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Morphological, Structural, Thermal, and Rheological Characteristics of Starches Separated from Apples of Different Cultivars

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The starches were separated from unripe apples of five cultivars (Criterion, Ruspippum, Red Spur, Skyline Supreme, and Granny Smith) and evaluated using scanning electron microscopy (SEM), gel permeation chromatography (GPC), X-ray diffraction, differential scanning calorimetry (DSC), and dynamic viscoelasticity. SEM showed the presence of round granules as well as granules that had been partially degraded, probably by amylases. The starch granules in different apple starches ranged between 4.1 and 12.0 μm. Debranching of starch with isoamylase and subsequent fractionation of debranched materials by GPC revealed the presence of an apparent amylose, an intermediate fraction (mixture of amylose and amylopectin), long side chains of amylopectin, and short side chains of amylopectin in the range of 28−35.2, 3.6−4.4, 20−21.3, and 39.9−47.1%, respectively. The swelling power of starches ranged between 14.4 and 21.3 g/g. X-ray diffraction of apple starches showed a mixture of A- and B-type patterns. All apple starches showed peak intensities lower than that observed for normal corn and potato starch, indicating the lower crystallinity. The transition temperatures (onset temperature, T_o; peak temperature, T_p; and conclusion temperature, T_c) and enthalpy of gelatinization (ΔH_{gel}) determined using DSC ranged between 54.7 and 56.2 °C, between 57.1 and 59.1 °C, between 60.2 and 63.5 °C, and between 3.3 and 4.2 J/g, respectively. The viscoelastic properties of starch from different cultivars measured during heating and cooling using a rheometer differed significantly. Red Spur and Criterion starches with larger granule size showed higher G' and G'' values, whereas those containing smaller size and amylolytically degraded granules showed lower G' and G''.

KEYWORDS: Starch; apple; amylose; amylopectin; scanning electron microscopy; SEM; X-ray; gel permeation chromatography; differential scanning calorimetry; viscoelasticity

INTRODUCTION

Starch is the main storage carbohydrate in unripe apples. It accumulates during apple growth and then progressively hydrolyses during ripening. When apples ripen, starch is hydrolyzed to sugars, and only a few cells have been reported to contain any starch (*1*). The starch present, although in very small quantity, has a significant importance in apple juice processing. The presence of starch in apple juice makes its processing difficult. In the beginning of the season apples contain a considerable amount of starch. A starch content of 15% in raw apples has been reported by Reed (*2*). Carrin et al. (*3*) reported nonpasteurized apple juice had as much as 8 g/L of starch. Ohmiya and Kakiuchi (*4*) studied changes in starch concentration during the growth and development of apples. The accumulation and degradation of starch in apples have been reported to vary with cultivar and to be dependent on climatic and growing conditions (*5*). Smith et al. (*6*) reported that during maturation, each cultivar exhibits a characteristic color pattern, measured by iodine staining, as the starch is lost from the fruit. A high temperature during summer favors starch accumulation and can delay the beginning of hydrolysis (*1*). A number of researchers have measured the disappearance of starch by using the starch−iodine test in apples and reported this test as the simplest for determining apple maturity (*6*). This has also been reported to correlate with the sensory quality of apples (*7*). The starch content in apples is being used as an indicator of maturation because of a close relationship between them. Efforts are also being made by various workers to develop a rapid method to evaluate the starch content of apples qualitatively. Cho and Gil (8) reported a rapid method consisting of staining an apple slice with iodine solution and analyzing the stained color image. Indication of starch hydrolysis by iodine staining is widely used in determining the optimum harvesting time of apples, yet few quantitative changes in apple starch have been reported. Several researchers have established tables with reference stages marking the progressive disappearance of starch from apples. Lau (*9*) proposes a scale of 1−9, where 1 corresponds to the generalized presence of starch and 10 to the

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absence of starch. Fan et al. (*10*) reduced this table to a scale of 1−6.

The structure and functional properties of starch present in cereals, legumes, and tubers have been extensively studied by many researchers (*11*−*16*). Recently, some works on the starches in nonconventional sources such as fruits have been initiated because of either their potential applications in the development of new products or their role in processing. The physicochemical and functional properties of mango (*17*, *18*), banana (*19*, *20*), and okenia (*21*) starches have been reported. Besides a few studies reported below on some characteristics of apple starches, no detailed study concerning the structural and functional characteristics of starch present in apples of different cultivars is available. Potter et al. (*22*) separated amylose and amylopectin of apple starch and reported an amylose content of 24.8%. They reported that the treatment of apple starch with a crystalline β-amylase hydrolyzed amylose and amylopectin to 90 and 63.5% maltose, respectively. Carrin et al. (*3*) reported the morphology and degradation of starch in the juice of Granny Smith apples by amylase. In the present study, we have characterized the morphological, structural, thermal, and rheological characteristics of starches separated from apples of different cultivars.

MATERIALS AND METHODS

Materials. Unripe apples of five cultivars (Criterion, Ruspippum, Red Spur, Skyline Supreme, and Granny Smith) were harvested from an apple orchard of the Regional Horticultural Research Station, Mashobra, India, 110 days after blooming (flowering) during the 2003 season. The station is located at 31.1° N longitude and 77.1° E and 2286 m above mean sea level in the Shimla Hills of Himachal Pradesh, India. The fruits (15 kg) of each cultivar were collected randomly from different trees (four to five) of the same cultivar, labeled, and packed in cardboard boxes for further starch separation. The starch was extracted within 8 h of harvesting in India.

Starch Separation. Apples from different cultivars were washed, peeled, and cut into small pieces (2cm × 2cm × 2cm), and their seeds were removed. The cut pieces were steeped in water containing 0.16% potassium metabisulfite. The steep water was drained off, and the apples were ground in a laboratory blender. The ground slurry was screened through nylon cloth (100 mesh). The material left over the nylon cloth was washed thoroughly with distilled water. The filtrate slurry was allowed to stand for 1 h. The supernatant was removed by suction, and the settled starch layer was resuspended in distilled water and centrifuged in wide-mouthed cups at 2800*g* for 5 min. The upper nonwhite layer was discarded. The white layer was resuspended in distilled water and recentrifuged four or five times. The starch was then collected and dried in a hot air circulatory oven at 40 °C for 10 h.

Scanning Electron Microscopy (SEM). Scanning electron micrographs were obtained with a scanning microscope (JEOL JSM-6100, JEOL Ltd., Tokyo, Japan). Starch samples were suspended in ethanol to obtain 1% suspension. One drop of the starch−ethanol suspension was applied on an aluminum stub, and the starch was coated with gold−palladium (60:40). An acceleration potential of 10 kV was used during micrographic observation.

Amylose Content, Gel Permeation Chromatography (GPC), and Swelling Power. Amylose content was determined using the method of Williams et al. (*23*). Starches were debranched with crystalline *Pseudomonas* isoamylase according to the method of Ikawa et al. (*24*). Debranched materials were fractionated by gel filtration first on a column of Toyopearl HW 55S (300 mm × 20 mm ϕ) and subsequently on three columns of Toyopearl HW 50S (300 mm × 20 mm ϕ) connected in series. Each fraction was divided according to the range of the wavelength at maximum absorption (λ_{max}) in the absorption spectra of the glucan−iodine complexes of each tube: fraction I, $\lambda_{max} \geq 620$ nm; intermediate fraction, 620 nm > $\lambda_{max} \geq$ 600 nm; fraction II, 600 nm > $\lambda_{max} \geq$ 540 nm; and fraction III, 540 nm > λ_{max}. The carbohydrate contents in each tube were determined by using the phenol−sulfuric acid method (*25*). Swelling determinations were carried out at 90 °C according to the method of Leach et al. (*26*). A 1% aqueous suspension of starch (100 mL) was heated in a water bath at 90 °C for 1 h with constant stirring. The suspension was cooled for 0.5 h at 30 °C. Samples were then poured into preweighed centrifuge tubes and centrifuged at 3000*g* for 10 min, and then the weight of sediments was determined.

X-ray Diffractometry. X-ray diffractograms of fully moistened starch granules (exposed to 100% relative humidity for 3 days) were recorded by an X-ray diffractometer (RINT 2000, Rigaku Co., Ltd., Tokyo, Japan) using the method of Ikawa et al. (*24*), that is, using Cu Kα (Ni filtered); voltage 40 kV; electric current, 40 mA; and scanning speed of goniometer, 4°/min.

Thermal Properties. The thermal properties of isolated starches were analyzed using a Micro-Differential scanning calorimeter (Setaram, France). Starch (200 mg) was weighed in a hastelloy cell. Distilled water was added with the help of a Hamilton microsyringe to prepare a starch−water suspension containing 70% water. Samples were hermetically sealed and allowed to stand for 1 h at 25 °C in DSC followed by heating at a rate of 1 °C/min from 25 to 110 °C and cooling to 5 °C at the same rate. Distilled water was used as reference. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and heat of gelatinization (ΔH_{gel}) were calculated automatically. The gelatinization temperature range (ΔT) was computed as ($T_c - T_o$) because the peaks were almost symmetrical.

Dynamic Rheometry. A Fluids Spectrometer RFS II (Rheometrics Co. Ltd., Piscataway, NJ) equipped with a parallel plate (25 mm diameter) was used. The gap size was set to 1.0 mm. The storage and loss shear moduli G' and G'' were measured at 1.0% strain, which is within the linear viscoelastic regime. Starch suspension of 20% (w/w) was loaded, after stirring for 30 min at room temperature on a magnetic stirrer, on the ram of the rheometer preheated to 50 °C and covered with a thin layer of low-density silicone oil to prevent evaporation. The starch samples were heated from 50 to 90 °C at a rate of 0.5 °C/min and held at 90 °C for 30 min. The frequency dependence of the viscoelastic moduli was also observed after heating at 90 °C for 30 min. The changes in both moduli during cooling from 90 to 50 °C at the same rate were also observed.

Reducing Capacity. A starch (50 mg dry wt basis) was suspended in 1.5 mL of deionized water and kept at 50 °C for 60 min in a water bath with constant shaking at a rate of 80/min (forward and backward). The starch suspensions were then centrifuged (1870*g*, 10 min, 5 °C). The reducing capacity of the supernatant was determined using the Park and Johnson (*27*) method as modified by Hizukuri et al. (*28*) using glucose as standard.

Statistical Analysis. The data reported are the average of triplicate observations and were subjected to analysis of variance using Minitab Statistical Software version 13 (Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

Scanning Electron Microscopy. The scanning electron micrographs in **Figure 1** show the presence of round granules. All of the starches showed the presence of granules partially degraded, probably by amylase; however, the extent of degradation varied (**Figure 1**). Skyline Supreme (**Figure 1E**) and Ruspippum (**Figure 1D**) starches showed higher degradation than other starches, the highest in the former. The starches showing a higher proportion of degraded granules also showed a higher reducing capacity determined as glucose equivalent. The degree of hydrolysis of starches has been reported to be dependent on the type of amylases and starches (*29*). Red Spur and Criterion showed the presence of large size granules, whereas Skyline Supreme starch showed the smallest size granules. Starch granules of Red Spur and Criterion ranged from 9.5 to 12.0 μm and from 5.9 to 10.9 μm, respectively, whereas Skyline Supreme, Ruspippum, and Granny Smith starch showed granule size ranges of 4.1−7.5, 5.4−8.6, and 5.7−9.4 μm,

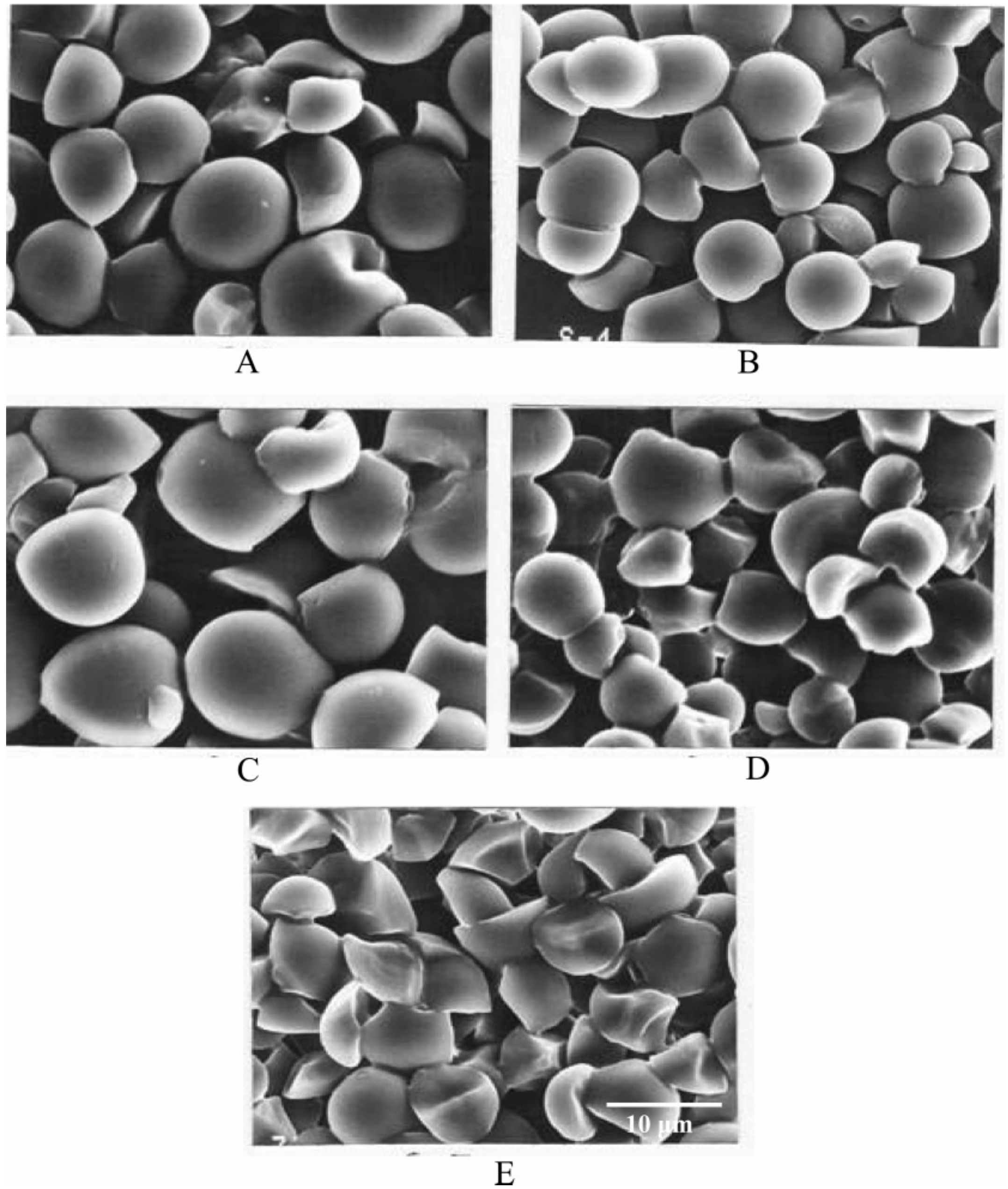

Figure 1. Scanning electron micrographs of starches separated from different apple cultivars (**A**, Criterion; **B**, Granny Smith; **C**, Red Spur; **D**, Ruspippum; **E**, Skyline Supreme).

respectively. Carrin et al. (*3*) reported the presence of spherical starch granules with a mean diameter of 9.21 μm. Kovacs and Eads (*30*) reported round starch granules of 10 μm size, the area and perimeter of which decreased and circularity increased with storage duration and ripeness of apples.

Gel Permeation Chromatography and Swelling Power. The amylose contents of Criterion, Ruspippum, Red Spur, Skyline Supreme, and Granny Smith were 26.1, 22.2, 24.1, 23.3, and 23.1%, respectively. Elution profiles of the debranched starches separated from different apple cultivars are shown in **Figure 2**, where fraction I represents apparent amylose, intermediate fraction stands for a mixture of amylose and long side chains of amylopectin, fraction II is long side chains of amylopectin, and fraction III is short side chains of amylopectin as reported by Ikawa et al. (*24*). The apparent amylose content of Criterion starch (35.2%) was the highest among the starches studied. Fraction I, intermediate fraction, fraction II, and fraction III ranged from 28.0 to 35.2%, from 3.6 to 4.3%, from 19.9 to 21.3%, and from 39.9 to 47.1%, respectively, in various apple starches (**Table 1**). Criterion starch with the highest content of fraction I showed the lowest content of fraction III (39.9%). Ruspipipum showed the lowest apparent amylose content (28.0%) and the highest content of fractions II (21.3%) and III (47.1%). Granny Smith starch showed the highest content of intermediate fraction. The amount of short-branch chain fractions observed in apple starches was lower than that observed for normal corn starch (*31*). On the other hand, the proportion of long-chain amylopectin in apple starches was higher than that in normal corn starch. All apple starches except Criterion also showed a higher amount of fraction III than sugary-2 corn starch (*32*). Skyline Supreme showed the lowest swelling power, whereas Granny Smith showed the highest swelling power. The swelling power observed for apple starches was lower than those observed for potato starches (*15*). All apple starches except the Skyline Supreme showed higher swelling powers than normal and sugary corn starches.

Table 1. Distribution of Isoamylase-Debranched Materials and Swelling Power of Starches Separated from Different Apple Cultivars[a]

	distribution of isoamylase branched materials					
cultivar	fraction I (%)	intermediate fraction (%)	fraction II (%)	fraction III (%)	fraction III/ fraction II	swelling power (g/g)
Criterion	35.2 d	4.0 b	20.9 bc	39.9 a	1.9 a	16.6 b
Ruspippum	28.0 a	3.6 a	21.3 c	47.1 d	2.2 b	17.0 b
Red Spur	32.0 c	4.0 b	20.3 b	43.7 b	2.2 b	16.7 b
Skyline Supreme	31.6 bc	4.0 b	19.9 b	44.5 bc	2.2 b	14.4 a
Granny Smith	31.2 b	4.3 c	20.0 b	44.5 c	2.2 b	21.3 c
Normal corn	29.4 ab	4.4 c	17.8 a	48.4 d	2.7 c	15.1 a

[a] Values with similar letters in a column do not differ significantly ($P < 0.05$). Fraction I, apparent amylose content; intermediate fraction, mixture of amylose and amylopectin; fraction II, long side chain of amylopectin; fraction III, short side chain of amylopectin.

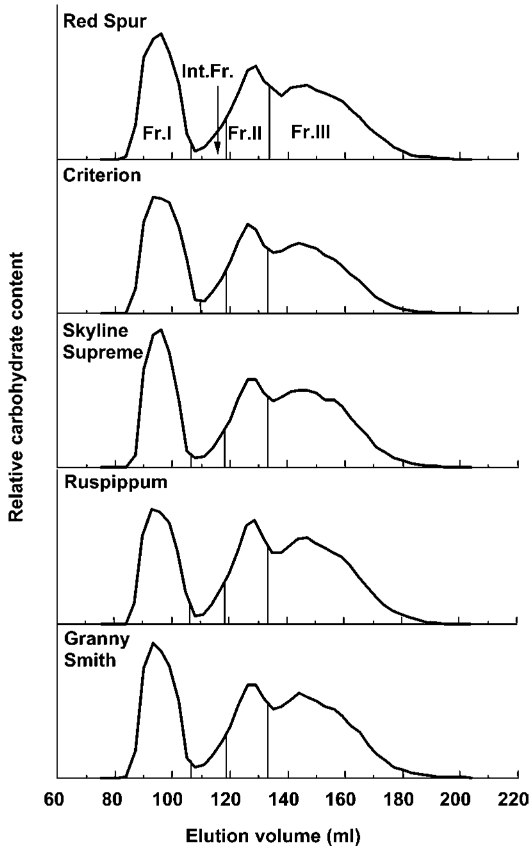

Figure 2. Gel permeation chromatograms of isoamylase-debranched apple starches.

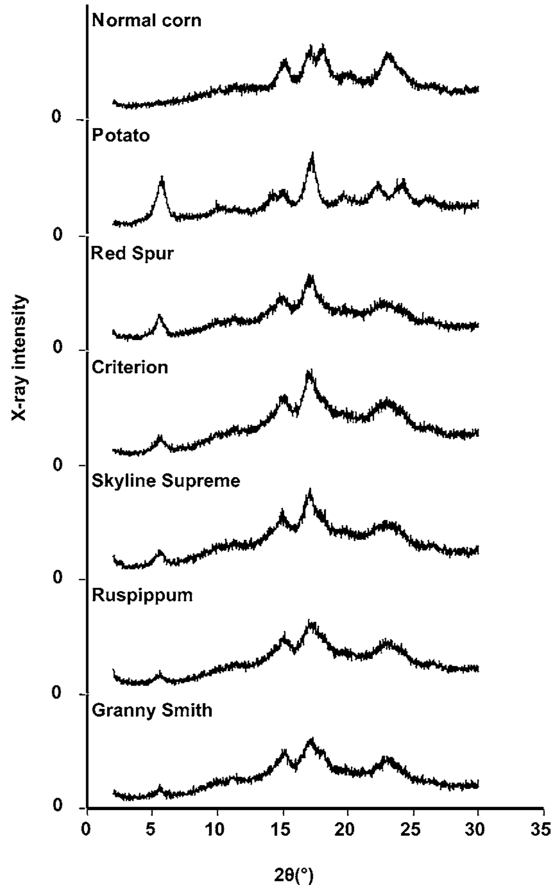

Figure 3. X-ray diffraction patterns of native starches separated from corn, potato, and apples.

X-ray Diffractogram. X-ray diffraction patterns of the apple starches were compared with those of potato and corn starches (**Figure 3**). Apple starches showed a small peak at $2\theta = 5.6°$ as observed for potato starch; however, the intensity was lower in the former. Potato starch showed dual peaks at $2\theta = 22.4-24.1°$, which appeared as a single broad peak in apple starches ($2\theta = 23.2°$). The single peak around $2\theta = 23.2°$ in corn starch is a characteristic of the A-type X-ray diffraction pattern. Potato starch showed two peaks at $2\theta = 14.1-15°$, whereas apple starch showed a single peak ($2\theta = 15°$). The X-ray diffraction of apple starch indicates the presence of some of the characteristics of both A- and B-type patterns. The intensity of the peak at $2\theta = 17°$ for apple starch was lower than that for potato starch. Corn starch showed dual peaks ($2\theta = 17$ and $18.1°$). In contrast to potato and corn starches, crystallinity was somewhat lower for apple starches. The lower crystallinity for apple starches may be attributed to lower amounts of short- and long-chain amylopectin and higher amylose content. It has been reported that the A-polymorph was favored by short chains, whereas the B-polymorph was favored by long chains (*33*, *34*). In general, legume starches and some tropical tuber starches have been reported to display the C-type pattern (*11*, *35*). Millan-Testa et al. (*18*) reported the C-type pattern for banana starch.

Thermal Properties. DSC heating curves of starches separated from unripe apples of different cultivars are illustrated in **Figure 4**. The transition temperatures (T_o, T_p, and T_c) and enthalpy of gelatinization (ΔH_{gel}) of different apple starches differed significantly (**Table 2**). T_o, T_p, and T_c ranged from 54.7 to 56.2 °C, from 57.1 to 59.1 °C, and from 60.2 to 63.5 °C, and ΔH_{gel} ranged from 3.3 to 4.2 J/g, respectively (**Table 2**). T_o, T_p, and T_c values observed for apple starches were close to those observed earlier for potato starches (*36*). Values of ΔH_{gel} observed for apples starches were lower than those observed for normal corn and potato starches (*36*). This may be attributed to the lower crystallinity in apple starches as compared to that in corn and potato starches. The ΔH_{gel} reflected primarily the loss of molecular (double-helical) order (*37*). High transition

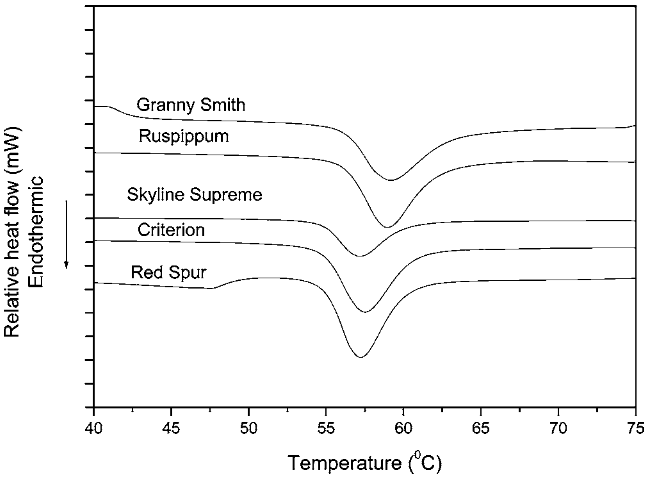

Figure 4. DSC heating curves of starches separated from different apple cultivars. Scan rate = 1 °C/min.

Table 2. Thermal Properties of Starches Separated from Apples of Different Cultivars[a]

cultivar	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g)	ΔT
Criterion	54.8 a	57.4 a	60.8 a	3.6 ab	6.0 b
Ruspippum	56.2 b	58.9 b	62.4 b	3.6 ab	6.2 b
Red Spur	54.9 a	57.2 a	60.3 a	4.2 c	5.4 a
Skyline Supreme	54.7 a	57.1 a	60.2 a	3.7 b	5.5 a
Granny Smith	56.1 b	59.1 b	63.5 b	3.3 a	7.4 c

[a] Values with similar letters in a column do not differ significantly ($P < 0.05$). T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, gelatinization temperature range, $\Delta T = T_p - T_o$; ΔH_{gel} = enthalpy of gelatinization (dwb, based on starch weight).

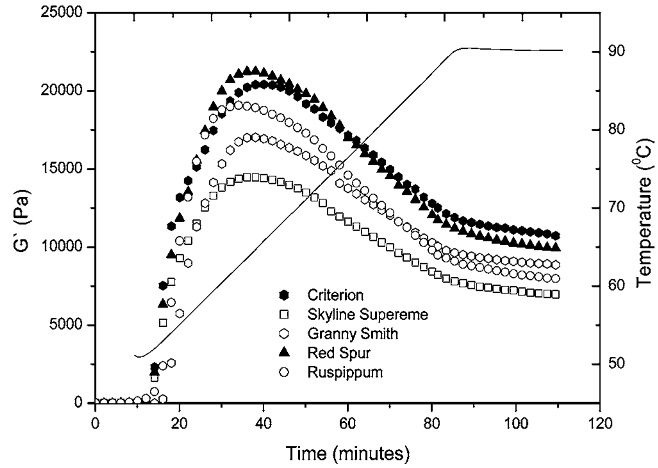

Figure 5. Changes in G' of starches separated from different apple cultivars during heating at 0.5 °C/min. Frequency = 1.0 rad^{-1}.

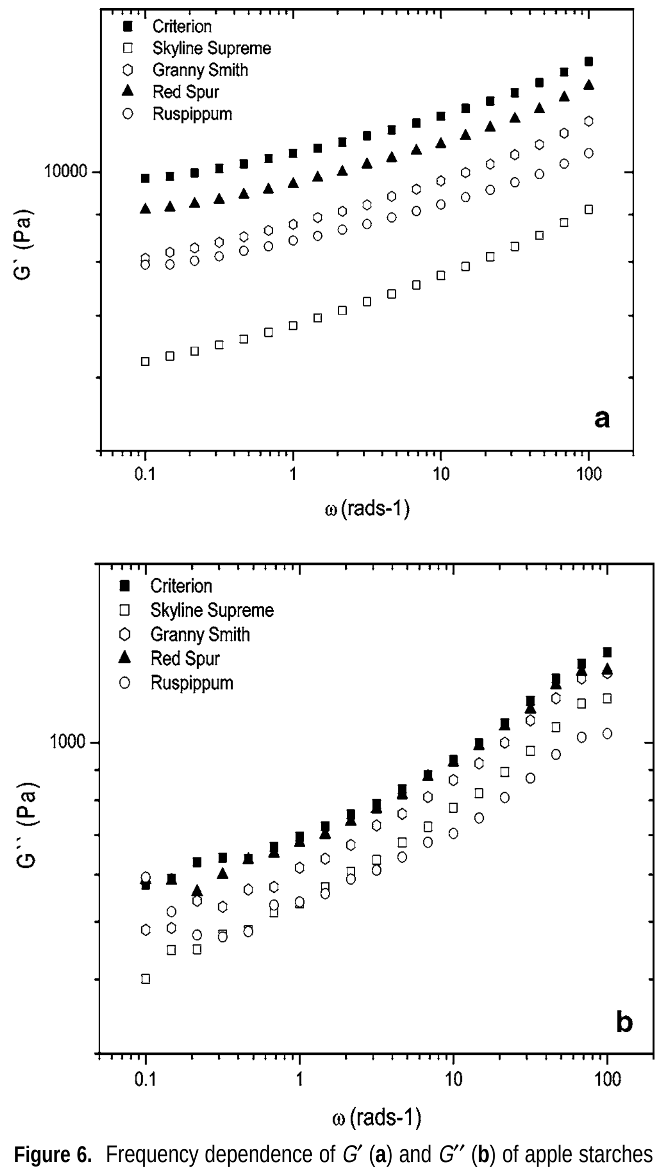

Figure 6. Frequency dependence of G' (**a**) and G'' (**b**) of apple starches at 90 °C. Strain = 1.0%.

temperatures have been reported to result from a high degree of crystallinity, which provided structural stability and made the granule more resistant to gelatinization (*38*). The difference in the ΔT values among starches from different apple cultivars may be due to the presence of crystalline regions within a starch granule composed of small crystallites having slightly different crystal strengths (*39*). The variation in T_o and ΔH_{gel} in starches from different cultivars might be due to the difference in amounts of longer chains in amylopectins. These longer chains require a higher temperature to dissociate completely than that required for shorter double helices (*40*). The ΔT of the gelatinization temperature for apple starches was narrow in comparison with those for normal corn and potato starches (*36*). The melting temperature of starch has been reported to be dependent on the degree of branching; the higher the degree of amylopectin branching, the wider the melting temperature range and vice versa (*41*).

Dynamic Rheometry. The storage and loss shear moduli G' and G'' of suspensions of starches separated from different apple cultivars as a function of temperature or frequency are illustrated in **Figures 5−8**. The storage modulus G' of all five apple starch suspensions increased during heating to a maximum and then decreased (**Figure 5**). Red Spur and Criterion showed higher peak G', whereas Skyline Supreme starch showed the lowest peak G'. Red Spur, Criterion, Ruspippum, Granny Smith, and Skyline Supreme starches showed peak G' values of 2.1×10^4, 2.0×10^4, 1.9×10^4, 1.7×10^4, and 1.4×10^4 Pa, respectively. During heating, the starch granules swell and amylose chains leach out, leading to an increase in G' to a maximum value, which may be attributed to the formation of a network of swollen starch granules (*42*). After reaching a maximum value between 66 and 69.5 °C, the modulus starts to decrease, indicating the destruction of gel structure (*43*). Red Spur, Criterion, Granny Smith, Ruspippum, and Skyline Supreme starches showed peak G' values at 68.1, 69.5, 67.5, 66, and 67.6 °C, respectively. Large granules in Red Spur and Criterion starches seem to be

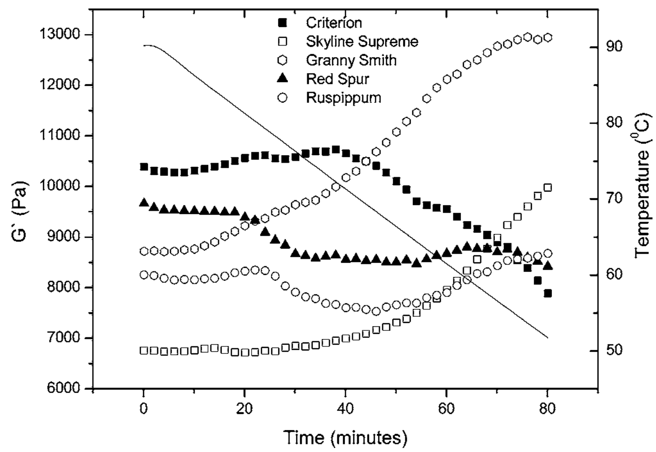

Figure 7. Changes in *G*′ of different starch pastes during cooling at 0.5 °C/min. Strain = 1.0%. Frequency = 1.0 rad^{-1}.

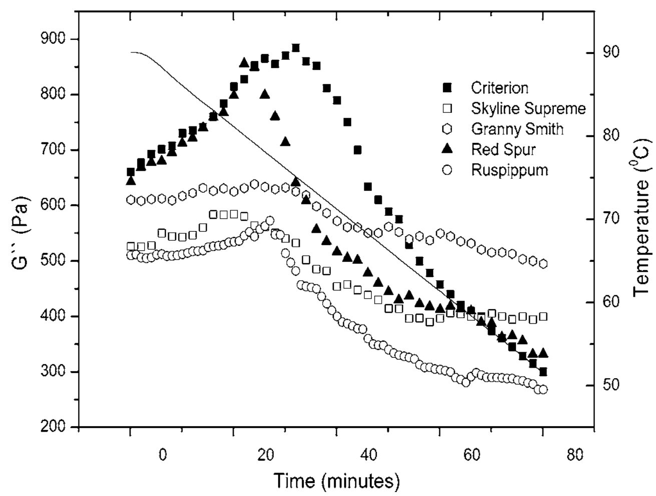

Figure 8. Changes in *G*″ of different starch pastes during cooling at 0.5 °C/min. Strain = 1.0%. Frequency = 1.0 rad^{-1}.

responsible for their higher *G*′ values, whereas smaller and partially degraded granules in Skyline Supreme may be responsible of the lowest *G*′. Skyline Supreme starch exhibited a more liquid-like behavior as indicated by higher tan δ, which may be attributed to the presence of smaller and amylolytically degraded granules. Between the Skyline Supreme and Ruspippum starches, which showed maximum degraded starch granules among the starches studied, the former starch showed a lower peak modulus. This may be attributed to more degradation of starch in Skyline Supreme by amylases because of its lower T_o. The difference in the storage modulus may be attributed to the difference in size of the granules, amylose content, and extent of degradation by amylases. The dependence of rheological parameters on granule size and amylose content in potato starch has been reported earlier (*15*, *44*). Red Spur starch showed a higher peak *G*′ than Criterion starch but had lower *G*′ than Criterion after heating for 30 min at 90 °C (**Figure 6**). Similarly, Ruspippum starch, which showed a higher peak *G*′ than Granny Smith, showed a lower *G*′ after heating for 30 min at 90 °C than the latter. Granny Smith starch with a higher amylose content than Ruspippum starch showed a lower breakdown in granules. After heating at 90 °C for 30 min, Criterion starch showed the highest *G*′ followed by Red Spur, Granny Smith, Ruspippum, and Skyline Supreme. The frequency dependence of *G*′ and *G*″ of starches from different cultivars is shown in **Figure 6**. Both moduli increased with increasing frequency. The storage modulus *G*′ was greater than the loss modulus *G*″ over the entire frequency range studied for all of the starches. During cooling, Granny Smith, Skyline Supreme, and Ruspippum starches showed an increase in moduli during cooling, whereas the reverse was observed in Criterion and Red Spur starches (**Figure 8**). Interestingly, this increase was observed only in the starches showing the presence of amylolytically degraded granules. It seems that sugars produced during amylolytic attack may have caused an increase in helix formation during cooling. Evagelious et al. (*45*) reported a large increase in moduli during cooling of starch gelatinized in the presence of sugars. To confirm the role of sugars and amylases, the reducing power of starch suspensions was determined after incubation at 50 °C. It was observed that the starches (Skyline supreme, Ruspippum, and Granny Smith) which showed an increase in *G*′ had higher reducing powers (82−7.8 mg/100 g as glucose equivalent) than Red Spur starch (4 mg/100 g as glucose equivalent), estimated using the sub-microdetermination procedure of Park and Johnson (*27*). Criterion starch did not show any reducing power.

Conclusions. Unripe apples displayed a mixture of A- and B-type patterns (C-pattern). The extent of starch damage varied significantly in all cultivars. All apple starches showed lower crystallinity than normal and potato starches. GPC results showed the presence of lower proportions of short-chain fractions of amylopectin in apple starches than in normal starch. The rheological properties of starches from different apple cultivars were observed to depend on amylose content, size, and extent of starch damage, probably induced by amylases.

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