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Structures and functional properties of apple (*Malus domestica* Borkh) fruit starch

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Abstract

Structures and functional properties of fruit starch of six apple cultivars (Gala, Golden Delicious, Granny Smith, Jerseymac, Jonagold and Royal Gala) were investigated. Apple starches exhibit C_A-type X-ray diffraction patterns, and granule diameters ranged from 2 to 12 μm. Immature apple fruit had 44–53% starch (dry basis). The apparent amylose content was high (40–48%), but the average branch chain-length of amylopectin was long (DP 27.9–29.6), resulting in the absolute amylose content of 26–29%. The weight-average molecular weight of amylopectin ranged from 4.6 to 11.1×10^8. The proportion of long-branch chains of amylopectin (DP≥37), determined by anion-exchange chromatography with a post-column amyloglucosidase reactor and a pulsed amperometric detector, ranged from 29.7 to 32.4%. The onset gelatinization temperature of the starch ranged 64–66 °C, and the ΔH of starch gelatinization was 16–18 J/g. The percentage retrogradation of the gelatinized starch, after being stored for 7 d at 4 °C, ranged 42–47%. Most distinctive characteristic of apple starch was that three cultivars had extremely low breakdown (<4 RVU) and high setback (>100 RVU) at 8% (w/w) starch concentration. Peak and final viscosities ranged 99–148 RVU and 144–224 RVU, respectively. Pasting temperature was around 70 °C.

Keywords: Apple starch; Starch structure; Starch function; Amylose; Amylopectin; *Malus*

1. Introduction

Starch is the main carbohydrate of plant storage organs. Starch in cereal, root and tuber crops has been extensively characterized, but little study has been done on characterization of fruit starches and its impact on fruit texture. Apples, like many other fruit crops, accumulate starch at early stages of maturation and progressively degrade starch to increase sweetness during ripening (Warrington, Fulton, Halligan, & de Silva, 1999). Some apples, such as Granny Smith and Jonagold, are considered good cooking apples and may be harvested immature when starch levels are still high. Starch content and properties have been shown to influence texture of squash fruit (Stevenson, 2003) and similar trends may apply for apples. Additionally, hail damage is a frequent cause of crop loss in the apple industry, resulting in premium apples being downgraded for juicing and other processing (Dodds, Penrose, Bower, & Nicol, 1994). Hail damage often occurs when fruits are immature and substantial amount of starch is present. Therefore, if apple starch possesses some unique characteristics, fruit may be more valuable for their starch used in niche industries rather than for juicing.

Apple starch was characterized by Potter, Hassid, and Joslyn (1949). The authors reported the amylose content to be 24% based on pentasol precipitation method, and 26.5% based on potentiometric iodine titration method. β-Amylolysis of amylose and amylopectin yielded 90 and 64% maltose, respectively. Starch content was not reported. The molecular weight of amylopectin was determined to be 1.2×10^6 by using acetylation of amylopectin. Average chain-length of amylopectin was 24 glucose residues, determined by periodate oxidation method. Average amylose chain-length was determined to be 530 glucose residues. Techniques and instrumentation of starch analyses have greatly advanced since then. Recently, studies on microscopy of apple starch during fruit

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development (Kovács & Eads, 1999) and amylases in apple juice (Carrin, Ceci, & Lozano, 2004) reported apple starch average granule diameter of 9.2 μm.

In this study we analyzed structures and functional properties of starch isolated from fruits of six apple cultivars to understand characteristics of apple starches and to determine differences between cultivars. We also correlated structures and functional properties of the starch to understand relationships between starch structures and functions.

2. Materials and methods

2.1. Materials

Immature fruits, determined on the basis of the starch index, Brix value, and color and size of the fruits, were harvested from trees of six apple (*Malus domestica* Borkh) cultivars on August 8 and 9, 2002, at the Iowa State University Horticultural Farm, Gilbert, Iowa. Cultivars studied were Gala, Golden Delicious, Granny Smith, Jerseymac, Jonagold and Royal Gala. Three replicates for each cultivar, each consisting of 20 apples were collected randomly from different trees, which included fruits from the center of trees and towards the end of branches, and varying tree canopy heights.

Isoamylase (EC 3.2.1.68, from *Pseudomonas amyloderamosa*) was a product of Hayashibara Biochemical Laboratories Inc. (Okayama, Japan). Amyloglucosidase (EC 3.2.1.3, from *Rhizopus*) was purchased from Sigma Chemical Co. (St Louis, MO). Total starch assay kit, consisting of α-amylase, amyloglucosidase, and glucose oxidase-peroxidase, was purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). Other chemicals were all reagent grade and used without further purification.

2.2. Starch isolation, starch content and water content of apple fruit

Starch was isolated from apple fruits using a method reported by Kasemsuwan, Jane, Schnable, Stinar, and Robertson (1995) with modification. On the same day as being harvested, apple fruits were sliced and blended in 0.3% (w/v) sodium metabisulfite using a blender (Oster® Designer® Slope Blender 14 speed, grind mode used, Sunbeam Products Inc., Boca Raton, FL). Apple starch puree was then filtered through a screen of 106 μm opens, and the filtrate was centrifuged at 10,500 *g* for 40 min to precipitate starch. To remove protein and chlorophyll pigments, the starch was washed under mechanical stirring for 1 h with 10% toluene in 0.1 M sodium chloride solution and allowed to stand for 4 h. This step was repeated 5–20 times until the supernatant was clear. The toluene/salt solution treated starch was then washed three times with distilled water, twice rinsed with ethanol, and then recovered by filtration using Whatman No. 4 filter paper. The purified starch was dried in a convection oven at 35 °C for 48 h.

The water content of the apple fruit was determined by using freeze-drying finely diced fruit. The total starch content of freeze-dried apple fruit powder, measured in duplicate, was determined by using total starch assay kit. An internal standard of cornstarch was added to the samples to check quantitative recovery of starch.

2.3. Starch granule morphology

Starch granules of each cultivar were spread on silver tape and mounted on a brass disk, then coated with gold/palladium (60/40). 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). Diameters of starch granules were determined by using a ruler to measure the diameters of individual granules on the basis of the scale bar provided on the captured scanning electron micrographs.

2.4. Starch crystallinity

Crystallinity of starch granules was determined using X-ray diffractometry. X-ray diffraction patterns of purified starch samples were obtained with copper, K_α radiation using a Siemens D-500 diffractometer (Siemens, Madison, WI). Analysis was conducted following the procedure reported by Song and Jane (2000). Percentage crystallinity was calculated following the method of Hayakawa, Tanaka, Nakamura, Endo, and Hoshino (1997). The following equation was used to determine percentage crystallinity:

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

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

2.5. Molecular weight 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 analysis, duplicate measurements of each replicate for all cultivars, was conducted 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 for the separation of amylose and amylopectin following the method of Yoo and Jane (2002b), except the flow rate being 0.3 mL/min and sample concentration 0.8 mg/mL.

2.6. Apparent and absolute amylose contents

Apparent and absolute amylose contents of starch were determined by measuring iodine affinities of defatted whole

starch and of amylopectin fraction using a potentiometric autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY) (Kasemsuwan et al., 1995). Starch samples were dissolved and defatted in 90% dimethyl sulfoxide (DMSO) solution and followed by alcohol precipitation. The absolute amylose content was determined by subtracting the iodine affinity of amylopectin from that of the defatted whole starch. The analysis was duplicated for each replicate of each apple cultivar.

2.7. Amylopectin branch chain-length distribution

Amylopectin was fractionated by selective precipitation of amylose using *n*-butanol as a complexing agent (Schoch, 1942). Amylopectin (1%) was dispersed in 90% DMSO and precipitated by adding excess alcohol and centrifuge, and then re-dispersed in 0.1 M sodium acetate, pH 4.5, and debranched using isoamylase following the procedure described by Jane and Chen (1992). Branch chain-length distribution of amylopectin was determined by using an HPAEC system (Dionex-300, Sunnyvale, CA) equipped with an amyloglucosidase post-column, on-line 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. The gradient profile of eluents and operating conditions were described previously (McPherson & Jane, 1999). HPAEC-ENZ-PAD analysis was duplicated for each replicate of each cultivar.

2.8. 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). Starch (2 mg, dry starch basis (dsb)) was accurately weighed in an aluminum pan, mixed with 6 mg of deionized water and sealed. The sample was allowed to equilibrate 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 the same process described for gelatinization (White, Abbas, & Johnson, 1989). All thermal analyses were conducted in triplicate for each replicate of each cultivar.

2.9. Pasting properties of starch

Starch pasting properties were analyzed using a Rapid Visco-Analyser (RVA-4, Foss North America, Eden Prairie, MN) (Jane et al., 1999). Starch suspension (8%, w/w, dsb), in duplicate for each replicate of each cultivar, was prepared by weighing starch (2.24 g, dsb) into a RVA canister and making up the total weight to 28 g with deionized water. Starch suspension was equilibrated at 30 °C for 1 min, heated at a rate of 6.0 °C/min to 95 °C, maintained at 95 °C 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 the first 10 s to disperse starch sample.

2.10. Statistical analysis

All statistical significance tests were calculated using SAS (1999) and applying Tukey difference test (Ramsey & Schafer, 1996) at the 5% level of significance. Correlations between apple 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 apple cultivars were correlated, with $n=6$ for all correlations.

3. Results and discussion

3.1. Starch content

Starch contents of apple fruits of different cultivars varied from 44.0 to 53.2% (dry weight basis) and were significantly different ($P=0.04$) (Table 1). Granny Smith apples had a significantly greater starch content than Royal Gala, which explains that Granny Smith apple is well suited for cooking, whereas Royal Gala is a good eating apple. There are few studies on the starch content of immature apple fruit found in the literature because most studies report starch content at harvest or measure starch index (color scale based on iodine staining of sliced fruit) during fruit development. An apple starch content of 28 mg/g fresh weight was previously reported for Royal Gala fruit during development (Brookfield, Murphy, Harker, & MacRae, 1997), which was less than the results obtained in this study (44% dry weight, or 48 mg/g fresh weight). Bowen and Watkins (1997) report 4% starch content (dry weight basis) of mature Fuji apples, which is substantially less than the values found in this study. The difference could be attributed to degradation of starch during maturation, which resulted in less starch content in mature apples. Besides some winter squash fruits, which have higher starch contents

Table 1
Fruit weight, water content and starch content of apple fruits

Cultivar	Average fruit weight (g)[a]	Water content (%)	Starch content (% dry weight)[b]
Gala	103	89.4	45.8ab
Golden Delicious	100	90.4	47.5ab
Granny Smith	114	88.3	53.2a
Jerseymac	159	89.9	44.3ab
Jonagold	123	88.5	51.0ab
Royal Gala	109	88.9	44.0b
		$P=0.18$	$P=0.04$

Values with different letters denote cultivar differences at the 5% level of significance for each comparison between cultivars in the respective column. *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

[a] Average of 60 fruits (20 fruits per replicate).

[b] Starch contents were averaged of two analyses from each of three replicates.

(up to 65% dry weight) (Hurst, Corrigan, Hannan, & Lill, 1995; Stevenson, Hurst, Yoo, & Jane, 2005) than apple fruit, the only other fruit containing substantial starch is tomato (20% dry weight) on 20 d post anthesis (Brampton, Asquith, Parke, Barraclough, & Hughes, 1994).

3.2. Starch granule morphology

Scanning electron micrographs of all the six apple-starches showed similar granule morphology and size distribution, with the diameters ranging between 2 and 12 μm (Fig. 1). The sizes of the granules were in agreement with the average starch granule size of 9.2 μm found in apple juice (Carrin et al., 2004). Starch granules were mainly spherical or dome-shaped and split, which were likely synthesized as compound starch. Apple starch had predominantly granule diameters of 6–10 μm, which was similar to squash fruit starch (Stevenson et al., 2005) and kiwifruit starch (Sugimoto, Yamamoto, Abe, & Fuwa, 1988). The spherical and dome-shaped starch granules found in apple starch have also been observed for the starch of squash fruit (Stevenson et al., 2005), pineapple stem, babassu, acorn (Jane et al., 1994) and kiwifruit (Sugimoto et al., 1988).

3.3. Starch crystalline structure

Apple starches all exhibited the C_A-type X-ray diffraction patterns (Fig. 2), with a less strong peak at $2\theta = 17.2°$ and a small peak at $2\theta = 5.5°$, showing a minor characteristic of the B-type starches, and a single peak at $2\theta = 22–24°$ and a shoulder peak at $2\theta = 18°$, characteristic of the A-type starch. The C-type X-ray diffraction pattern of apple starch is similar to that of banana fruit starch (Jane et al., 1999). Percentage crystallinity of Gala, Golden Delicious, Granny Smith, Jerseymac, Jonagold, and Royal Gala apple starches, calculated based on X-ray diffractograms, was 43.7, 47.3, 41.3, 45.7, 40.6 and 46.4, respectively.

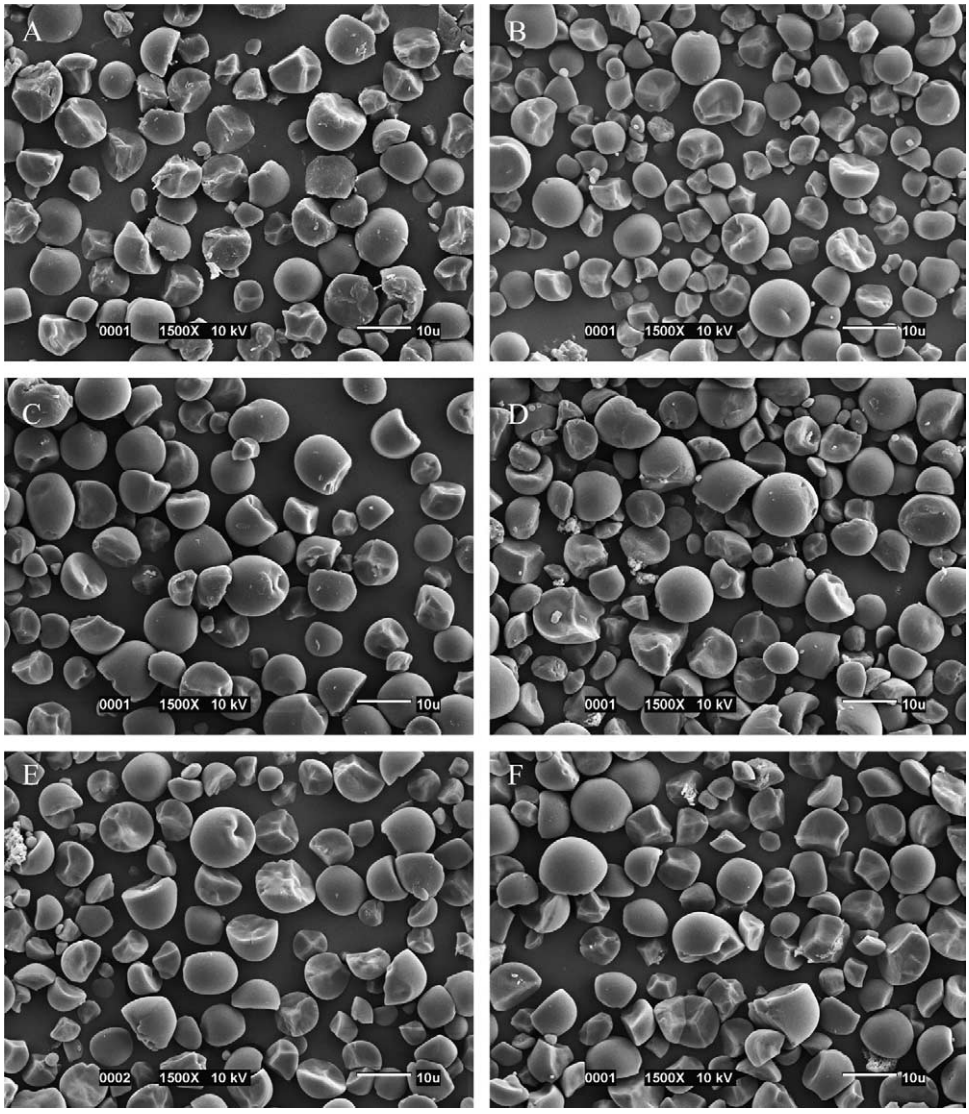

Fig. 1. Scanning electron micrographs of Gala (A), Golden Delicious (B), Granny Smith (C), Jerseymac (D), Jonagold (E) and Royal Gala apple fruit starches (scale bar = 10 μm).

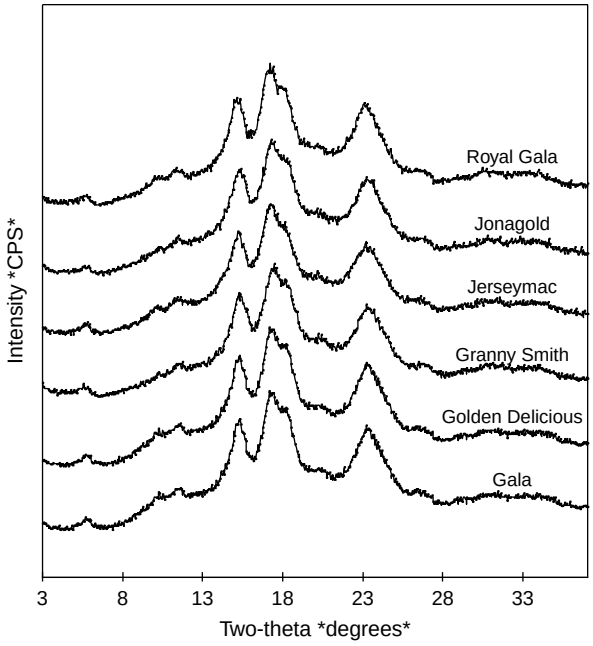

Fig. 2. X-ray diffraction patterns of Gala, Golden Delicious, Granny Smith, Jerseymac, Jonagold and Royal Gala apple fruit starches.

3.4. Iodine affinity and amylose content

The iodine affinities of the defatted whole starches and the corresponding apparent amylose contents are shown in Table 2, with the apparent amylose content of Jerseymac starch significantly greater than that of Gala starch. The absolute amylose content, calculated by subtracting the iodine affinity of amylopectin from that of the defatted whole starch, was not significantly different among the apple cultivars. The iodine affinities of apple whole starch and of amylopectin were larger than that of most native starches reported. The large iodine affinity of the amylopectin suggested that the amylopectin molecules consisted of long branch-chains. The absolute amylose contents of apple starches (26.0–29.3%) were considerably larger than that reported for starch from normal corn (21.4–22.5%) (Hizukuri, 1993; Jane et al., 1999), potato (16.9–19.8%) (Jane et al., 1999; Suzuki, 1993), rice (20.5%) (Jane et al., 1999) and wheat (21.6–25.8%) (Akashi, Takahashi, & Endo, 1999; Hizukuri, 1993; Jane et al., 1999; Suzuki, 1993).

Table 2
Iodine affinities, apparent amylose and absolute amylose contents of defatted apple fruit Starchesa

Cultivar	Iodine affinity		Apparent amylose content (%)[a]	Absolute amylosecontent (%)[b]
	Whole starch	Amylopectin fraction		
Gala	7.92b	2.12	39.8b	29.1
Golden Delicious	9.16ab	3.59	45.4ab	28.0
Granny Smith	8.62ab	2.80	43.3ab	29.3
Jerseymac	9.57a	3.77	48.1a	29.1
Jonagold	9.02ab	3.84	46.1ab	26.0
Royal Gala	8.42ab	3.13	42.4ab	26.6
	$P=0.05$	$P=0.09$	$P=0.05$	$P=0.95$

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column. P represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

[a] Apparent amylose contents were averaged from two analyses for each of three 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 three 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.

3.5. Molecular weight and gyration radius of amylopectin

Weight-average molecular weight (M_w), polydispersity, and gyration radius (R_z) of apple starch amylopectin are shown in Table 3. The M_w of apple amylopectin ranged from 4.63×10^8 to 1.11×10^9 g/mol for the six cultivars, which was larger than that of most starches reported (Yoo & Jane, 2002b). The polydispersity (M_w/M_n) of molecular weight of Granny Smith, Jonagold and Royal Gala amylopectin was much smaller than that of most other native starches reported (Stevenson, 2003) and was significantly smaller than that of Gala amylopectin. Differences in amylopectin polydispersity between Gala and Royal Gala are intriguing because of their genetic similarity (Hansen & Zanon, 1982; Kruczynska, Rutkowski, & Czynczyk, 2001). Gyration radius of apple amylopectin ranged from 406 to 435 nm, which were larger than that of most starch amylopectin reported in the literature (Yoo & Jane, 2002b). Despite the larger gyration radius of apple amylopectin for all the cultivars, the densities of the dispersed molecules were comparable or larger than that of other C-type starches, such as lotus root and green banana starch (Yoo & Jane, 2002b).

Table 3
Average amylopectin molecular weight, polydispersity, gyration radius and density of dispersed apple amylopectin[a]

Cultivar[b]	$M_w\times10^8$ (g/mol)[c]	Polydispersity (M_w)	R_z (nm)[d]	ρ (g/mol/nm^3)[e]
Gala	4.63	3.16a	406	6.9
Golden Delicious	7.20	2.14ab	422	9.6
Granny Smith	11.10	1.47b	435	13.5
Jerseymac	6.41	1.80ab	419	8.7
Jonagold	6.47	1.54b	413	9.2
Royal Gala	7.79	1.51b	428	9.9
	$P=0.79$	$P=0.02$	$P=0.94$	$P=0.83$

Values with different letters denote differences at the 5% level of significance for each. comparison between cultivars in the respective column. P represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

[a] Data were obtained from two analyses of three replicates.

[b] 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.

[c] Weight-average molecular weight.

[d] z-average radius of gyration.

[e] Dispersed amylopectin density is equal to M_w/R_z^3.

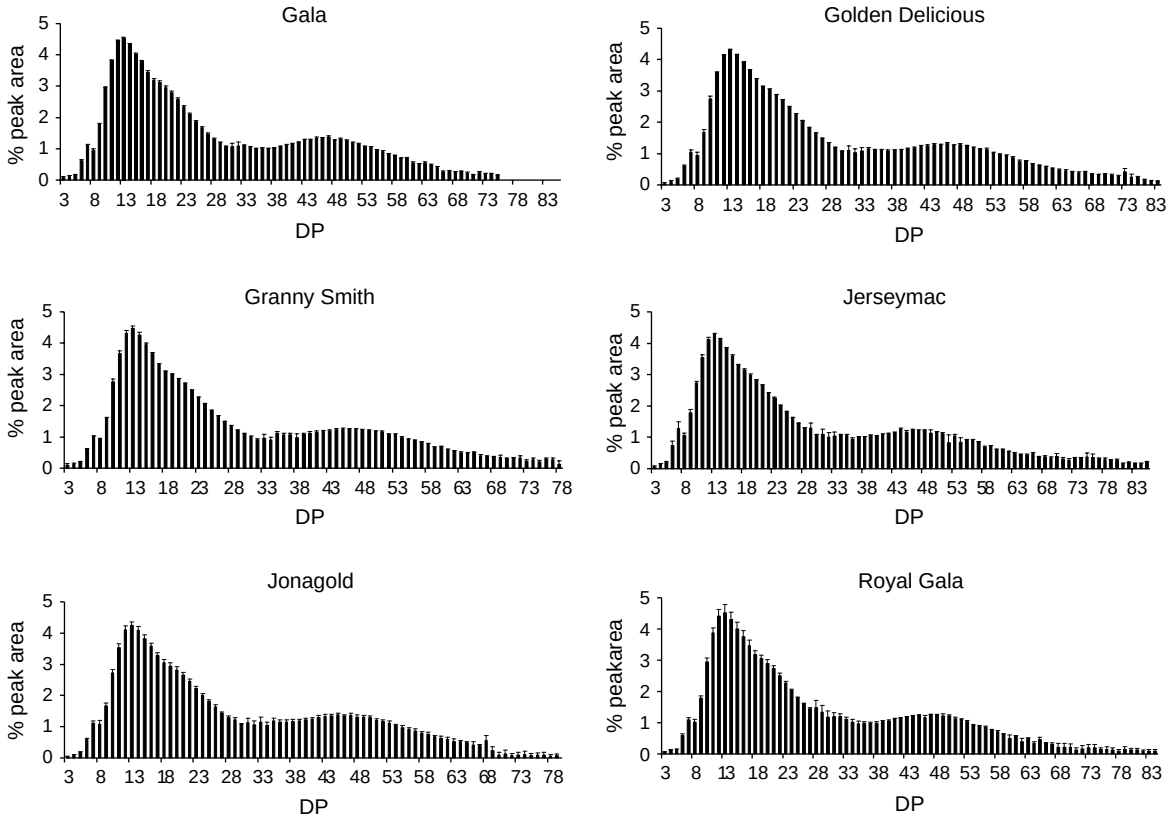

Fig. 3. Relative peak area distributions of Gala, Golden Delicious, Granny Smith, Jerseymac, Jonagold and Royal Gala apple fruit amylopectin branch chain-lengths analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from two analyses of three replicates. DP = Degree of polymerization.

3.6. Amylopectin branch chain-length distribution

Amylopectin branch chain-length distributions for the starches of apple cultivars are shown in Fig. 3 and summarized in Table 4. The most notable characteristic of all apple amylopectins was the very large proportion (29.7–32.4%) of long branch-chains (DP≥37) (Table 4, Fig. 3), which far exceeded the proportion reported previously for any other starch of C-type polymorphism (24% or less) and also larger than that of the B-type starches reported (26.1–29.5%) using the same analytical method (Jane et al., 1999). Average chain-lengths of apple amylopectins (DP 27.9–29.6) were longer than that of other C-type starches (DP 25.4–26.7) but were comparable to that of the B-type starches (DP 28.9–30.7) because B-type starch had less (8.5–12.3%) short branch chains (DP 6–12) (Jane et al., 1999) than the apple starch (14.7–15.9%). The peak chain lengths of long branch-chains (peak II) of apple amylopectin (DP 45.3–47) were shorter than that of other B- or C-type starch (DP 48–53), but were comparable with that of the A-type starch (DP 41–51) (Jane et al., 1999). The peak chain lengths of the short branch chains (peak I) (DP 12.7–13) were also similar to that of the A-type starch (DP 12–14) (Jane et al., 1999). The larger proportions of short branch chains of DP 6–12 and the shorter peak chain lengths of amylopectin resulted in the C_A type polymorphism of apple starch despite its very long average branch-chain length.

Table 4
Branch chain-length distributions of apple fruit amylopectins[a]

Cultivar	Peak DP		Average	Percent distribution					
	I	II	CL	DP 3–6	DP 6–9	DP 6–12	DP 13–24	DP 25–36	DP≥37
Gala	13.0	46.3	27.9	1.0	4.6	15.9	39.0	15.0	29.7
Golden Delicious	13.0	45.7	29.1	0.9	4.3	14.7	38.0	14.4	31.8
Granny Smith	13.0	46.3	29.2	1.0	4.2	14.9	38.2	14.0	31.9
Jerseymac	13.0	45.3	29.6	1.1	4.9	15.3	37.5	14.1	32.1
Jonagold	13.0	45.3	29.1	0.9	4.5	14.9	37.2	15.2	32.4
Royal Gala	12.7	47.0	28.4	0.9	4.4	15.6	38.5	15.7	29.9
			$P=0.39$	$P=0.45$	$P=0.57$	$P=0.61$	$P=0.76$	$P=0.69$	$P=0.53$

P represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.
[a] Values were calculated from two injections for each of the three replicates.

Table 5
Thermal properties of native apple starches

Cultivar[a]	T_o (°C)[b]	T_p (°C)	T_c (°C)	Range (°C)[c]	ΔH (J/g)
Gala	66.1a	70.9	77.1a	11.0b	17.1
Golden Delicious	64.7bc	70.0	76.2ab	11.4ab	17.7
Granny Smith	66.5a	70.1	75.1b	8.5c	15.8
Jerseymac	64.2bc	70.0	76.9a	12.8a	17.3
Jonagold	64.1c	70.3	77.2a	13.1a	17.4
Royal Gala	65.5ab	70.7	77.3a	11.8ab	16.5
	$P=0.0001$	$P=0.14$	$P=0.007$	$P<0.0001$	$P=0.68$

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column. P represents the probability of F-statistic exceeding expected 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] Values were calculated from three analyses for each of three replicates.

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

The apparent amylose content of apple starch was correlated to the average branch chain-length of amylopectin ($r=0.92$, $P=0.008$) and to the proportions of amylopectin branch-chains of DP 13–24 ($r=-0.92$, $P=0.008$) and of DP≥37 (Table 8). Results of correlation coefficients are shown in Table 8 if not given in the text.

3.7. Thermal properties

Thermal properties of the apple starches are shown in Table 5. Onset gelatinization temperatures (T_o) of Gala and Granny Smith starch were significantly higher than that of Golden Delicious, Jerseymac and Jonagold ($P=0.0001$). Peak gelatinization temperatures (T_p) were not significantly different between cultivars. The conclusion gelatinization temperature (T_c) of Granny Smith was significantly lower than that of other cultivars except Golden Delicious ($P=0.007$). Thus, Granny Smith starch had a narrower gelatinization temperature range (8.5 °C) than others. Gelatinization temperatures of apple starches closely resembled that of cornstarch, but the ΔH of apple starch was similar to that of potato starch (Jane et al., 1999).

The apparent amylose content of the apple starch was negatively correlated to T_o and T_p (Table 8). This agreed with previous report that the starch with a larger apparent-amylose content displays a lower T_o (Demenke, Hucl, Abdel-Aal, Båga, & Chibbar, 1999; Inouchi et al., 1993; Visser, Suurs, Steeneken, & Jacobsen, 1997). T_o was also correlated to the proportion of amylopectin chains of DP 13–24 ($r=0.82$, $P=0.05$). The correlation coefficients between the T_p and branch chain lengths of the apple amylopectin (Table 8) showed a reverse trend to that reported for other starches. Because the T_p of the apple cultivar starches were quite similar, it was difficult to determine if the relationships were meaningful. The M_w of apple amylopectin was negatively correlated to T_c ($r=-0.81$, $P=0.05$) and ΔH ($r=-0.79$, $P=0.05$).

Thermal properties of retrograded apple starches, shown in Table 6, had lower onset temperature of dissociation of retrograded starch than that of other starches reported (Jane et al., 1999), except squash fruit starches (Stevenson et al., 2005). Peak, and conclusion temperature, enthalpy change (ΔH_R) and percentage retrogradation of retrograded apple starches were similar to that reported for other C-type retrograded starches (Jane et al., 1999). There were no significant differences in starch retrogradation properties among apple cultivars.

3.8. Pasting properties

Pasting properties of apple starches are shown in Fig. 4 and summarized in Table 7. Gala, Granny Smith, and Royal Gala starch displayed significantly greater peak and breakdown viscosity (peak viscosity–trough viscosity) than the other three starches ($P<0.0001$). Golden Delicious, Jerseymac and Jonagold starch exhibited very little breakdown, a distinctive feature only previously reported for green leaf canna starch (Jane et al., 1999). These three starches also displayed large

Table 6
Thermal properties of retrograded apple starch

Cultivar[a]	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	% Retrogradation
Gala	36.9	53.3	64.3	7.1	41.6
Golden Delicious	36.1	52.4	63.3	7.4	42.3
Granny Smith	37.5	53.4	64.5	7.1	45.2
Jerseymac	36.5	53.1	64.4	7.8	46.1
Jonagold	36.5	52.8	63.5	7.9	45.5
Royal Gala	37.6	53.1	64.7	7.5	47.3
	$P=0.34$	$P=0.20$	$P=0.21$	$P=0.88$	$P=0.95$

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

[a] Same starch samples after gelatinization (see Table 5) were stored for 7 days at 4 °C and rescan using DSC.

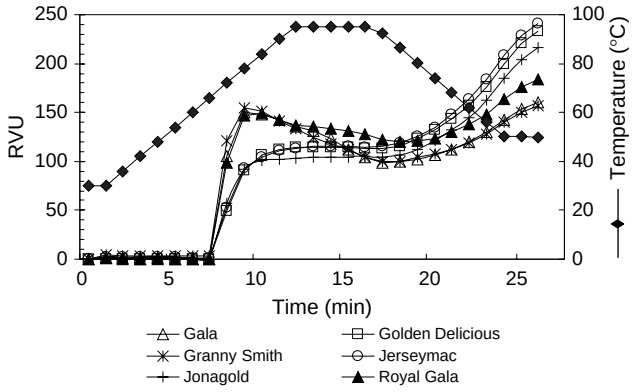

Fig. 4. Rapid Visco-Analyser pasting profiles of Gala, Golden Delicious, Granny Smith, Jerseymac, Jonagold and Royal Gala apple fruit starches (8.0% dsb, w/w).

final viscosity and unusually great setback viscosity of over 100 RVU (Table 7). The correlation analysis showed that the apparent amylose content of apple starch was negatively correlated to the peak and breakdown viscosities and positively correlated to the final and setback viscosities (Table 8). These results agreed with previous reports that larger apparent amylose contents resulted in lower peak viscosity (Jane et al., 1999; Kuno, Kainuma, & Takahashi, 2000; Wang, White, & Pollak, 1993). The proportion of amylopectin branch-chains of DP 13–24 was correlated to the peak viscosity ($r=0.91$, $P=0.01$), breakdown viscosity ($r=0.81$, $P=0.05$), and setback viscosity ($r=-0.79$, $P=0.05$). The peak viscosity was also negatively correlated to the proportion of amylopectin chains of DP≥37 (Table 8), which agreed with previous reports (Han & Hamaker, 2001; Li, Vasanthan, Rossnagel, & Hoover, 2001). These results indicated that long amylopectin chains restrict starch from swelling and reduce the peak viscosity. Pasting temperature of around 70 °C for apple starch was higher than that of potato starch (63.5 °C) but considerably lower than that of cereal starch from rice (79.9 °C), corn (82.0 °C) and wheat (88.6 °C) (Jane et al., 1999) because of the lack of lipids in the apple starch.

The results of correlation coefficients showed that the low breakdown viscosity of Golden Delicious, Jerseymac and

Table 7
Pasting properties of apple starches measured by Rapid Visco-Analyser[a,b]

Cultivar	Peak viscosity (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Setback (RVU)	Peak time (min)	Pasting temperature (°C)
Gala	147.8a	45.6a	161.5bc	59.3b	9.5b,c	70.3ab
Golden Delicious	113.1b	4.2b	216.1a	107.2a	11.6a,b	70.7ab
Granny Smith	141.5a	51.3a	144.0c	53.8b	9.0c	69.9b
Jerseymac	112.9b	2.1b	224.0a	113.1a	12.2a	70.6ab
Jonagold	99.3b	2.7b	198.8ab	102.2a	11.8a,b	71.3a
Royal Gala	148.3a	37.2a	179.7abc	68.6b	9.8b,c	70.6ab
	$P<0.0001$	$P<0.0001$	$P=0.0008$	$P<0.0001$	$P=0.002$	$P=0.01$

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column. P represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

[a] 8% (w/w) starch suspension measured in duplicate for all three replicates.

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

Table 8
Correlation coefficients ($r\times100$) among apple fruit starch of selected structural and functional properties

	St	Ap_A	Ab_A	T_o	T_p	ROG	ΔH	ΔH_R	DP_{6-12}	$DP_{\geq37}$	PV	BK	FV	SB	PT
St	100														
Ap_A	5	100													
Ab_A	0	−14	100												
T_o	22	−83*	47	100											
T_p	−37	−83*	−18	44	100										
ROG	−51	51	−57	−88**	2	100									
ΔH	37	42	−25	−77*	−14	76*	100								
ΔH_R	−18	78*	−59	−92**	−33	87*	52	100							
DP_{6-12}	−65	−62	16	36	85*	10	−13	−25	100						
$DP_{\geq37}$	56	85*	−4	−56	−90**	14	21	51	−86*	100					
PV	−21	−85*	35	89**	65	−62	−68	−78*	66	−83*	100				
BK	16	−86*	36	97**	58	−77*	−81*	−83*	49	−64	93**	100			
FV	−44	79*	−26	−93**	−49	79*	82*	76*	−30	44	−76*	69	100		
SB	−22	87*	−27	−96**	−61	76*	80*	80*	−47	63	−89**	−99***	97**	100	
PT	−12	50	−80*	−86*	−7	88**	71	85*	−24	32	−76*	−78*	68	71	100

St, starch content; Ap_A, apparent amylose content; Ab_A, absolute amylose content; T_o, onset gelatinization temperature; T_p, peak gelatinization temperature; ROG, range of gelatinization temperature; ΔH, enthalpy change of gelatinization; ΔH_R, enthalpy change of retrograded thermal transition, DP_{6-12}, proportion of amylopectin branch chain-lengths DP 6–12; $DP_{\geq37}$, proportion of amylopectin branch chain-lengths DP≥37; PV, peak viscosity; BK, breakdown; FV, final viscosity; SB, setback and PT, pasting temperature. *0.05, **0.01 and ***0.001 level of significance.

Jonagold starch pastes are results of the amylopectin with more branch-chains of DP $\geq$ 37 and less branch chains of DP 13–24 and the larger apparent amylose content, which help hold the granule together during swelling. This result agreed with that rice starch granules composed of higher proportions of amylose and long branch-chain amylopectins resist swelling and disintegration when heated in water under shear (Reddy, Ali, & Bhattacharya, 1993).

4. Conclusion

Starch granules of six apple cultivars had diameters ranging from 2 to 12 μm, and starches exhibited C_A-type X-ray diffraction patterns. Apparent amylose content ranged 40–48%, absolute amylose content ranged 26–29%, and amylopectin molecular weight ranged $4.6–11.1 \times 10^8$. Average amylopectin branch chain-length was very long (DP 27.9–29.6), and this contributed to the distinctive pasting properties of very low breakdown viscosity ($\leq$4 RVU) and very high setback viscosity ($>$100 RVU) observed for three cultivars of the apple starch. Onset gelatinization temperatures of the starches were 64–66 °C and ΔH was high (16–18 J/g).

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