (b) Granule size distribution of soursop starch.

Table 3
Granule size analysis of sweetsop and soursop starches.

Particle characteristics	Sweetsop	Soursop
Particle number	31	40
Maximum diameter (μm)	6.42	7.77
Minimum diameter (μm)	2.92	2.76
Average granule size (μm)	4.84	4.72
Length/diameter, L/D	1.20	1.23
Roundness	0.73	0.70

gelatinization process was investigated by determining the gelatinization parameters: Onset (T_o), peak (T_p), completion (T_c) and enthalpy change (ΔH) by differential scanning calorimetry. The endothermic heat flow and the results of analysis are presented in Fig. 4 and Table 4, respectively. Sweetsop starch started gelatinizing at a temperature of 64.12 °C and completed at 72.99 °C with an endothermic enthalpy of 10.62 J/g while in soursop gelatinization started at 65.67 and completed at 75.30 °C with an endothermic enthalpy of 4.58 J/g. The gelatinization parameters indicate weaker intra-granular bonds strength or a lesser crystalline structure in sweetsop starch compared with soursop starch. When compared with some regular starches (Table 4), the gelatinization temperature peaks of sweetsop and soursop were higher than those of potato and cassava but close to sago and waxy corn, with sweetsop similar to waxy corn. Starch gelatinization is affected by the degree of crystallinity, amylose/amylopectin ratio, molecular mass, granule architecture and amylose–lipid complexes. Goni et al. (2008) have reported gelatinization peak temperatures of 63.7–65.2 °C and enthalpies of 14.8–15.9 J/g for cherimoya fruit starch during ripening. Fuke and Matsuoka (1984) reported a gelatinization temperature of 72 °C for kiwi starch while Kwok et al. (2006) reported gelation temperatures for varieties of passion fruit (yellow, 58.5–67.0 °C; purple, 58.5–66.5 °C).

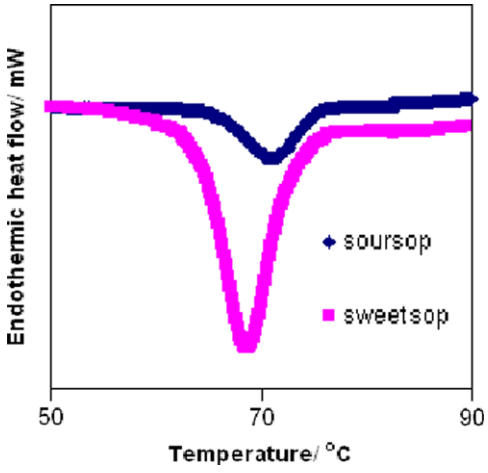

Fig. 4. DSC thermograms of 10% dispersions of sweetsop and soursop starches.

3.3. Swelling power and solubility

Swelling power and solubility of starches provide evidence of the crystalline nature of the granules (Leach, McCowen, & Schoch, 1959). From Table 5, the swelling power of sweetsop (42.37 g/g starch) was higher than of soursop (27.40 g/g starch) and the solubility of sweetsop (16.75%) was higher than of soursop (13.99%); with both parameters being significantly different for the two starches ($p < .05$). This indicates that sweetsop has a weaker granule structure than soursop. When starch dispersions are heated in water, the granules acquire thermal energy; the resulting thermal agitations weaken the intra-granular bonds in proportion to the strength of the binding forces. Weaker forces relax first causing the granules to absorb water and swell, the lower molecular weight amylose solubilizes and leaches out of the granule into the surrounding medium. Increased thermal agitation causes significant swelling of the granules and the internal bonds become fragile. At a critical stress point, the swollen envelope ruptures becoming a ghost, releasing the majority of the internal starch molecules, while a majority of the starch polymers remain trapped by the collapsed ghost (Atkin, Abeysekera, & Robards, 1998). Previous workers have demonstrated that high swelling and solubility is due to the existence of a loose granule structure and low molecular weight amylose which leaches out of the amorphous domains of the starch granules (Balagopalan, Padmaja, Nanda, & Moorthy, 1988; Nwokocha et al., 2009). The swelling and solubility of starch granules have been reported to be affected by amylose/amylopectin ratio, the characteristics of each fraction in terms of molecular weight, length/degree of branching and the physical manner in which these constituents are arranged within the starch granules together with the presence of naturally occurring non carbohydrate impurities (Leach, 1965). Amylose has been reported to inhibit swelling, especially in the presence of lipids which can form insoluble complexes with some of the amylose during swelling and gelatinization (Leach et al., 1959).

3.4. Paste viscosity

The pasting characteristics are shown in Table 6 while the pasting curves are shown in Fig. 5a and b. Sweetsop starch started pasting at 74.75 °C and attained a viscosity peak of 578.25 RVU in 4.30 min. On holding at 95 °C, the viscosity thinned down to 247.25 RVU and on cooling to 50 °C retrograded to a final viscosity of 317.25 RVU. For soursop starch, pasting occurred at a higher temperature (77.55 °C), attained a viscosity peak at 428.92 RVU in 4.69 min, and on holding at 95 °C, the viscosity dropped to 284.67 RVU and retrograded to a final viscosity of 375.67 RVU on cooling to 50 °C. Sweetsop starch paste suffered more viscosity breakdown (331 RVU) than soursop starch paste (144 RVU) indicating a poor capacity of the starch paste to withstand severe

Table 4
Gelatinization properties of sweetsop and soursop starches.

Gelatinization parameters	Sweetsop	Soursop	Sago*	Waxycorn*	Potato*	Cassava*
Onset temperature, T_o (°C)	64.12	65.67				
Peak temperature, T_p (°C)	68.62	70.96	69.3–70.1	68.1	63.1	66.3
Completion temperature, T_c (°C)	72.99	75.30				
Gelatinization range, ($T_c - T_o$) (°C)	8.87	9.63				
Endothermic enthalpy, ΔH (J/g)	10.62	4.58	15.1–16.7	13.9	17.8	15.1

* Ahmad, Williams, Doublier, Durand, and Buleon (1999).

Table 5
Swelling power and solubility of sweetsop and soursop starches.

Parameter	Sweetsop	Soursop
Swelling power (g/g)	42.37 ± 0.1[a]	27.40 ± 0.1[b]
Solubility (%)	16.75 ± 0.2[a]	13.99 ± 0.1[a]

Averages are means of three determinations ± SD.
[a,b] Values in the same row with different superscripts are significantly different ($p < .05$).

Table 6
RVA paste viscosity analysis of sweetsop and soursop starches.

Pasting property	Sweetsop	Soursop
Pasting temperature (°C)	74.75	77.55
Peak viscosity (RVU)	578.25	428.92
Pasting time (min)	4.30	4.69
Viscosity trough (RVU)	247.25	284.67
Breakdown viscosity (RVU)	331.00	144.25
Final viscosity (RVU)	317.25	375.67
Setback viscosity (RVU)	70.00	91.00

processing conditions. The very low setback of the starch pastes especially sweetsop indicates a potential for use in frozen foods (Pal, Singhal, & Kulkarni, 2002).

3.5. Paste clarity and freeze–thaw stability

From Fig. 6, sweetsop had higher paste clarity than soursop at all starch concentrations. The stability of a given starch paste to retrogradation and its suitability for use in frozen foods is determined by the level of syneresis when subjected to freeze–thaw cycles. A starch paste is freeze–thaw stable if it releases little or no exudate when subjected to freeze–thaw cycles. From Fig. 7, sweetsop starch survived three freeze–thaw cycles before any noticeable syneresis occurred and achieved a maximum exudate of 15.6% after eight freeze–thaw cycles. Soursop starch survived the first freeze–thaw cycle and achieved maximum separation of 43% after eight freeze–thaw cycles. These values indicate that sweetsop has good freeze–thaw stability. It also has a lower retrogradation tendency than soursop. The freeze–thaw stability of sweetsop is comparable to that of waxy corn starch (Takeiti, Fakhouri, Ormenese, Steel, & Collares, 2007). High freeze–thaw stability has been reported for *Amaranthus hypochondriacus* starch paste which survived four freeze–thaw cycles before significant syneresis occurred (Yanez, Messinger, Walker, & Rupnow, 1986), however, positive syneresis has been reported for starch isolated from plantain fruits (Nwokocha & Williams, 2009; Perez-Sira, 1997). There is no available data on freeze–thaw stability of sop starches.

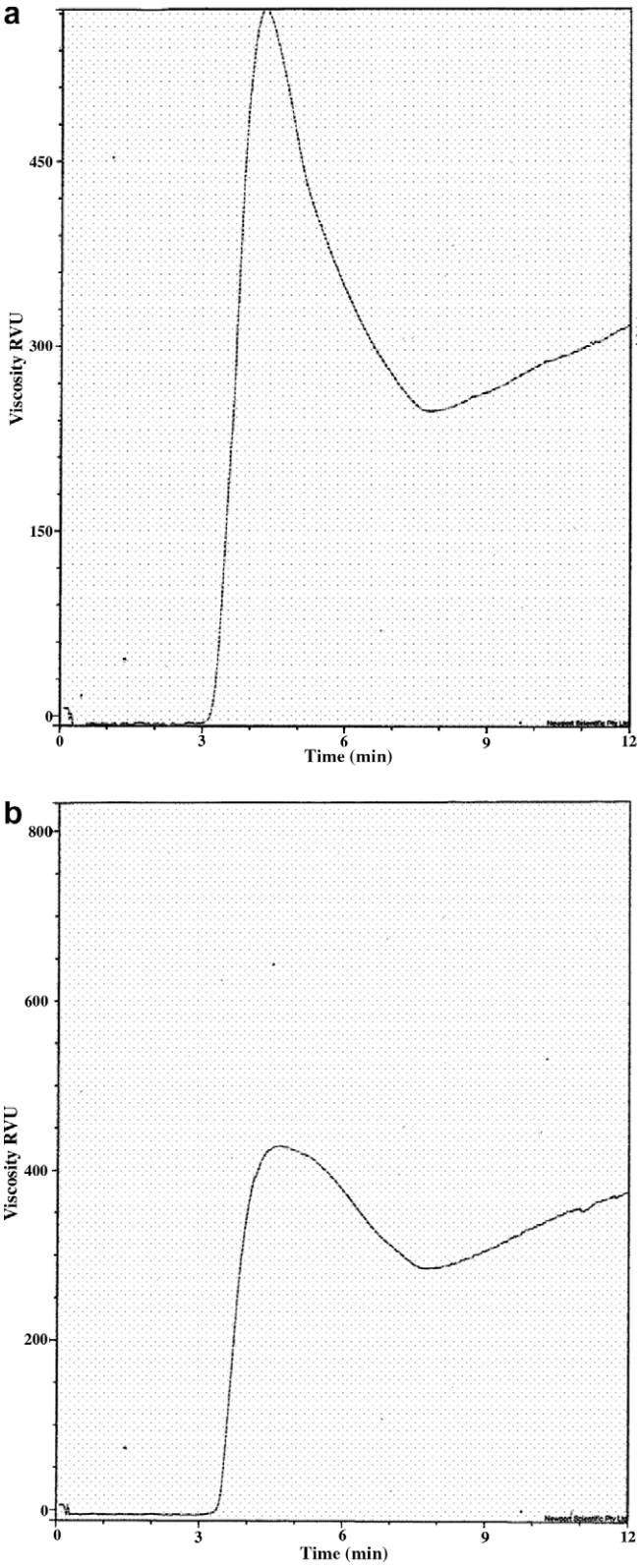

Fig. 5. (a) RVA pasting curve for sweetsop starch. (b) RVA pasting curve for soursop starch.

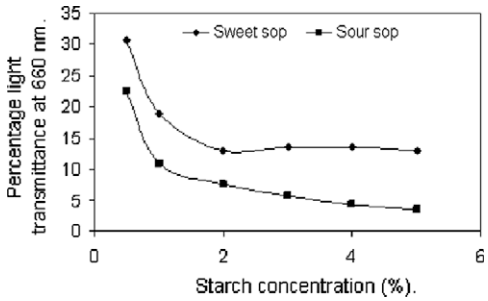

Fig. 6. Paste clarity of sweetsop and soursop starches.

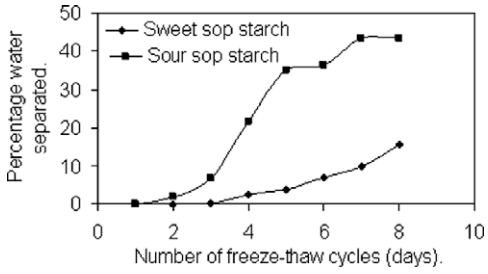

Fig. 7. Freeze–thaw stability of sweetsop and soursop starches.

3.6. Rheological properties

The viscosity of 8% gels of sweetsop and soursop starch pastes are shown as a function of shear rate in Fig. 8. The curves are almost superimposed and show that the viscosity decreases with increasing shear rate. The rate index, *N*, is low (0.24 and 0.28 for sweetsop and soursop, respectively), confirming the pastes are highly shear thinning in common with other starches such as corn (Malumba, Massaux, Deroanne, Masimango, & Bera, 2009). Fig. 9 presents the mechanical spectra for the two starches. The storage modulus, G′, is significantly greater than the loss modulus, G″, for the two samples and both moduli exhibit some frequency dependence indicating weak gel characteristics. G′ and G″ values have been reported to be a function of starch molecular weight (Della Vale, Buleon, Carreau, Lavoie, & Vergnes, 1998).

4. Conclusion

Starch isolated from sweetsop and soursop fruits has been characterized. Both starches had small granules (2.49–2.76 μm), similar amylose composition (∼19%) and gelatinization temperatures (sweetsop, 64.12–72.99 °C; soursop, 65.67–75.30 °C). The sweetsop starch showed higher swelling power and solubility, lower pasting temperature, higher viscosity peak, higher viscosity breakdown and lower setback; higher paste clarity and freeze–thaw stability compared to soursop starch. Both starches indicated weak

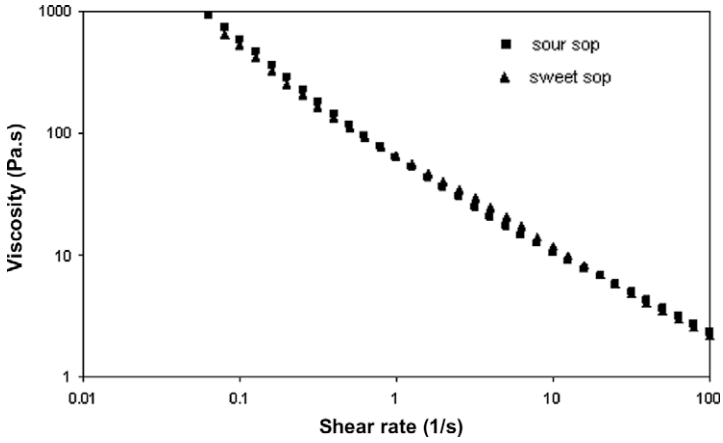

Fig. 8. Viscosity-shear rate profiles of 8% gels of soursop and sweetsop starches.

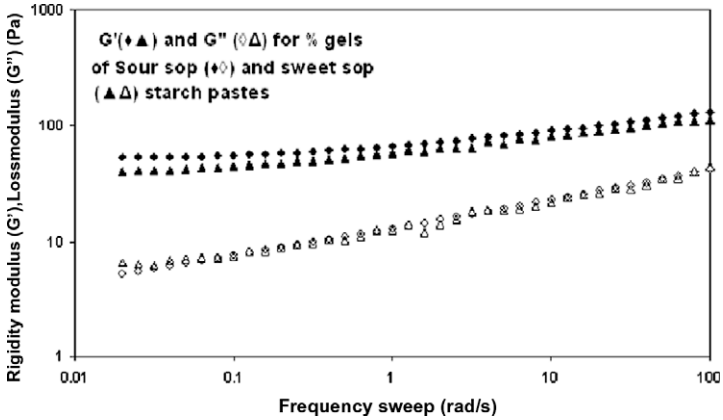

Fig. 9. Frequency sweep showing G′ (♦▲) and G″ (◇△) for % gels of soursop (♦◇) and sweetsop (▲△) starch passtes.

gel characteristics. The functionality of sweetsop starch is comparable to those of waxy corn and *A. hypochondriacus* starches, making it a candidate for use in instant or frozen foods.

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