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Structural and physicochemical characteristics of winter squash (*Cucurbita maxima* D.) fruit starches at harvest

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Abstract

Structures and physicochemical properties of starch isolated from fruit (pepo) of seven winter squash cultivars (*Cucurbita maxima* D.) were studied. Squash starches exhibited the B-type X-ray diffraction pattern and had most granules with diameters of 1.5–2.5, 6–8, and 11–13 μm. Squash amylopectin had more long branch chains (25.4–29.3% DP ≥ 37), and squash starch had relatively small absolute amylose contents (12.9–18.2%). Squash starch amylopectins had weight-average molecular weights ranging from 2.03 to 3.22×10^8 g/mol, gyration radii of 294–337 nm, and unusually low polydispersity (1.21–1.81). Isoamylase-debranched amylopectins showed average chain-length varied from DP 26.5 to 28.1. Squash starches had onset gelatinization temperature ranging from 60.6 to 63.5 °C and enthalpy changes (ΔH) from 15.9 to 17.4 J/g. Retrogradation rate for squash starches ranged from 41 to 55% after 7 days at 4 °C. Squash starch pastes, measured by using a Rapid Visco-Analyzer, had peak viscosity of 174–233 RVU, final viscosity of 193–244 RVU, and setback of 79–100 RVU, with pasting temperature of 65.6–68.8 °C.

Keywords: Starch; Starch structure; Starch function; Amylose; Amylopectin; Winter squash; Pumpkin; Cucurbit

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1. Introduction

Starch is the major carbohydrate in plant storage organs. Starch structures and physicochemical properties have been characterized for storage organs of many plant families. However, Cucurbitaceae, which includes squash and pumpkins, have had little published results on fruit (pepo) starch characteristics, despite the fruit dry matter starch content ranging from <3% (Corrigan, Hurst, & Potter 2001; Schales & Isenberg, 1963) to >60% (Hurst, Corrigan, Hannan, & Lill, 1995; Merrow & Hopp, 1961). Studies have shown that texture of squash fruit is related to its starch content and possibly starch properties (Corrigan et al., 2001; Corrigan, Irving, & Potter, 2000; Culpepper & Moon, 1945; Cumarasamy, Corrigan, Hurst, & Bendall, 2002; Daniel, Brecht, Sims, & Maynard, 1995; Harvey, Grant, & Lammerink, 1997; Hurst et al., 1995; Merrow & Hopp, 1961; Schales & Isenberg, 1963; Smittle, Hayes, & Williamson, 1980).

Sugimoto, Yamashita, Oomori, et al. (1998), investigated fruit starch properties of one cultivar each of *Cucurbita maxima* D. and *C. moschata* D. during development. Squash starch was shown to exhibit a B-type X-ray pattern, granule diameter of 2.9–8.8 μm, amylose content ranging from 14 to 23%, and onset gelatinization temperature of 62–66 °C. Starch granules were also reported to have a larger diameter range (3–35 μm) for the same *C. maxima* D. cultivar (Yoshida, 1989). Starch content and granule size variation between external and internal cellular layers of the fruit were also reported (Yoshida, 1989).

There are few cultivars of squash with limited starch properties characterized. To our knowledge, there has been no report on starch retrogradation, phosphorus content

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of starch, absolute amylose content, and amylopectin structures such as molecular size, gyration radii, and amylopectin branch chain-length distribution in any *Cucurbita* L. sp. starch. In this study, we characterized structures and physicochemical properties of starch isolated from fruit of seven cultivars of *C. maxima* D., grown in the same year, at the same location and using identical procedures. Structures and physicochemical properties of starch will be related to their textural and eating qualities reported elsewhere.

2. Materials and methods

2.1. Plant material

Seven squash cultivars were planted in summer of 1998 at an Iowa State University farm site 1.7 miles south of Ames, Iowa (geographical location 41°58′57.5″N, 93°38′22.9″W), in a completely randomized block (3.05 × 3.05 m blocks) with 18 replicates (four plants/replicate). Normal crop husbandry was followed as required. Five replicates of each cultivar were randomly selected for analysis of starch characteristics. Squash cultivars studied, with range of fruit weight observed in parentheses, were three buttercups [Delica (0.79–2.68 kg), Kurijiman (0.75–2.13 kg) and Sweet Mama (1.61–3.25 kg)], one Halloween-type [Prizewinner (13.7–70.2 kg)], one Hubbard-type [Warren Scarlet (2.70–8.12 kg)], one Crown-type [Whangaparoa Crown (1.82–6.03 kg)] and one Native American Indian squash [Lakota (1.24–3.78 kg)] (Coyne, Reiser, Sutton, & Graham, 1995). Pictures of winter squash fruits for all the cultivars have been reported previously (Stevenson, 2003). Seeds of Kurijiman, Warren Scarlet and Whangaparoa Crown were purchased from Webling and Stewart Ltd, Petone, New Zealand; Delica seeds from Yates New Zealand Ltd, Onehunga, New Zealand; Prizewinner seeds from King's Seeds, Auckland, New Zealand; Sweet Mama seeds from Henry Field Seed and Nursery Co., Shenandoah, IA, and Lakota seeds from W. Atlee Burpee and Co., Warminster, PA. Squash fruit maturity was adjudged when stalks became woody (Hawthorne, 1990), and this stage had been previously shown to have the highest starch content (Irving, Hurst, & Ragg, 1997).

2.2. Starch isolation

Starch was isolated from squash fruit by using the method reported by Badenhuizen (1964) with slight modifications (Kasemsuwan, Jane, Schnable, Stinar, & Robertson, 1995). Two fruits per replicate, peeled and deseeded, were used for starch isolation. Squash pulp was ground in 0.01 M $HgCl_2$ and then filtered through 106 μm mesh. The starch residue was washed with 10% toluene in 0.1 M NaCl, washed three times with distilled water, twice with ethanol, and then recovered by filtration using Whatman No. 4 filter paper. Purified starch cake was dried in a convection oven at 35 °C for 24 h.

2.3. Starch granule morphology

Starch granules, spread on silver tape and mounted on a brass disk, were coated with gold/palladium (60/40) for all five replicates of each cultivar. Sample images were observed at 1500 × magnification under a scanning electron microscope (JOEL model 1850, Tokyo, Japan), following the method of Jane, Kasemsuwan, Leas, Zobel, and Robyt (1994).

2.4. Crystalline structure

Crystallinity of starch granules was studied using X-ray diffractometry. X-ray diffraction patterns were obtained with copper, Kα radiation using a Siemens D-500 diffractometer (Siemens, Madison, WI). Analysis was conducted following the procedure of Song and Jane (2000). Degree of crystallinity was calculated based on the method of Hayakawa, Tanaka, Nakamura, Endo, and Hoshino (1997). The following equation was used to determine percent crystallinity:

$$\text{Crystallinity }(\%) = A_c/(A_c + A_a)100$$

where A_c is the crystalline area on the X-ray diffractogram and A_a is the amorphous area on the X-ray diffractogram.

2.5. Molecular weight distribution and gyration radius of amylopectin

Weight-average molecular weight and *z*-average gyration radius of amylopectin were determined using high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors (HPSEC-MALLS-RI). Starch samples, duplicate measurements of each replicate, were prepared as described by Yoo and Jane (2002a). The HPSEC system consisted of a HP 1050 series isocratic pump (Hewlett Packard, Valley Forge, PA), a multi-angle laser-light scattering detector (Dawn DSP-F, Wyatt Tech. Co., Santa Barbara, CA) and a HP 1047A refractive index detector (Hewlett Packard, Valley Forge, PA). To separate amylopectin from amylose, Shodex OH pak KB-G guard column and KB-806 and KB-804 analytical columns (Showa Denko K.K., Tokyo, Japan) were used. Operating conditions and data analysis are described by Yoo and Jane (2002b).

2.6. Phosphorus content

The phosphorus content of the starch was determined following the method of Smith and Caruso (1964) except that 5 g of starch was used and all glassware was soaked

24 h in 0.625% sodium molybdate (w/v) in 1.75 N H_2SO_4:10% ascorbic acid (w/v) mixture (4:1) to remove residual phosphorus. Duplicate analysis of each replicate was conducted.

2.7. Amylose contents

Apparent amylose was determined following the method of Takeda and Hizukuri (1987), and the iodine affinity was determined by using a potentiometric autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY). The absolute amylose content of starch was calculated by subtracting iodine affinity of amylopectin fraction from that of the defatted whole starch, following the procedure of Lu, Jane, Keeling, and Singletary (1996). Amylose content of a 90% dimethyl sulfoxide (DMSO)-defatted starch sample was also determined by treating the starch sample with concanavalin A (Yun & Matheson, 1990) and measuring glucose content, by the glucose oxidase method, of the supernatant using amylose/amylopectin assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland). Determination of amylose content, by both methods, was duplicated for each squash cultivar replicate.

2.8. Amylopectin branch chain-length distribution

Amylopectin was fractionated using *n*-butanol as a complexing agent (Schoch, 1942). Amylopectin (10 mg/ml) was debranched using isoamylase (EC 3.2.1.68 from *Pseudomonas amyloderamosa*) (EN102, Hayashibara Biochemical Laboratories Inc., Okayama, Japan), following the method of Jane and Chen (1992). Branch chain-length distribution of amylopectin was determined by using an HPAEC system (Dionex-300, Sunnyvale, CA) equipped with an on-line amyloglucosidase (EC 3.2.1.3, from *Rhizopus* mold, A-7255, Sigma Chemical Co., St Louis, MO) post-column reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) (Wong & Jane, 1997). PA-100 anion exchange analytical column (250×4 mm, Dionex, Sunnyvale, CA) and a guard column were used for separating debranched amylopectin samples. Gradient profile of eluents and operating conditions were described previously (McPherson & Jane, 1999). Debranched amylopectin was also analyzed by using a HPSEC equipped with a RI detector to determine the presence of extra-long branch chains (Yoo & Jane, 2002a). HPAEC-ENZ-PAD and HPSEC analysis was duplicated for each replicate of each cultivar.

2.9. Thermal properties of starch

Thermal properties of starch were determined by using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) (Jane et al., 1999). Approximately 2 mg of starch (dry starch basis, dsb) was precisely weighed in an aluminum pan, mixed with 6 mg of deionized water and sealed. The sample was equilibrated for 2 h and scanned at a rate of 10 °C/min over a temperature range of 10–100 °C. An empty pan was used as the reference. The rate of starch retrogradation was determined using the same gelatinized samples, stored at 4 °C for 7 d, and analyzed using DSC as described previously (White, Abbas, & Johnson 1989). Analysis of starch thermal properties was carried out in triplicate for each replicate of each cultivar.

2.10. Starch pasting properties

Starch pasting properties were analyzed using a Rapid Visco-Analyser (RVA-4, Newport Scientific, Sydney, Australia) (Jane et al., 1999). Starch suspension (8%, w/w, dsb, 28 g total weight) was equilibrated at 30 °C for 1 min, heated at a rate of 6.0 °C/min to 95 °C, maintained at that temperature for 5.5 min, and then cooled to 50 °C at a rate of 6.0 °C/min. Constant paddle rotating speed (160 rpm) was used throughout entire analysis, except for rapid stirring at 960 rpm for first 10 s to disperse sample. Analysis was in duplicate for each replicate of each cultivar.

2.11. Data analysis

All statistical significance tests were calculated by using SAS (SAS Institute Inc., 1999) and applying Tukey difference test (Ramsey & Schafer, 1996). Correlations between squash starch structures and functional properties were conducted using SAS (1999) and the PROC CORR function specifying use of the Pearson correlation coefficient. A 5% level of significance was used to discriminate correlations of importance. Means of the squash cultivars were correlated, with $n=6$ or 7 depending on whether Prizewinner provided sufficient starch for the analysis.

3. Results and discussion

3.1. Starch granule morphology

Scanning electron micrographs show that squash starches of all cultivars have granules ranging mainly from 1.5–2.5, 6–8 and 11–13 μm in diameter (Fig. 1). Squash starches exhibited a mixture of spherical and polyhedral granules with some dome-shaped granules that had larger diameters on average. Yoshida (1989) reported that starch granules in the endocarp region of squash fruit flesh had compound starch granules, which was not found in the remainder of the fruit. The dome-shaped indented starch granules in our study were more likely resulted from shrinkage of swollen granules than from compound starch. Squash starch granules displayed many surface indentations. Large granules of Whangaparoa Crown (Fig. 1G), Lakota (Fig. 1C), Prizewinner (Fig. 1D) and Sweet Mama (Fig. 1E) had less incidence of indentations, whereas Delica (Fig. 1A), Kurijiman (Fig. 1B)

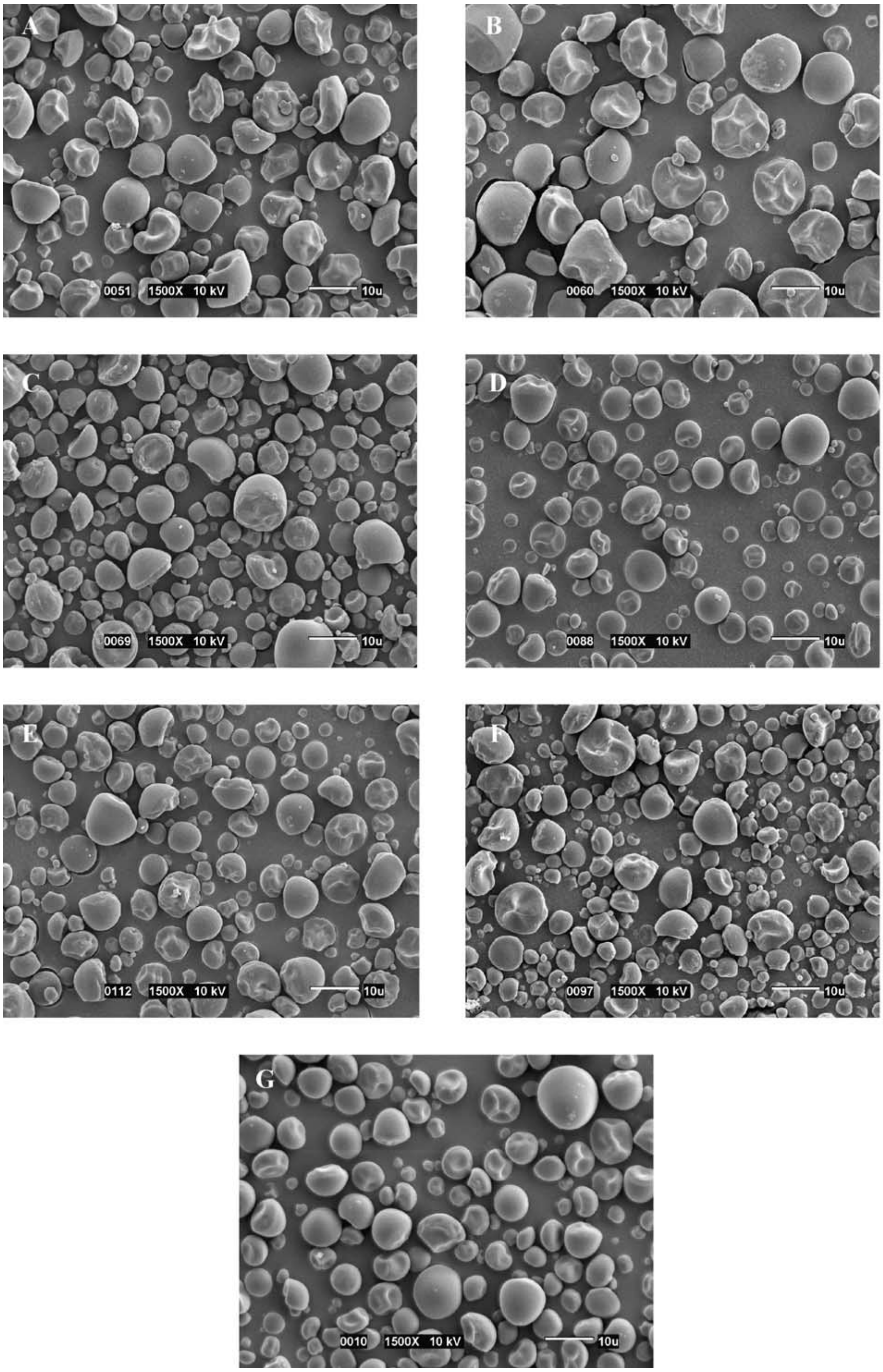

Fig. 1. Scanning electron micrographs of Delica (A), Kurijiman (B), Lakota (C), Prizewinner (D), Sweet Mama (E), Warren Scarlet (F) and Whangaparoa Crown (G) squash fruit starches (scale bar = 10 μm).

and Warren Scarlet (Fig. 1F) frequently showed indentations. Whangaparoa Crown and Prizewinner had a high incidence of indentations in medium-size starch granules. Granule indentations could be due to non-uniform growth within starch granules or collapse during drying. The granule size distribution observed in our study was in agreement with that reported by Sugimoto, Yamashita, Suzuki, Morishita, and Fuwa (1998b). Yoshida (1989) reported starch granule diameters up to 35 μm, but we did not observe granule diameter above 14 μm.

3.2. Crystalline structure

Squash starches all exhibited B-type X-ray diffraction patterns (Fig. 2). Prizewinner, Warren Scarlet, Whangaparoa Crown, Kurijiman, and Delica all had an additional

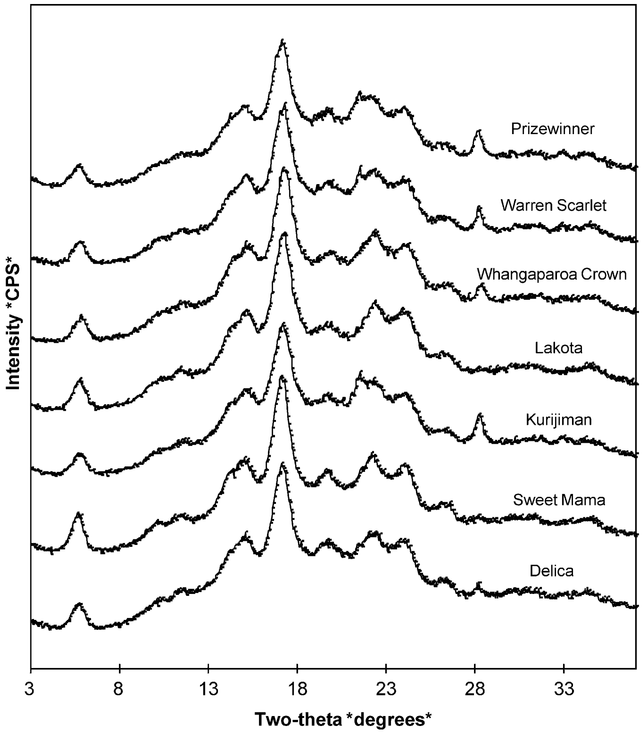

Fig. 2. X-ray diffraction patterns of Delica, Kurijiman, Lakota, Prizewinner, Sweet Mama, Warren Scarlet and Whangaparoa Crown squash fruit starches.

peak at $2\theta = 28.2°$ that was not observed in other cultivars. The nature of the peak is not known. The percentage crystallinity of Prizewinner, Warren Scarlet, Whangaparoa Crown, Lakota, Kurijiman, Sweet Mama and Delica squash starches, calculated based on X-ray diffractograms, was 48.0, 54.0, 42.9, 43.9, 55.7, 45.7 and 35.0, respectively. The three buttercup cultivar squash starches, Delica, Kurijiman and Sweet Mama, showed different degrees of crystallinity. The percentage crystallinity of squash starch (35–55.7%) is greater than other native starches, such as 20–28% reported for a variety of A- and B-type starches (Cooke & Gidley, 1992), 25–44% reported for wheat starches (Fujita, Yamamoto, Sugimoto, Morita, & Yamamori, 1998), and 38% for normal maize (Keppel, 2001).

3.3. Amylose content

Iodine affinities for defatted whole starch and amylopectin, and corresponding apparent and absolute amylose contents are shown in Table 1. Both apparent and absolute amylose contents were significantly different between the starches of squash cultivars. Iodine affinities of the squash amylopectin were greater than that of most other native starches, indicating more long-branch chains of the amylopectin, which resulted in substantially less absolute amylose contents than the apparent amylose content counterparts.

Concanavalin A method resulted in less amylose contents than the absolute amylose content counterparts obtained by the iodine-affinity method, but both sets of results were in the same trend. The lower amylose content obtained by using concanavalin A method could be due to co-precipitation of amylose with amylopectin, resulting in amylose lost when amylopectin was removed by centrifugation.

3.4. Phosphorus content

Phosphorus contents of squash starches ranged from 0.022 (Whangaparoa Crown) to 0.026% (Lakota) (w/w, dsb) and were not significantly different between cultivars (other data are not shown). Phosphorus contents of all squash starches were higher than that reported for all tuber and root starches, except potato

Table 1
Iodine affinities, apparent amylose, absolute amylose contents, and amylose content measured using concanavalin A (Con A) for squash fruit defatted starches

Cultivar	Iodine affinity		Apparent amylose content (%)[a]	Absolute amylose content (%)[b]	Amylose (%) content measured by Con A[c]
	Whole starch	Amylopectin fraction			
Delica	5.51ab	1.89ab	27.8ab	18.2	12.4
Kurijiman	5.63a	2.35ab	28.3a	16.5	12.6
Lakota	4.45b	1.65b	22.3b	14.0	10.2
Prizewinner	5.31ab	2.73a	26.4ab	12.9	7.9
Sweet Mama	5.02ab	2.33ab	24.9ab	13.2	9.1
Warren Scarlet	5.41ab	1.84ab	27.1ab	17.9	11.4
Whangaparoa Crown	5.48ab	1.96ab	27.9ab	18.0	11.8
	$P=0.03$[d]	$P=0.02$	$P=0.03$	$P=0.007$[e]	$P=0.07$

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[a] Apparent amylose contents were averaged from two analyses for each of five replicates. Values were calculated from dividing iodine affinity by a factor of 0.199.

[b] Absolute amylose contents were averaged from two analyses for each of five replicates. Values were calculated by subtracting iodine affinity for the amylopectin fraction from the iodine affinity for the whole starch, divided by a factor of 0.199.

[c] Amylose contents measured by concanavalin A were averaged from two analyses for each of five replicates.

[d] P represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

[e] Conservative Tukey test was unable to detect differences between individual cultivars.

(Jane, Kasemsuwan, Chen, & Juliano, 1996; Kasemsuwan & Jane, 1996; Lim, Kasemsuwan, & Jane, 1994).

3.5. Amylopectin molecular weight and size

Average molecular weight, gyration radii, and dispersed density of squash starches are shown in Table 2. Squash starch weight-average molecular weight (M_w) ranged from 2.03 to 3.22×10^8 g/mol for the seven squash cultivars; there was no significant difference between cultivars. Squash amylopectin M_w is comparable with other starches of the B-type polymorphism, ranging from 1.7 to 3.4×10^8 g/mol (Yoo & Jane, 2002b). Squash amylopectins displayed substantially lower polydispersity (M_w/M_n) than did amylopectin of other sources (Stevenson, 2003). The polydispersity of Lakota, Prizewinner, Whangaparoa Crown and Sweet Mama amylopectin ranged between 1.21 and 1.30 (Table 2), indicating highly uniform in size of the amylopectin molecules. Gyration radius of amylopectin was not significantly different between the squash cultivars. Prizewinner and Whangaparoa Crown amylopectins, dispersed in aqueous solutions, were somewhat denser than other squash cultivars, but differences were not significant (Table 2). The density of the dispersed squash amylopectin (10.1–12.2 g/mol/nm^3) was larger than that of the B-type non-waxy amylopectin (2.9–5.3 g/mol/nm^3) but was comparable to that of the ae-waxy maize amylopectin (11.2 g/mol/nm^3) (Yoo & Jane, 2002b).

3.6. Amylopectin branch chain-length distribution

HPAEC-ENZ-PAD chromatogram of debranched amylopectin of each squash cultivar is shown in Fig. 3. Chromatograms show that all squash starches have peak chain-length at DP 13–14. The chain-length distribution of amylopectin is summarized in Table 3. Average amylopectin chain lengths varied from DP 26.5 to 28.1 for the squash cultivars and were not significantly different. However, proportions of branch chains of different chain-lengths were significantly different (Table 3). The different chain-length distributions correlated with properties of the starches as discussed in Section 3.9. The greater contents of short branch chains (DP 6–12, 12.6–16.7%) of squash amylopectin than that of other B-type amylopectin (8.5–12.3%) (Jane et al., 1999) may contribute to the greater density of dispersed squash amylopectin. HPSEC chromatograms of debranched squash amylopectin showed only two distinct peaks of short and long branch chains; there were no extra-long branch chains (DP ~ 720), as in cereal amylopectin (Yoo & Jane, 2002a) detected (Stevenson, 2003).

Table 2
Average amylopectin molecular weight, polydispersity, gyration radius and density of squash fruit starches

Cultivar[a]	$M_w\times10^8$ (g/mol)[b]	Polydispersity (M_w)	R_z (nm)[c]	ρ (g/mol/nm^3)[d]
Delica	2.41	1.44	310	8.1
Kurijiman	2.03	1.81	294	8.0
Lakota	3.16	1.21	324	9.3
Prizewinner	3.22	1.23	317	10.1
Sweet Mama	2.54	1.30	311	8.4
Warren Scarlet	2.91	1.35	337	7.6
Whangaparoa Crown	2.70	1.29	304	9.6
	$P=0.12$[e]	$P=0.27$	$P=0.11$	$P=0.15$

Data were obtained from two injections each of all five replicates.

[a] Starch samples were dissolved in 90% DMSO solution and precipitated with 5 vol. ethanol. Freshly prepared starch aqueous solution (100 μl; 0.8 mg/ml) was injected to HPSEC system.

[b] Weight-average molecular weight.

[c] z-Average radius of gyration.

[d] Density is equal to M_w/R_z^3.

[e] P represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

3.7. Thermal properties

Thermal properties of native squash starches are shown in Table 4. Three buttercup squash cultivars, Delica, Kurijiman and Sweet Mama, showed similar onset gelatinization temperatures (T_o), which were higher than others. The ranges of gelatinization temperature (T_c-T_o) (ROG) of squash starches were the smallest comparing with other starches (Jane et al., 1999). Biliaderis, Maurice, and Vose (1980) suggested that the greater the degree of amylopectin branching, the wider the melting temperature range. Our results demonstrated a negative relationship between the ROG and the long branch chains of DP$\geq$37. The reduction in ROG by increasing number of branch chains of DP$\geq$37 can be attributed to cooperative gelatinization induced by the long branch chains that extended through multiple clusters. Enthalpy changes of gelatinization for all squash starches were similar to that of other B-type starches (Jane et al., 1999), and there were no significant differences between cultivars.

Squash starches, after being stored at 4 °C for 7 days, showed greater degrees of retrogradation than other starches (Table 5) (Jane et al., 1999). Among the squash starch cultivars, Lakota, Sweet Mama and Warren Scarlet displayed the greatest degrees of retrogradation. Prizewinner had the lowest percentage retrogradation, which agreed with its largest content of short branch chains (16.7% DP 6–12 and 1% DP 3–5).

3.8. Pasting properties

Pasting properties of squash starches are shown in Table 6. Peak viscosity of Lakota and Prizewinner starches were higher than that of others. Pasting temperature of squash starches was only 4–6 °C higher than respective T_o, which could be attributed to the absence of phospholipids and the low amylose content, facilitating the granule swelling after gelatinization. The final viscosity of the squash starch was similar to that of potato starch, but was higher than that of

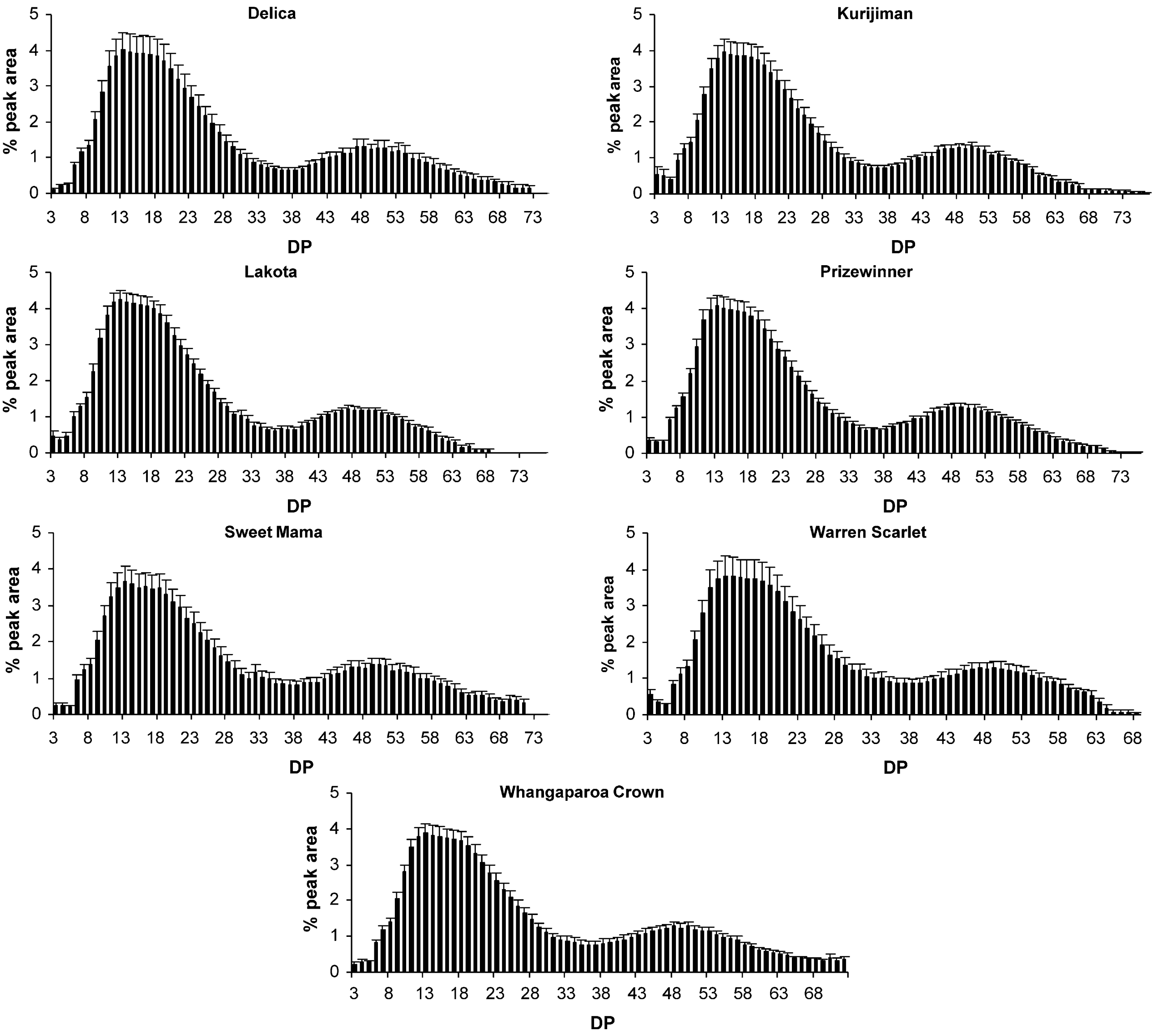

Fig. 3. Relative peak area distributions of Delica, Kurijiman, Lakota, Prizewinner, Sweet Mama, Warren Scarlet and Whangaparoa Crown squash fruit amylopectins analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from two analyses of five replicates. DP, degree of polymerization.

Table 3
Branch chain-length distributions of squash fruit amylopectins

Cultivar	Peak DP		Average CL	Percent distribution						Highest detectable DP
	I	II		DP 3–5	DP 6–9	DP 6–12	DP 13–24	DP 25–36	DP$\geq$37	
Delica	13	50	28.1	0.61	5.0b	14.9a	40.6b	14.7abc	29.3	72
Kurijiman	13	48	27.4	1.25	5.4ab	15.6a	41.0ab	14.2bc	28.4	76
Lakota	14	49	27.0	0.80	3.8c	12.6b	44.7a	16.5a	25.4	69
Prizewinner	13	49	26.5	1.01	6.1a	16.7a	41.9ab	14.1c	26.2	76
Sweet Mama	13	48	27.9	1.00	5.3ab	15.2a	40.1b	15.0abc	28.8	74
Warren Scarlet	14	49	26.9	1.43	5.4ab	15.4a	40.4b	16.0ab	27.1	68
Whangaparoa Crown	13	49	27.2	0.74	5.5ab	15.9a	41.2ab	14.6bc	27.3	72
			$P=0.26$[a]	$P=0.46$	$P<0.0001$	$P<0.0001$	$P=0.01$	$P=0.003$	$P=0.13$	

Grouping of degree of polymerization (DP) numbers followed that of Hanashiro, Abe, and Hizukuri (1996). Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[a] P represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 4
Thermal properties of native squash fruit starches

Cultivar[a]	Native starch				
	T_o (°C)	T_p (°C)	T_c (°C)	Range (°C)[b]	ΔH (J/g)
Delica	63.4a	66.5a	69.7ab	6.3b	17.3
Kurijiman	63.5a	66.5a	69.8a	6.3b	16.9
Lakota	62.9ab	66.4a	70.4a	7.5ab	16.8
Prizewinner	60.9bc	65.0ab	69.7a	8.8a	15.9
Sweet Mama	63.5a	66.2a	69.8a	6.3b	17.4
Warren Scarlet	61.7abc	65.7ab	70.4a	8.7a	16.4
Whangaparoa Crown	60.6c	64.0b	67.7b	7.1ab	16.3
	$P=0.0001$[c]	$P=0.001$	$P=0.006$	$P<0.0001$	$P=0.26$

Values were calculated from three analyses for each of five replicates. Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[a] Starch samples (~2.0 mg, dsb) and deionized water (~6.0 mg) were used for the analysis; T_o, T_p, T_c and ΔH are onset, peak, conclusion temperature, and enthalpy change, respectively.

[b] Range of gelatinization is equal to $T_c - T_o$.

[c] P represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

most A-type starches (Jane et al., 1999). Setback viscosity of all squash starches is higher than that of most other starches, with exceptions of cattail millet, mungbean and green leaf canna starches (Jane et al., 1999). High setback indicates that amylose forms network with amylopectin.

3.9. Structure–property correlations

Correlation coefficients among selected squash starch structures and functional properties are shown in Table 7. Correlation coefficients are given in the text when not included in Table 7. Apparent amylose content of squash starches was negatively correlated to amylopectin chain lengths of DP 13–24 and DP 25–36 and pasting peak viscosity, breakdown, final viscosity and setback viscosity. But it is positively correlated to onset gelatinization temperature of retrograded starch (T_{oR}) ($r=0.73$, $P=0.05$), which reflected the participant of amylose in the retrogradation and co-crystallized with amylopectin. Absolute amylose content was positively correlated to amylose content measured by concanavalin A, but negatively correlated to pasting peak viscosity, breakdown and final viscosity. The negative relationship between the apparent amylose content and peak and final viscosity is in agreement with previously reported (Blennow, Bay-Smidt, & Bauer, 2001; Collado & Corke, 1997; Jane et al., 1999; Kuno, Kainuma, & Takahashi, 2000; Wang, White, & Pollak, 1993).

Squash amylopectin M_w was negatively correlated to polydispersity, average amylopectin branch chain-length, and amylopectin branch chains of DP≥37, but positively

Table 5
Thermal properties of retrograded squash fruit starches

Cultivar[a]	Native starch				
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	% Retrogradation
Delica	36.3	54.0	64.5	8.1ab	46.6ab
Kurijiman	36.6	54.5	64.8	7.8ab	45.8ab
Lakota	35.2	53.3	65.4	8.9a	53.3a
Prizewinner	36.6	54.8	64.3	6.5b	40.8b
Sweet Mama	35.1	52.4	65.0	9.5a	54.7a
Warren Scarlet	36.0	52.6	65.5	8.8ab	53.4a
Whangaparoa Crown	35.6	53.3	65.0	8.2ab	49.8ab
	$P=0.25$[b]	$P=0.31$	$P=0.59$	$P=0.008$	$P=0.002$

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[a] Same starch samples after gelatinization (see Table 4) were left for 7 days at 4 °C and rescan using DSC.

[b] P represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 6
Pasting properties of squash fruit starches measured by Rapid Visco-Analyzer

Cultivar[a]	Peak viscosity[b]	Breakdown[b]	Final viscosity[b]	Setback[b]	Pasting temperature (°C)
Delica	174.8b	52.2	206.9	84.3	68.8
Kurijiman	179.2ab	63.1	195.3	79.2	67.8
Lakota	232.8a	88.7	243.8	99.7	67.2
Prizewinner	225.1a	76.7	244.3	95.8	66.5
Sweet Mama	205.2ab	66.6	231.6	93.0	68.4
Warren Scarlet	176.8b	52.6	217.5	93.2	68.0
Whangaparoa Crown	173.7b	65.4	193.1	84.6	65.6
	$P=0.01$[c]	$P=0.26$	$P=0.49$	$P=0.70$	$P=0.16$

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[a] 8% (w/w) starch suspension.

[b] Viscosity measured in Rapid Visco-Analyzer units (RVU), 1 RVU = 12 centipoise.

[c] P represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 7
Correlation coefficients ($r \times 100$) for selected squash starch structural and functional properties

	Ap_A	Ab_A	A_{con}	M_w	P_{Mw}	R_z	DS	CL	DP_{13-24}	DP_{25-36}	$DP_{\geq 37}$	T_o	ROG	ΔH	PV	BK	FV	SB
Ap_A	100																	
Ab_A	68	100																
A_{con}	55	89**	100															
M_w	−58	−44	−68	100														
P_{Mw}	59	39	65	−86**	100													
R_z	−44	−8	−33	73*	−61	100												
DS	11	−25	−41	38	−38	−29	100											
CL	17	28	43	−72*	34	−44	−44	100										
DP_{13-24}	−72*	−39	−24	57	−36	22	21	−46	100									
DP_{25-36}	−69	−1	0	46	−42	74*	−53	−13	49	100								
$DP_{\geq 37}$	57	39	46	−85**	56	−52	−36	87**	−80*	−46	100							
T_o	−21	−9	26	−59	49	−25	−72*	72*	−6	14	53	100						
ROG	−14	−17	−47	81*	−51	74*	27	−89**	25	25	−74*	−72*	100					
ΔH	−15	5	31	−61	32	−28	−64	92**	−23	13	69	91**	−84*	100				
PV	−84*	−90**	−82*	68	−55	30	27	−44	72*	28	−67	−1	34	−17	100			
BK	−78*	−75*	−59	58	−44	6	41	47	87**	25	−75*	−11	21	−25	89**	100		
FV	−80*	−83*	−87**	76*	−66	57	9	−40	51	39	−58	−1	48	−14	92**	66	100	
SB	−83*	−65	−76*	88**	−81*	77*	5	−49	55	62	−71	−19	61	−25	81*	62	93**	100
PT	−2	8	22	−45	35	11	−83*	66	−37	18	62	83*	−40	78*	−19	−47	3	−10

Ap_A, apparent amylose content; Ab_A, absolute amylose content; A_{con}, amylose content measured by concanavalin A; M_w, weight-average amylopectin molecular weight; P_{Mw}, polydispersity (M_w/M_n); R_z, gyration radius; DS, density; CL, average amylopectin branch chain-length; DP_{13-24}, proportion of amylopectin branch chain-lengths DP 13–24; DP_{25-36}, proportion of amylopectin branch chain-lengths DP 25–36; $DP_{\geq 37}$, proportion of amylopectin branch chain-lengths $DP \geq 37$; $DP_{\geq 26}$, proportion of amylopectin branch chain-lengths $DP \geq 26$ measured by HPSEC; T_o, onset gelatinization temperature; ROG, range of gelatinization temperature; ΔH, enthalpy change of gelatinization; PV, peak viscosity; BK, breakdown; FV, final viscosity; SB, setback; PT, pasting temperature. *0.05 and **0.01 level of significance.

correlated to R_z, ROG, final viscosity, and setback viscosity. The negative correlation observed between M_w and average amylopectin branch chain-length and proportion of branch chains of DP ≥ 37 agreed with findings reported by Yoo and Jane (2002b). The correlations indicated that squash starches with smaller amylopectin molecular weights and R_z possessed more long branch chains of DP ≥ 37 and, thus, displayed narrower ROG. The negative correlation between the M_w and polydispersity indicated that reduction in the molecular weight of amylopectin was resulted from molecular breakdown.

Average amylopectin branch chain-length was positively correlated to T_o (Jane et al., 1999; Li, Vasanthan, Rossnagel, & Hoover, 2001) and ΔH (Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2001), but negatively correlated to ROG. The percentage of branch chains of DP 13–24 was positively correlated to peak viscosity and breakdown, and that of branch chains of DP 25–36 was positively correlated to T_{cR} ($r = 0.86$, $P = 0.01$), percent retrogradation ($r = 0.77$, $P = 0.04$) and R_z. Long amylopectin branch chains (DP ≥ 37) were negatively correlated to peak viscosity, breakdown, and setback viscosity, which agree with previous reports (Han & Hamaker, 2001; Li et al., 2001). However, increasing peak viscosity with increasing amylopectin branch chain-length has also been reported (Sasaki & Matsuki, 1998; Shibanuma, Takeda, & Hizukuri, 1996). These conflict results are attributed to that long amylopectin chains can restrict swelling but also prevent breakdown of swollen granules. T_o, T_p and ΔH were positively correlated to pasting temperature ($r = 0.86$, $P = 0.01$ for T_p) and ROG was negatively correlated to ΔH.

4. Conclusion

Squash starches displayed B-type X-ray diffraction patterns, consisted of low absolute amylose (12.9–18.2%) and moderate phosphorus content (0.022–0.026%). All squash starches had long amylopectin branch chain-lengths. Distinctive attributes of squash starches were low polydispersity, narrow range of gelatinization, and high retrogradation rate. Squash starches had pasting temperature 4–6 °C higher than the onset gelatinization temperature.

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