

Relationship of Granule Size Distribution and Amylopectin Structure with Pasting, Thermal, and Retrogradation Properties in Wheat Starch

SANDEEP SINGH

Department of Food Science and Technology, Guru Nanak Dev University, Amritsar-143005, India

NARPINDER SINGH*

Department of Food Science and Technology, Guru Nanak Dev University, Amritsar-143005, India

NAOTO ISONO

Graduate School of Bioresources, Mie University, Tsu 514-8507, Japan

TAKAHIRO NODA

National Agricultural Research Center for Hokkaido Region, Memuro, Hokkaido 082-0081, Japan

Starches separated from 18 Indian wheat varieties were evaluated to see relationship of granule size distribution and amylopectin structure with pasting, thermal, and retrogradation properties. Average diameter of A-, B-, and C-granules among different starches varied between 23.0 and 28.5, 10.0 and 12.0, and 2.3 and 2.7 μ m, respectively. Amylopectin chain length distribution varied significantly, short length chains (DP 6–12) and long length chains (DP >24) ranged between 44.5 and 52.4% and 3.7 and 6.5%, respectively, whereas amylose content ranged between 18.2 and 28.8%. Short length chains of amylopectin had inverse relationship with starch gelatinization temperatures $T_{\rm o}$, $T_{\rm p}$, and $T_{\rm c}$. Starches with higher crystallinity had higher enthalpy of gelatinization and lower swelling power. Paste characteristics were mainly dependent upon granule type and all pasting parameters except pasting temperature, showed significant positive correlations with A-granules and negative with the proportion of B- and C-granule.

KEYWORDS: Granule size; retrogradation; amylopectin; wheat starch

INTRODUCTION

Different size and shapes of starch granules build up in the endosperm during the development of grain. The mature endosperm in wheat consists of two distinct types of starch granules that are large, disk-shaped, and termed A-granules as well as small and spherical, termed as B-granules. Bechtel et al. (1) and Raeker et al. (2) reported for wheat starch having another distinct class of small C-granules that initiated 21 days after anthesis. The different granule types show significant differences in chemical composition and functional properties such as amylose, lipid content, and gelatinization characteristics (3, 4).

Starch is composed of two glucose polymers, amylose and amylopectin. Amylopectin with its multiple branched chains of $(1-4)-\alpha$ -glucans interlinked by $(1-6)-\alpha$ -linkages is usually the major component of starch, with the essentially unbranched amylose making up the minor fraction. The molecular architecture of amylopectin and its molecular arrangement within the granule is

related to the granule size (5). The ratio of long branch chains to short branch chains affects the shape of the amylopectin molecules, which affects their packing and, in turn, the morphology and size of the starch granule (6). The functional properties of starch are affected by amylose content, branched chain-length distribution of amylopectin (7, 8), phosphate monoester, phospholipid, and lipids content (9, 10), starch granule size distribution (2, 11), crystalline structures (12), and granular architecture (13). In wheat starches, amylopectin is considered to contribute to water absorption, swelling, and pasting of starch granules, whereas amylose and lipids tend to retard these processes (14). Jane and Chen (15)concluded that the amylopectin chain length distribution and amylose molecular size produce synergistic effects on the viscosity of starch pastes. The present study assesses the characteristics of starches from different Indian wheat varieties with regard to structural, physicochemical, granule size distribution and proportion, pasting, thermal, and retrogradation properties. The objective of our study was to investigate the structural and physicochemical features that provide diverse functional properties to wheat and to develop a relationship between these properties.

^{*}To whom correspondence should be addressed. Phone: 0183-2258802 ext 321. Fax: +91-183-2258820. E-mail: narpinders@yahoo.com.

MATERIALS AND METHODS

Materials. Thirteen common wheat varieties (HPW 147, HPW 155, HPW 184, HPW 42, HPW 89, HS 240, HS 295, HS 420, PBW 343, PBW 502, PBW 509, PBW 527, and VL 616) and five durum varieties (PBW 34, PDW 215, PDW 233, PDW 274, and PDW 291) were procured from Palampur University, Himachal Pradesh, and Punjab Agricultural University, Punjab. Grains of each variety were milled by using a Brabender Quadrumat junior mill (Germany) to obtain wheat flour.

Starch Isolation. Stiff dough was prepared by mixing flour (100 g) with distilled water (45-55 mL) in a laboratory pin mixer (National Mfg. Co., Lincoln, NE) for 3 min at slow speed. The dough ball was kept covered with moist cheesecloth at 30 °C for 1 h. Starch was washed from a dough ball by kneading by hand under a stream of distilled water over a sieve with mesh openings of 70 μ m fitted over a 2 L vessel. The starch slurry was wet sieved twice through the bolting cloth to remove bran and endosperm cell-wall impurities. The material retained on the cloth was discarded. Starch slurry was then centrifuged at 2500g for 10 min. The upper pigmented layer (tailings) was carefully removed using a small spatula, mixed with water (20 mL), and decanted from any more starch, which had settled after 30 min. The starch fraction along with starch from decanting step was purified by resuspending in distilled water and centrifugation (16). Four such purification cycles were carried out to obtain pure starch. The starch was finally dried at 40 °C in a convection drier. The starch recovery was between 45-48% (flour wt basis).

Morphology of Starch. Scanning electron micrographs were taken by a JEOL JSM-6100 scanning electron microscope (JEOL Ltd., Tokyo, Japan). Starch samples were suspended in ethanol (1% w/w), mounted on aluminum stub using double-sided sticky tape, and coated with gold/ palladium (60/40). An accelerating potential of 10 kV was used during micrography.

Particle Size Analysis. Particle size distribution of the starches was measured by laser scattering on triplicate samples using a Malvern Mastersizer Hydro QS-MU (Malvern Instruments Ltd., UK). The sample was added to the sample port until the instrument read ~40% obscuration. The size distribution was expressed in terms of the volumes of equivalent spheres. The selected criteria were the percent volume (% vol) of granules with a diameter lower than 50 μ m and the parameters d (0, 1), d (0, 5), and d (0, 9) expressed in micrometers.

Amylose Content and Amylopectin Chain Length Distribution. Amylose content of the isolated starches was determined by iodine binding method as described by Williams et al. (17). The starch sample (20 mg, db) was dispersed in 10 mL KOH (0.5 M) and made up to 100 mL with distilled water. To an aliquot (10 mL) of the dispersion, 5 mL of HCl (0.1 M) and 0.5 mL of iodine solution (0.1%) were added and made up to 50 mL. Absorbance was measured at 625 nm. The quantity of amylose was determined from a standard curve developed using amylose and amylopectin blends. The absorbance was read on three replications per sample and averaged.

Unit chains of amylopectin between DP 6 and 30 were analyzed by fluorophore-assisted capillary electrophoresis as described previously (18). Starch was debranched with isoamylase and labeled with 8-amino-1,3,6-pyrenetrisulfonic acid (APTS) according to Edwards et al. (19). The labeled sample was diluted water and electrophoresis was conducted on an ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA). The POP-6 polymer and 36 cm capillary (Applied Biosystems) were used. Electrophoresis was performed with a genetic analyzer buffer (Applied Biosystems) at 15 kV for 2 h, and then data were collected and analyzed using Genescan 3.7 software (Applied Biosystems). The amylopectin chains were classified into three fractions according to the chain length, short length chains with degree of polymerization (DP) 6-12, medium length chains with DP 13–24, and long chains with DP greater than 24.

Crystalline Structure. X-ray diffractograms of starch samples (equilibrated at 100% relative humidity, at 25 °C for 24 h) were recorded using an analytical diffractometer (Pan Analytical, Phillips, Holland), Cu K α radiation with a wavelength of 0.154 nm operating at 45 kV and 40 mA. XRD diffractograms were acquired at 25 °C over a 2 θ range of 4–30° with a step size of 0.02° and sampling interval of 10 s. Relative crystallinity was estimated from the ratio of the area of peaks to the total area of a diffractogram (20).

Swelling Power. Swelling power (g/g) was determined in triplicate using 2% aqueous suspension (w/w) of starch at 90 °C by the method of Leach et al. (21). The suspension was stirred at 90 °C for 30 min, cooled, and centrifuged at 1800g for 10 min. Swelling power was calculated as the ratio of weight of residue to the weight of starch.

Pasting Properties. Pasting properties of isolated wheat starches were studied by using a Rapid visco analyzer (Newport Scientific Pty Ltd., Warriewood NSW 2102, Australia). Viscosity profiles of starches from different wheat varieties were recorded using starch suspensions (3:25 w/w; starch:water). The temperature—time conditions included a heating step from 50 to 95 °C at 6 °C/min (after an equilibration time of 1 min at 50 °C), a holding phase at 95 °C for 1.5 min, a cooling step from 95 to 50 °C at 6 °C/min, and a holding phase at 50 °C for 2 min. Pasting temperature, peak viscosity, trough viscosity, breakdown, final viscosity, and setback were obtained.

Thermal Properties of Starch and Amylose-Lipids (AMLs). Thermal properties were analyzed using a DSC-822 (Mettler Toledo, Greifense, Switzerland), equipped with a thermal analysis data station. Starch sample (3.5 mg, db) was weighed into a 40 μ L capacity aluminum pan (Mettler, ME-27331), and distilled water was added with the help of Hamilton microsyringe to achieve a starch water suspension containing 70% water (w/w). Pans were sealed and allowed to stand for 1 h at room temperature before heating in DSC. The analyzer was calibrated using indium, and an empty aluminum pan was used as reference. Sample pans were heated at a rate of 10 °C/min from 40 to 110 °C and cooled at the same rate to 40 °C. The heating and cooling cycles were repeated three times. Onset temperature (T_{o}), peak temperature (T_{p}), conclusion temperature (T_{c}), and enthalpy of gelatinization (ΔH_{gel}) were calculated for endotherms and the exotherms using Star^e Software for thermal analysis Ver. 8.10.

Retrogradation Properties. The sample pans were kept at 4 °C for one week to study retrogradation properties. The pans were heated at a rate of 10 °C/min from 40–110 °C and cooled at the same rate to 40 °C. Enthalpy of retrogradation (ΔH_{ret}) was calculated for the endotherms and the exotherms.

Statistical Analysis. The data reported here is an average of duplicate observations except that of amylose and swelling power. Pearson correlation and analysis of variance (ANOVA) by Duncan's test (p < 0.05) were conducted using Minitab statistical software (State College, PA).

RESULTS AND DISCUSSION

Granule Characteristics. Scanning electron micrographs revealed the presence of large disk-like or lenticular shaped Agranules and small, roughly spherical, B- and C-granules in all the starches (Figure 1). PBW 527 starch showed larger A-granules and lower proportion of B- and C-granules. HPW 155 starch showed smaller A-granules and higher proportion of B- and Cgranules (Figure 1A,D). Starches from different wheat varieties showed significant variation in the size distribution as well as in the percent volume of the granules (Table 1). To highlight the differences in starch size distribution and their effects on other properties, granule size distribution was classified into A- (>15 μ m), B- (5–15 μ m), and C-granules (< 5 μ m) by the method of Bechtel et al. (1). Average diameter of A-, B-, and C-granules ranged between 23.0 and 28.5 μ m, 10.0 and 12.0 μ m, and 2.3 and $2.7 \,\mu\text{m}$, respectively. Among all the starches, A-granules had the highest contribution to the total volume (45.6 to 73.2%), followed by B- (14.0-37.0%) and C-granules (10.5-17.5%). PBW 527 starch showed the highest proportion of A-granules and the lowest proportion of B- and C-granules, while HPW 155 starch showed the lowest proportion of A- and the highest of B- and Cgranules (Figure 2). All the PBW and PDW varieties showed higher proportion of A-granules and lower proportion of B- and C-granules compared to that of HPW and HS varieties. The variation in size and proportion of starch granules may be due to the difference in genotype (2).



Figure 1. Scanning electron micrographs (SEM) of starches separated from different wheat varieties: (A) PBW 527, (B) PDW 291, (C) HS 295, and (D) HPW 155.

Table 1. Granule Size Distributions (Volume %) of Starches from Different Wheat Varieties a

variety	A-type	B-type	C-type
Talloty	(* 10 µ)	(0 10 pm)	(10 µ)
PBW 343	$64.7\pm1.14\text{ef}$	$23.4\pm1.05\mathrm{c}$	$11.8\pm0.76\mathrm{ab}$
PBW 502	$64.9\pm2.67\text{ef}$	$23.8\pm0.30\mathrm{c}$	$11.3\pm0.80\mathrm{ab}$
PBW 509	71.0 ± 1.41 gh	$17.2 \pm 1.46 a$	$11.8\pm0.76\mathrm{ab}$
PBW 527	$73.2\pm0.34h$	$16.4 \pm 1.52{ m a}$	$10.5\pm1.00\mathrm{a}$
HPW 42	$55.6\pm0.66\mathrm{c}$	$30.2\pm1.35\mathrm{e}$	$14.2\pm0.70\mathrm{c}$
HPW 89	$51.3\pm2.21\mathrm{b}$	$34.3\pm1.57\mathrm{fg}$	$14.4\pm0.95\mathrm{c}$
HPW 147	$59.5\pm1.54\text{d}$	$27.6\pm1.50\text{d}$	$12.9\pm0.92\text{bc}$
HPW 155	$45.6\pm1.06a$	$37.0\pm1.74\mathrm{g}$	$17.5\pm0.89\text{d}$
HPW 184	$52.5\pm1.34\mathrm{b}$	$32.5\pm1.25\mathrm{f}$	$15.1\pm0.89\text{cd}$
HS 240	$56.4\pm1.80\mathrm{c}$	$29.3\pm0.75\mathrm{e}$	$14.3\pm0.94\mathrm{c}$
HS 295	$59.4\pm0.82\text{d}$	$26.7\pm0.91~\text{d}$	$13.9\pm1.04\mathrm{c}$
HS 420	$56.2\pm0.58\mathrm{c}$	$29.5\pm1.20\mathrm{e}$	$14.3\pm0.89\mathrm{c}$
VL 616	$62.6\pm1.34\mathrm{e}$	$24.0\pm1.18\mathrm{c}$	$13.4\pm0.95\text{bc}$
PBW 34	$69.2 \pm 2.57 \mathrm{g}$	$18.7\pm0.82\mathrm{ab}$	$12.1\pm0.55\mathrm{b}$
PDW 215	$70.8\pm2.79\mathrm{gh}$	$17.9\pm1.35\mathrm{ab}$	$11.3\pm0.90\mathrm{ab}$
PDW 233	$66.6 \pm 2.71{ m f}$	$21.6\pm1.89\text{bc}$	$11.8\pm0.37\mathrm{ab}$
PDW 274	$67.5\pm2.75\mathrm{fg}$	$20.0\pm1.21\text{b}$	$12.5\pm0.44\text{b}$
PDW 291	$73.0\pm2.19h$	$14.0 \pm 1.51 a$	$10.6\pm0.68a$

 a Values are means \pm SD. Means with similar letters in a column do not differ significantly (p > 0.05).

Structural Properties. Amylose content of starches from different wheat varieties ranged between 18.2% (PBW 509) and 28.8% (VL 616) as shown in **Table 2**. Hung et al. (22) reported amylose content of 25.6% for normal wheat starch against 28.0 to 36.9% for high amylose Australian wheat varieties. Amylose did not show any correlation with the proportion of A-, B-, and



Figure 2. Granule size distribution of starches from different wheat varieties.

C-granules. However, high amylose content of A- than B-granules in normal and partial waxy wheat has been reported (23). In addition, A- and B-granules have also been reported to differ in ratio of amylose to lipid content (24, 25).

The chain length distribution of amylopectins showed significant variation among the starches from different wheat varieties (**Figure 3**). Starches from all the varieties exhibited a smooth polymodal distribution with the peak maxima at DP 11. The most abundant side chains of DP 11 ranged between 10.9% and 12.4%, which were consistent with the results obtained in our earlier studies (26). PDW 291 showed the lowest (10.9%) and HPW 147 showed the highest (12.4%) proportion of DP 11. The short

Table 2. Physicoche	mical and Pasting Prope	erties of Starches Separat	ed from Different Wheat Varieties ^a
---------------------	-------------------------	----------------------------	--

variety	amylose content (%)	swelling power (g/g)	peak viscosity (cP)	trough viscosity (cP)	breakdown viscosity (cP)	final viscosity (cP)	setback viscosity (cP)	pasting temperature (°C)
DBW/ 242	22.1 ± 0.02 bo	$14.2 \pm 0.04.2$	2552 ± 44.0 c	$2007 \pm 00.7 h$	455 ± 17.9 bo	$2865 \pm 02.2 \text{ ab}$	769 ± 62 2 ab	96 2 ⊥ 1 12 od
PBW 502	23.1 ± 0.93 bc 21.5 + 1.64 h	$14.2 \pm 0.94 a$ 166 + 140 h	2552 ± 44.90 2747 + 113 0 d	2097 ± 90.7 b 2244 ± 92.3 c	$433 \pm 17.8 \text{ bc}$ 503 ± 29.6 c	$2003 \pm 93.2 \text{ ab}$ 3083 $\pm 139.6 \text{ b}$	$700 \pm 03.3 \text{ ab}$ $839 \pm 50.4 \text{ b}$	85.5 ± 0.99 c
PBW 509	$182 \pm 1.61a$	17.6 ± 1.30 bc	3271 ± 651 gh	$2666 \pm 53.0 e$	605 ± 28.00	3992 + 187 0 de	$1326 \pm 52.3 \text{ e}$	$83.9 \pm 0.62 \text{ h}$
PBW 527	27.0 ± 0.86 d	$14.4 \pm 0.55 \text{ ab}$	3077 ± 14.2 fg	$2460 \pm 61.1 \mathrm{d}$	617 ± 59.7 de	3636 ± 47.8 cd	$1176 \pm 55.3 d$	83.9 ± 0.66 b
HPW 42	23.2 ± 1.49 bc	24.9 ± 1.11 d	$3433 \pm 41.0 \text{ h}$	$2842 \pm 33.9 \mathrm{f}$	$591 \pm 52.7 \mathrm{d}$	3976 ± 195.0 de	1134 ± 99.7 d	83.8 ± 0.63 b
HPW 89	19.0 ± 1.52 a	$15.1 \pm 1.67 \text{ab}$	2838 ± 122.1 de	2351 ± 101.1 cd	487 ± 41.7 c	3292 ± 103.8 bc	$941 \pm 43.2 \text{ bc}$	83.2 ± 1.00 ab
HPW 147	21.0 ± 1.37 b	22.4 ± 1.36 d	$2722 \pm 70.3 \text{d}$	$2203 \pm 90.2 \text{ bc}$	$519 \pm 38.1{ m c}$	3162 ± 24.9 b	$959\pm83.5\mathrm{bc}$	85.6 ± 1.30 c
HPW 155	28.0 ± 1.34 d	$13.1 \pm 0.32 a$	2264 ± 52.6 a	$1932 \pm 64.6 a$	$332 \pm 21.6 a$	$2624 \pm 98.7 a$	$692 \pm 48.3 a$	$89.6\pm1.01\mathrm{e}$
HPW 184	$25.7\pm0.85\mathrm{c}$	$13.5 \pm 1.81 a$	$2420\pm61.8\mathrm{b}$	$1975 \pm 50.5 a$	$445\pm46.3\mathrm{b}$	$2726 \pm 84.1 a$	$751 \pm 58.8 a$	$84.6\pm0.65\mathrm{bc}$
HS 240	$24.5\pm1.39\mathrm{c}$	$20.2\pm1.54\mathrm{c}$	$3321\pm105.8\mathrm{h}$	$2662\pm84.8\mathrm{e}$	$659\pm33.7\mathrm{e}$	$3759\pm76.3\mathrm{d}$	$1097\pm68.9\mathrm{cd}$	$82.4\pm0.93\mathrm{a}$
HS 295	$20.0 \pm 1.04 \text{ ab}$	$21.2\pm1.46\text{cd}$	$2866 \pm 39.4 \text{ e}$	$2277 \pm 31.3{ m c}$	$589\pm26.1\mathrm{d}$	$3297\pm188.0\mathrm{bc}$	$1020\pm58.4\mathrm{c}$	$86.3\pm1.16\mathrm{cd}$
HS 420	$26.8\pm1.52\text{cd}$	$16.8\pm1.43\mathrm{b}$	$2819\pm29.2\mathrm{de}$	$2405\pm24.9\mathrm{d}$	$414\pm31.0\mathrm{b}$	$3386 \pm 132.7{ m c}$	$981\pm71.0\mathrm{c}$	$86.3\pm0.70\mathrm{cd}$
VL 616	$28.8\pm1.25\text{d}$	$15.6\pm1.48\mathrm{ab}$	$2840\pm60.9\mathrm{de}$	$2344\pm50.2\text{cd}$	$496\pm42.6\mathrm{c}$	$3342\pm156.6\text{bc}$	$998\pm69.2\mathrm{c}$	$87.2\pm1.74\mathrm{d}$
PBW 34	$28.2\pm1.46\text{d}$	$15.8\pm1.74\mathrm{b}$	$3007\pm111.7\mathrm{f}$	$2443\pm90.8\mathrm{d}$	$564\pm27.3\mathrm{d}$	$3890\pm93.7\mathrm{d}$	$1447\pm67.4\mathrm{ef}$	$82.3\pm0.86\mathrm{a}$
PDW 215	$22.4\pm0.48\mathrm{bc}$	$19.7\pm0.98\mathrm{c}$	$3258\pm128.2\mathrm{gh}$	$2638\pm103.8\mathrm{e}$	$620\pm25.3\mathrm{de}$	$4455\pm151.4\mathrm{e}$	$1817 \pm 72.7{ m g}$	$83.1\pm1.12\mathrm{ab}$
PDW 233	$25.0\pm1.15\mathrm{c}$	$16.6\pm1.39\mathrm{b}$	3204 ± 130.3 g	$2690\pm60.1\mathrm{e}$	$514\pm37.1\mathrm{c}$	$4278\pm177.6\mathrm{e}$	$1588 \pm 90.1 { m f}$	$84.0\pm1.26\text{b}$
PDW 274	$22.7\pm0.49\text{bc}$	$19.0\pm1.55\mathrm{c}$	$3167\pm129.0\mathrm{g}$	$2647\pm107.8\mathrm{e}$	$520\pm21.3\mathrm{c}$	$4320\pm181.8\mathrm{e}$	$1673\pm73.0\mathrm{fg}$	$86.4\pm0.75\mathrm{cd}$
PDW 291	$26.5\pm1.32\text{cd}$	$16.0\pm1.08\text{b}$	$3039\pm91.0\text{f}$	$2301\pm68.9\mathrm{c}$	$738\pm22.1~\text{f}$	$3092\pm137.0b$	$791\pm18.9~{ m ab}$	$85.8\pm0.48\text{c}$

^a Values are means \pm SD. Means with similar letters in a column do not differ significantly (p > 0.05).



Figure 3. Chain length distribution of amylopectin of starches from different wheat varieties.

length chains (DP 6–12), medium length chains (DP 13–24), and long chain (DP >24) proportions ranged between 44.5 and 52.4%, 43.8 and 50.5%, and 3.7 and 6.5%, respectively. In HPW 147, short length chains were increased while chains of DP 14–30 were reduced compared to rest of the starches. The long length chains showed weak positive relationship with the proportion of A-granules and negative with that of B-and Cgranules. Amylopectin molecules of the A-granule starch consist of more long chains but lesser short chains while those of Bgranule starch consist of more short chains but lesser long chains (27).

X-ray Diffractometry. X-ray diffractograms of wheat starches showed a typical A-type pattern of cereal starch (**Figure 4**). The starches showed strong reflections at $2\theta = 15^{\circ}$, 17°, 18°, and 23°. HS 240, HPW 89, and HPW 155 showed stronger A-type patterns with higher peak intensities compared to other starches, indicating greater crystallinity while HPW 147 and HS 295 showed weaker peak intensities, indicating lower crystallinity. Percent relative crystallinity of the starches ranged between 28.2% (HPW 147) and 36.5% (HS 240). An additional peak at $2\theta = 20^{\circ}$ was observed in all the starches, corresponding to the presence of crystalline V-type amylose—lipid complexes (*28*). The intensity



Figure 4. X-ray diffraction patterns of starches from different wheat varieties.

of amylose–lipid peak was observed to be higher in PBW 527 and PBW 34 starches having full width at half-maximum (fwhm) of 0.80 and 0.74, respectively, than the other starches studied.

Swelling Power. Swelling power can be used to assess the extent of interaction between starch chains, within the amorphous and crystalline domains of the starch granule. Starches from



Figure 5. Pasting profiles of starches separated from different wheat varieties.

different wheat varieties showed swelling power values between 13.1 g/g (HPW 155) and 24.9 g/g (HPW 42) and are shown in Table 2. Swelling power was observed to be dependent upon the amylopectins. A negative correlation of swelling power with amylose content (r = -0.444, p = 0.06) was observed, which indicated suppression of granules swelling in the presence of amylose. Swelling behavior of cereal starches has been reported to be affected by their amylopectin content wherein amylose contributes as an inhibitor, especially in the presence of lipids (29). A positive relation between swelling power and short chains of amylopectin (r = 0.506, $p \le 0.05$) was consistent with the effect of these chains on destabilization of lamellar structure and an inverse relationship with long chains (r = -0.622, $p \le 0.05$) indicated formation of a stronger crystalline network by these chains within the starch granules (30). Starches with higher crystallinity showed lower swelling power. A stronger crystalline structure reduced the swelling of starch. The differences in the amylose and the lipid content as well as the granule organization results in the difference in the swelling behavior of the starches among different botanical sources or cultivars. The extent of this interaction is influenced by the amylose to amylopectin ratio and by the characteristics of amylose and amylopectin in terms of molecular weight/distribution, degree, and length of branching and conformation (31).

Paste Characteristics. The RVA profiles of wheat starches showed a wide diversity among the pasting characteristics (Figure 5 and Table 2). Peak viscosity ranged between 2264 cP (HPW 155) and 3433 cP (HPW 42). Peak, trough, and final viscosity showed significant positive correlations with the swelling power (r = 0.592, 0.579, and 0.468, respectively, $p \le 100$ 0.05). The increase in viscosity with temperature is due to the removal of water from the exuded amylose by the granules during swelling. Pasting properties are dependent on the rigidity of starch granules, which in turn affect the granule swelling potential (32) and amount of amylose leaching out in the solution (33). Pasting viscosities (peak, breakdown, final, and setback) showed significant positive correlations with Agranules ($r = 0.519, 0.637, 0.512, \text{ and } 0.537, \text{ respectively}, p \le 1000$ 0.05) and negative with B- (r = -0.524, -0.631, -0.550) and -0.560, respectively, $p \le 0.05$) and C-granules (r = -0.470, -0.616, respectively, $p \le 0.05$; -0.416 and -0.418, respectively, $p \le 0.09$). A-granules, which are larger in size, would possess a loose packing ability and occupy a relatively larger volume compared to B- and C-granules at similar concentration. Starch rheology is mainly influenced by particle size, and suspensions of large size particles tend to be more viscous compared to those of the counterpart smaller size (34). Hence, starch suspensions with high proportions of A-granules would be expected to exhibit higher viscosity than starch suspensions with higher contents of B- and C-granules (35). Pasting temperature indicates temperature at which the viscosity begins to increase during the heating process. HPW 155 starch showed the highest pasting temperature and PBW 34 showed the least. The starches with higher pasting temperature showed lower pasting viscosities, i.e., peak, trough, breakdown, setback, and final viscosity (r = -0.649, -0.598, -0.566, -0.485, and -0.569, respectively, $p \le 0.05$). Starches with higher amylose content showed lower pasting viscosities, although the correlations were statistically insignificant. A decrease in peak and final viscosity with increase in amylose content has been reported (36). Setback was positively correlated to peak, trough, and final viscosity (r = 0.710, 0.759 and 0.953, respectively, $p \le 0.005$). This suggests that amylose association is not the only factor responsible for setback. The structure of amylose and amylopectin has been reported to play an important role in the pasting properties of starches. Starches with high molecular weight amylose and amylopectins had lower amount of long branch chains as well as lower degree of branching in amylopectins, which resulted into an increase in peak viscosity and breakdown while decreasing in setback and final viscosity (37). Yuryev et al. (38) proposed that the increasing amount of amylose decreased the melting temperature of starch granules by disrupting crystallinity in the granular structure, causing a decrease in peak viscosity. Trough viscosity ranged between 1932 cP and 2842 cP among different starches. The breakdown and setback viscosity ranged between 414 cP and 738 cP and 751 cP and 1817 cP, respectively, PDW 291 showed the highest breakdown. Breakdown viscosity showed highly significant positive relationship with proportion of A-granule $(r = 0.637, p \le 0.005)$ and negative with B- and C-granules $(r = -0.656, p \le 0.005 \text{ and } -0.616, p \le 0.05, \text{ respectively})$. The results clearly indicated that the starches with higher A-type granules were less stable toward the shearing as compared to those with the higher B- and C-type granules. Setback is the recovery of the viscosity during cooling of the heated starch suspension. High setback of the PDW 215 (1817 cP) starch may be due to the amount and the molecular weight of the amylose leached from the granules and the remnants of the gelatinized starch (39). Final viscosity increased upon cooling may be due to the aggregation of the amylose molecules (40). Final viscosity varied from 2624 cP (HPW 155) to 4455 cP (PDW 215). Pasting properties of starch has been reported to be affected by concentration of starch, amylose, and branching architecture of amylopectin, the ratio of amylose/amylopectin, and the presence of lipids that can complex with amylose (14). The relationship between granule type and starch pasting properties suggested that granule size contributes significantly to pasting properties, although additional physicochemical differences along with amylopectin chain length distribution may also account for the difference in pasting properties (41).

Thermal Properties of Starch and AMLs. DSC thermograms of starches obtained during three successive heating and cooling cycles are shown in **Figures 6** and **7**, respectively. All the starches exhibited distinct biphasic endotherms and an exotherm during the first cycle of heating and cooling. The first endotherm indicating melting of starches showed transition temperature $(T_{o}, T_{p} \text{ and } T_{c})$ values ranging between 55.6 and 57.3 °C, 60.6 and 62.1 °C, and 65.3 and 67.5 °C, respectively (**Table 3**). HPW 89



Figure 6. DSC thermograms showing endotherms of a starch sample during repeated heating cycles (1, 2, and 3).



Figure 7. DSC thermograms showing exotherms of a starch sample during repeated cooling cycles (1, 2, and 3).

starch showed the highest $T_{\rm o}$ (57.3 °C) and $T_{\rm p}$ (62.1 °C). High transition temperatures have been related to high degree of crystallinity, which provide structural stability and make the granule more resistant to gelatinization (42). HS 295 starch showed the lowest T_p (60.6 °C) and T_c (65.3 °C). The gelatinization range $(T_c - T_o)$ was 8.7–10.7 °C for all the starches. The difference in the transition temperatures among the starches could be attributed to the difference in the presence of crystalline regions within a starch granule composed of small crystallites having slightly different crystal strength (43). An inverse relationship was observed between short length chain amylopectin and starch gelatinization temperatures with $T_{\rm o}$ ($r = 0.469, p \le 0.05$), $T_{\rm p}$ ($r = 0.481, p \le 0.05$), and $T_{\rm c}$ ($r = 0.489, p \le 0.05$). Nakamura et al. (44) observed increase in onset temperature of gelatinization with the decrease in proportion of chains with $DP \leq 10$ to those of DP \leq 24 in amylopectin molecules. Noda et al. (45) also observed a negative correlation between the amount of amylopectin short chains (DP 6-12) with $T_{\rm o}$ and $T_{\rm p}$. Shorts chains are known to be located on the external part of the crystalline structure (46). It seems that short chains of amylopectin were unable to form stable double-helical structures because of their shorter lengths, hence, easily disrupted by the heat at lower temperature. Medium and long chain amylopectins showed weak positive relations with T_0 , T_p , and T_c , may be due to stronger crystalline network formation within starch granules. Enthalpy (ΔH_{gel}) is an important parameter during gelatinization for determining the energy input, which reflects primarily the loss of molecular (double-helical) order (47). HS 420 and HS 240 starches showed higher ΔH_{gel} (10.8 and 10.7 J/g, respectively) compared to PBW 343 and HPW 147 starches (8.00 J/g). Starches with higher ΔH_{gel} showed higher crystallinity and vice versa. $\Delta H_{\rm gel}$ showed significant negative correlation with short length chains of amylopectin (r = -0.571, $p \le 0.05$) and positive correlation with medium length chains of amylopectin (r =0.602, respectively, $p \le 0.05$). T_c also showed similar relationship with short- (r = -0.489, $p \le 0.05$) and long-chain amylopectins $(r = 0.519, p \le 0.05)$. Gidley and Bulpin (48) reported that the presence of short chains with $DP \le 10$ decreases the stability of double helix in amylopectin molecules, which lowers the gelatinization temperature as well as enthalpy. The variation in $T_{\rm c}$ and $\Delta H_{\rm gel}$ in starches from different varieties might be due to differences in amounts of longer chains in amylopectins, which require a higher temperature to dissociate completely than that for shorter double helices (49). Endothermic DSC parameters did not show correlation with amylose content which agrees with the results, reported earlier (45).

The second endotherm peak characterized as dissociation of AMLs during the first heating cycle showed values of peak temperature and enthalpy ranging between 97.7 and 100.7 °C and 0.5 and 1.6 J/g, respectively (**Table 4**). The lipids and amylose form complexes (AMLs) during biosynthesis of native starch as well as during heating of starch slurries at gelatinization temperature and above. The AMLs have a negative effect on physicochemical properties of starch, as they reduce water binding by starch granules and retard granule swelling (50). The starch with lower and less perfect crystallinity has lower T_o and $\Delta H_{\rm gel}$ (51).

During cooling, an exotherm indicating reassociation of AMLs showed peak temperature ranging between 85.9 °C (PDW 291) and 88.9 °C (HS 420). Exothermic T_p at 86 °C has been attributed to the reforming and possible more stable form of AMLs in wheat starch (52). Enthalpies of AMLs reassociation ranged between 0.8 and 1.6 J/g (**Table 4**). PDW 291 starch showed wider temperature range of dissociation and reassociation of AMLs compared to starches from other varieties. Exotherm with T_o between 85.6 and 87.9 °C for the starches from wheat exposed to water stress has been observed earlier (53). The starches with higher amylose content had higher temperature of reassociation (exotherm). This indicates that more stable amylose-complexed lipids were generated in the starches with higher amylose content.

The successive heating and cooling cycles for all the starches exhibited respective dissociation and reassociation of AMLs only. The peak temperatures corresponding to the AMLs dissociation increased to higher values ranging between 103.0 and 104.3 °C during the second heating cycle (Table 5) and between 103.2 and 103.9 °C during the third. The increase in temperature of AML dissociation during second and third heating cycles may be attributed to the structural changes among AMLs after each successive heating cycle. More stable, crystalline polymorphs dissociate at higher temperatures than amorphous AMLs that are more susceptible to degeneration (26). The enthalpies (0.4-0.9 J/g) and temperature range (6.2-8.3 °C) of AML dissociation range decreased during the second heating cycle and further during the third. The shape of the endotherms changed and lower dissociation temperature range indicated generation of more stable crystalline form of AMLs (54). The corresponding AML reassociation peak values decreased to 86.1-88.7 °C and 86.1-87.9 °C, respectively, during the second and third cooling cycle. The results confirmed the amorphous character of the AMLs formed and the values of dissociation and reassociation showed that smaller and still smaller fraction of complexes dissociated and reassociated during the successive cycle (Figure 8). $T_{\rm o}$, $T_{\rm p}$, and $T_{\rm c}$ of AML dissociation and

Table 3. Thermal and Retrogradation Properties of Starches Separated from Different Wheat Varieties^a

	thermal				retrogradation				
variety	<i>T</i> ₀ (°C)	T _p (°℃)	T _c (°℃)	$\Delta H_{ m gel}$ (J/g)	<i>T</i> ₀ (°C)	T _p (°℃)	7 _c (°℃)	$\Delta H_{\rm ret}$ (J/g)	
PBW 343	$57.2\pm0.44~\mathrm{c}$	$61.6\pm0.42\text{b}$	$66.4\pm0.51~\mathrm{b}$	$8.0\pm0.34\mathrm{a}$	$47.8\pm0.05\text{cd}$	$50.5\pm0.21\mathrm{a}$	$57.9\pm0.24\mathrm{bc}$	$2.9\pm0.15\mathrm{c}$	
PBW 502	$57.2\pm0.24\mathrm{c}$	$61.6\pm0.47\mathrm{b}$	$66.9\pm0.52\mathrm{bc}$	$8.9\pm0.44\text{b}$	$47.4\pm0.30\mathrm{c}$	$52.8\pm0.01\text{bc}$	$56.9\pm0.31\mathrm{b}$	$0.7\pm0.32\mathrm{a}$	
PBW 509	$56.9\pm0.32\mathrm{bc}$	$61.9\pm0.48\mathrm{b}$	$67.5\pm0.53\mathrm{c}$	$10.2\pm0.38\text{cd}$	$46.4\pm0.10\text{bc}$	$53.1\pm0.24\mathrm{c}$	$58.3\pm0.32\mathrm{c}$	$1.6\pm0.36\mathrm{ab}$	
PBW 527	$56.8\pm0.35\text{bc}$	$61.8\pm0.31\mathrm{b}$	$66.4\pm0.33\mathrm{b}$	$8.2\pm0.36\mathrm{a}$	$48.2\pm0.20~\text{d}$	$53.4\pm0.15\text{cd}$	$57.9\pm0.27~{ m bc}$	$0.9\pm0.28\mathrm{a}$	
HPW 42	$56.0 \pm 0.12 a$	$60.9 \pm 0.47 \mathrm{a}$	$66.3\pm0.51\mathrm{b}$	$8.2\pm0.42\mathrm{a}$	$48.0\pm0.02\text{cd}$	$53.9\pm0.37\mathrm{d}$	$59.2\pm0.25\text{cd}$	$1.8\pm0.41\mathrm{b}$	
HPW 89	$57.3\pm0.48\mathrm{c}$	$62.1\pm0.74\mathrm{b}$	$67.2\pm0.50\mathrm{c}$	$10.4\pm0.32\text{cd}$	$47.8\pm0.37\text{cd}$	$53.8\pm0.20\text{cd}$	$58.5\pm0.16\mathrm{c}$	$1.7\pm0.20\mathrm{b}$	
HPW 147	$56.0 \pm 0.53 \mathrm{a}$	$61.3\pm0.63\mathrm{bc}$	$65.8\pm0.67\mathrm{ab}$	$8.0\pm0.23\mathrm{a}$	$47.8\pm0.15\text{cd}$	$53.9\pm0.33\text{d}$	$59.3\pm0.09\text{cd}$	$2.1\pm0.04\text{bc}$	
HPW 155	$55.6 \pm 0.43 \mathrm{a}$	$60.9\pm0.38\mathrm{a}$	$66.3\pm0.41\mathrm{b}$	$8.7\pm0.36\text{b}$	$49.7\pm0.10\mathrm{e}$	$54.4\pm0.21\text{de}$	$58.7\pm0.31\mathrm{c}$	$1.1\pm0.26\mathrm{a}$	
HPW 184	$56.2\pm0.43a$	$61.4\pm0.25\mathrm{b}$	$66.7\pm0.27\mathrm{bc}$	$9.3\pm0.51\mathrm{bc}$	$49.7\pm0.31\mathrm{e}$	$54.9\pm0.27\mathrm{e}$	$59.6\pm0.21\mathrm{d}$	$1.5\pm0.31\mathrm{ab}$	
HS 240	$56.7\pm0.64\mathrm{b}$	$61.6\pm0.49~\mathrm{b}$	$66.6\pm0.53\mathrm{bc}$	10.7 ± 0.44 d	$49.7\pm0.12\mathrm{e}$	$54.9\pm0.15~\mathrm{e}$	$59.7\pm0.33\text{d}$	$1.6\pm0.09\mathrm{b}$	
HS 295	$56.1 \pm 0.51 \mathrm{a}$	$60.6 \pm 0.35 \mathrm{a}$	$65.3 \pm 0.38 \mathrm{a}$	$8.9\pm0.35\mathrm{b}$	$49.5\pm0.24\mathrm{e}$	$54.8\pm0.18\text{de}$	$59.1\pm0.26\text{cd}$	$1.2\pm0.32\mathrm{ab}$	
HS 420	$56.1 \pm 0.46 \mathrm{a}$	$61.1\pm0.46\mathrm{bc}$	$65.4 \pm 0.50 \mathrm{a}$	$10.8\pm0.32\text{d}$	$50.5\pm0.07\mathrm{e}$	$52.9\pm0.09\mathrm{c}$	$59.8\pm0.27\mathrm{d}$	$3.0\pm0.21\mathrm{c}$	
VL 616	$56.7\pm0.47\mathrm{b}$	$61.6\pm0.63\text{b}$	$67.0\pm0.69\mathrm{c}$	$10.2\pm0.35\text{cd}$	$50.2\pm0.32\mathrm{e}$	$55.6\pm0.30\mathrm{e}$	$60.2\pm0.11d$	$1.7\pm0.24\mathrm{b}$	
PBW 34	$57.2\pm0.40~\mathrm{c}$	$61.4\pm0.62\mathrm{b}$	65.9 ± 0.66 ab	$8.4\pm0.33\mathrm{ab}$	44.2 ± 0.24 a	$51.3\pm0.12\mathrm{ab}$	$57.7\pm0.16~{ m bc}$	$1.4\pm0.27\mathrm{ab}$	
PDW 215	$55.9 \pm 0.21 \mathrm{a}$	$60.9\pm0.46\mathrm{a}$	$66.3\pm0.50\mathrm{b}$	$9.8\pm0.26\mathrm{c}$	$44.6\pm0.45a$	$50.9\pm0.35\mathrm{a}$	$51.7 \pm 0.25 a$	$1.0\pm0.16\mathrm{a}$	
PDW 233	$56.3\pm0.45\mathrm{ab}$	$60.8 \pm 0.66 \mathrm{a}$	$66.2 \pm 0.71{ m b}$	$8.7\pm0.35b$	$45.5\pm0.15\mathrm{b}$	$51.9\pm0.33b$	$57.2\pm0.40\mathrm{b}$	1.2 ± 0.41 ab	
PDW 274	$56.5\pm0.30~\mathrm{b}$	$61.4\pm0.54\mathrm{b}$	$67.1 \pm 0.59 \ \mathrm{c}$	$10.1\pm0.30\mathrm{c}$	$47.0\pm0.20~\mathrm{c}$	$52.7\pm0.19\text{bc}$	$57.6\pm0.26~{ m bc}$	$1.0\pm0.35\mathrm{a}$	
PDW 291	$56.6\pm0.32\text{b}$	$61.5\pm0.32b$	$66.5\pm0.34\text{b}$	$9.2\pm0.32\text{bc}$	$46.3\pm0.10\text{bc}$	$52.4\pm0.23\text{bc}$	$57.2\pm0.19b$	$1.0\pm0.22a$	

^a Values are means \pm SD. Means with similar letters in a column do not differ significantly (p > 0.05).

Tuble 4. Characteristics of the Statemet from Different wholes adding DOOT not syste of froating and Sooning	Table 4.	Characteristics of	AMLs of the	Starches from	Different Wheat	Varieties during	DSC First C	ycle of Heating	and Cooling	Ja
--	----------	--------------------	-------------	---------------	------------------------	------------------	-------------	-----------------	-------------	----

	AML dissociation				AML reassociation			
variety	<i>T</i> ₀ (°C)	<i>Т</i> _р (°С)	<i>T</i> _c (°℃)	ΔH (J/g)	<i>T</i> ₀ (°C)	T _p (°C)	T _c (°C)	∆H (J/g)
PBW 343	$92.5\pm0.21\mathrm{bc}$	$99.4\pm0.45\mathrm{b}$	$104.4\pm0.70\text{bc}$	$1.2\pm0.09\text{b}$	$89.2\pm0.20ab$	$87.7\pm0.07\text{bc}$	$84.8\pm0.32\text{cd}$	$-0.9 \pm 0.12{ m ab}$
PBW 502	$93.4\pm0.42\mathrm{c}$	$99.6\pm0.25\mathrm{b}$	$104.6\pm0.50\text{bc}$	$0.9\pm0.54\mathrm{bc}$	$89.2\pm0.00\text{ab}$	$87.6\pm0.15\mathrm{b}$	$82.7\pm0.20b$	$-0.9\pm$ 0.26 ab
PBW 509	$96.4\pm0.65\mathrm{e}$	$99.4\pm0.60b$	$102.3 \pm 0.57\mathrm{a}$	$1.1\pm0.38b$	$88.7\pm0.31\mathrm{a}$	$87.1\pm0.19\mathrm{b}$	$84.2\pm0.36\mathrm{c}$	$-1.1\pm0.25\mathrm{b}$
PBW 527	$94.8\pm0.47\text{d}$	$100.1\pm0.44\mathrm{bc}$	$104.4\pm0.65\mathrm{bc}$	$0.7\pm0.15a$	$89.1\pm0.24\mathrm{ab}$	$87.6\pm0.27\mathrm{b}$	$84.5\pm0.20\text{cd}$	$-0.8 \pm 0.25{ m a}$
HPW 42	$94.2\pm0.72\text{d}$	$99.9\pm0.50\text{bc}$	$104.1\pm0.15\text{b}$	$0.9\pm0.02bc$	$89.3\pm0.09\mathrm{b}$	$87.8\pm0.34\text{bc}$	$84.9\pm0.10\text{cd}$	-0.9 ± 0.21 ab
HPW 89	$93.7\pm0.44\text{cd}$	$99.5\pm0.29\mathrm{b}$	$104.2\pm0.45\text{bc}$	$0.9\pm0.32bc$	$89.2\pm0.31\mathrm{ab}$	$87.4\pm0.24\mathrm{b}$	$83.7\pm0.32\text{bc}$	$-1.3\pm0.20\mathrm{bc}$
HPW 147	$94.5\pm0.45\text{d}$	$99.9\pm0.70\text{bc}$	$104.4\pm0.10\text{bc}$	$0.7\pm0.23a$	$88.9\pm0.36\text{ab}$	$87.2\pm0.21\mathrm{b}$	$83.9\pm0.36\mathrm{c}$	$-0.8\pm0.25\mathrm{a}$
HPW 155	$94.8\pm0.59\text{d}$	$99.7\pm0.23\mathrm{bc}$	$103.7\pm0.14b$	$0.5\pm0.36a$	$89.5\pm0.13\mathrm{b}$	$87.4\pm0.42\mathrm{b}$	$83.5\pm0.27\mathrm{bc}$	$-1.1\pm0.15\mathrm{b}$
HPW 184	$93.9\pm0.18\text{cd}$	$100.7\pm0.57\mathrm{c}$	$104.5\pm0.50\text{bc}$	$0.8\pm0.55\text{bc}$	$90.0\pm0.25\text{bc}$	$88.3\pm0.33\mathrm{c}$	$85.2\pm0.42\text{d}$	$-1.1\pm0.26\mathrm{b}$
HS 240	$93.6\pm0.75\text{cd}$	$99.5\pm0.61\mathrm{b}$	$103.9\pm0.50b$	$0.8\pm0.44\text{bc}$	$89.7\pm0.46\mathrm{b}$	$88.1\pm0.13\text{bc}$	$84.5\pm0.51\text{cd}$	$-1.0\pm23\mathrm{ab}$
HS 295	$93.2\pm0.54\mathrm{c}$	$99.2\pm0.70\mathrm{b}$	$104.3\pm0.29\mathrm{bc}$	$1.1\pm0.35\mathrm{b}$	$89.6\pm0.53\mathrm{b}$	$87.9\pm0.12\mathrm{bc}$	$84.7\pm0.63\text{cd}$	-0.9 ± 20 ab
HS 420	$94.1\pm0.71\text{cd}$	$100.0\pm0.25\text{bc}$	$104.6\pm0.70\mathrm{c}$	$0.7\pm0.32a$	$90.4\pm0.27\mathrm{c}$	$88.9\pm0.25\mathrm{c}$	$86.3\pm0.22\text{d}$	$-0.7\pm25\mathrm{a}$
VL 616	$94.4\pm0.33\text{d}$	$99.5\pm0.57\mathrm{b}$	$103.3\pm0.23\mathrm{bc}$	$0.7\pm0.13a$	$89.3\pm0.15\mathrm{b}$	$87.6\pm0.46\mathrm{b}$	$84.1\pm0.10\mathrm{c}$	$-0.8\pm12a$
PBW 34	$92.7\pm0.23\text{bc}$	$99.0\pm0.15b$	$104.2\pm0.47\text{bc}$	$1.0\pm0.33b$	$89.3\pm0.18\mathrm{b}$	$87.8\pm0.35\text{bc}$	$84.0\pm0.04\mathrm{c}$	$-1.0\pm0.06\mathrm{ab}$
PDW 215	$92.4\pm0.10\text{bc}$	$99.0\pm0.10b$	103.0 \pm 0.31 bc	$0.9\pm0.26bc$	$88.7\pm0.22\text{ab}$	$87.1\pm0.51\mathrm{b}$	$83.5\pm0.37\text{bc}$	$-1.6\pm0.25\mathrm{c}$
PDW 233	$93.7\pm0.67\text{cd}$	$98.9\pm0.50\text{ab}$	$103.8\pm0.70\text{b}$	$0.9\pm0.05\text{bc}$	$88.8\pm0.27\text{ab}$	$87.2\pm0.21\mathrm{b}$	$84.0\pm0.41\mathrm{c}$	$-1.2\pm0.15\mathrm{b}$
PDW 274	$91.9\pm0.81\mathrm{b}$	$98.9\pm0.36\text{ab}$	$103.7\pm0.50b$	$1.1\pm0.30b$	$88.7\pm0.34\text{ab}$	$86.4 \pm 0.27 \mathrm{a}$	$82.0\pm0.24ab$	$-1.3\pm0.36\mathrm{bc}$
PDW 291	$88.7\pm0.46a$	$97.7\pm0.60a$	$103.1\pm0.45\text{bc}$	$1.6\pm0.54\text{c}$	$88.2\pm0.41a$	$85.9\pm0.15a$	$81.3\pm0.27a$	$-1.5\pm0.10\mathrm{c}$

^a Values are means \pm SD. Means with similar letters in a column do not differ significantly (p > 0.05).

reassociation showed significant positive relationship with the corresponding values of the successive heating and cooling cycles.

Retrogradation Properties. The retrogradation properties of starch gels were measured using DSC after seven days of refrigerated storage. Enthalpy of retrogradation (ΔH_{ret}) for the starches varied between 0.7 J/g for PBW 502 and 3.0 J/g for HS 420 (**Table 3**). ΔH_{ret} provides a quantitative measure of the energy transformation that occurs during the melting of reassociated amylopectin (55). The higher the ΔH_{ret} of a starch, the higher is the tendency to retrograde. ΔH_{ret} is an indication of the unraveling and melting of double helices formed during storage, influenced by the amylopectin unit chain length distribution (56). T_o and T_p of retrograded starches ranged between 44.2 and 50.5 °C and 50.5 and 55.6 °C, respectively. PBW 343 had the lowest T_p , while VL 616 showed the highest. T_o , T_p , and T_c showed significant negative correlations with A-granules proportion (r = -0.672, $p \le 0.005$; -0.587 and -0.534, respectively, $p \le 0.05$) and

positive correlations with those of counterpart B- and C-granules. In addition, T_o and T_c of retrograded starches showed significant negative correlations with final viscosity (r = -0.581 and -0.464, respectively, $p \le 0.05$) and setback viscosity (r = -0.652 and -0.581, $p \le 0.05$). Pasting temperature showed positive correlation with T_o (r = 0.505, $p \le 0.05$). Transition temperatures and $\Delta H_{\rm ret}$ of stored starch pastes were significantly lower than the transition temperatures of gelatinization and $\Delta H_{\rm gel}$ of starch dispersions. Morikawa and Nishinari (57) reported that starch molecule recrystallization occurs in a less-ordered manner in stored starch gels than in native starches.

Conclusion. The results of this work bear the importance of starch size distribution and amylopectin chain length as critical parameters that significantly affect the rheological properties of the wheat starch. Amylose did not show any correlation with the proportion of A-, B-, and C-granules or starch thermal properties. Short length chain amylopectin showed an inverse

Table 5.	Characteristics of	AMLs of the Starche	s from Different Wheat	Varieties during DSC	Second Cycle of	Heating and Cooling
----------	--------------------	---------------------	------------------------	----------------------	-----------------	---------------------

		AML diss	ociation		AML reassociation				
variety	<i>T</i> ₀ (°C)	T _p (°C)	T _c (°℃)	ΔH (J/g)	<i>Т</i> _о (°С)	T _p (°C)	<i>Т</i> _с (°С)	ΔH (J/g)	
PBW 343	$98.8\pm0.02\mathrm{bc}$	$103.7 \pm 0.21\mathrm{b}$	$106.3\pm0.13\mathrm{bc}$	$0.7\pm0.01\mathrm{ab}$	$88.8\pm0.13\mathrm{ab}$	$87.6\pm0.32\mathrm{bc}$	$84.2\pm0.02\mathrm{c}$	-0.9±0.11 a	
PBW 502	$97.9 \pm 0.13 a$	$104.0\pm0.34\mathrm{c}$	$106.2 \pm 0.25\mathrm{b}$	$0.7\pm0.06\mathrm{ab}$	$89.1\pm0.32\mathrm{b}$	$87.4\pm0.16\mathrm{b}$	$83.0\pm0.20\mathrm{b}$	$-0.9 \pm 0.23 \mathrm{a}$	
PBW 509	$98.8\pm0.21\mathrm{bc}$	$103.4\pm0.33\mathrm{ab}$	105.7 \pm 0.16 ab	$0.5 \pm 0.20 a$	$88.5 \pm 0.20 a$	$87.0 \pm 0.13 \text{b}$	$84.2 \pm 0.13 c$	$-1.0 \pm 0.05 a$	
PBW 527	$98.4\pm0.20\mathrm{b}$	$104.0\pm0.05\mathrm{c}$	$105.9\pm0.19b$	$0.5\pm0.12a$	$89.0\pm0.13\mathrm{b}$	$87.4\pm0.20\mathrm{b}$	$84.3\pm0.41\mathrm{c}$	$-0.8\pm0.09\mathrm{a}$	
HPW 42	$98.3\pm0.18\mathrm{ab}$	$103.6 \pm 0.15 \text{b}$	$106.2\pm0.29\mathrm{b}$	$0.8\pm0.15\mathrm{b}$	89.2 ± 0.15 b	$87.6\pm0.01\mathrm{bc}$	$84.9\pm0.20~\text{cd}$	$-0.9 \pm 0.16 a$	
HPW 89	$98.5\pm0.24\mathrm{b}$	$103.4\pm0.05\text{ab}$	$105.7\pm0.33\text{ab}$	$0.8\pm0.20b$	$89.2\pm0.36\mathrm{b}$	$87.1\pm0.06\mathrm{b}$	$83.6\pm0.30\text{bc}$	$-1.2\pm0.33\mathrm{ab}$	
HPW 147	$98.5\pm0.32\mathrm{b}$	$103.7 \pm 0.16\text{b}$	$106.5 \pm 0.26{ m c}$	$0.7\pm0.13\mathrm{ab}$	$89.2\pm0.15\mathrm{b}$	$87.2\pm0.09\mathrm{b}$	$83.8\pm0.25\mathrm{bc}$	$-0.9 \pm 0.12 a$	
HPW 155	$98.7\pm0.13\mathrm{b}$	$103.8\pm0.19\text{bc}$	$106.1\pm0.32b$	$0.7\pm0.09\mathrm{ab}$	$89.5\pm0.06\text{bc}$	$87.4\pm0.06\mathrm{b}$	$83.5\pm0.12\text{bc}$	$-1.0 \pm 0.01 a$	
HPW 184	$99.0\pm0.05\text{bc}$	$104.3\pm0.25\mathrm{c}$	$106.1\pm0.20b$	$0.5\pm0.16\mathrm{a}$	$89.7\pm0.19\mathrm{c}$	$88.2\pm0.26\mathrm{c}$	$84.3\pm0.05\mathrm{c}$	$-1.0 \pm 0.32 \mathrm{a}$	
HS 240	$98.7\pm0.35\mathrm{b}$	$103.6\pm0.28\mathrm{b}$	$105.9\pm0.25\mathrm{b}$	$0.4\pm0.13a$	$89.4\pm0.28\mathrm{bc}$	$87.6\pm0.32\mathrm{bc}$	$83.8\pm0.00\text{bc}$	$-1.0 \pm 0.25 \mathrm{a}$	
HS 295	$98.7\pm0.16\mathrm{b}$	$103.9\pm0.35\text{bc}$	$106.0\pm0.27b$	$0.5\pm0.28\mathrm{a}$	$90.1\pm0.24\mathrm{c}$	$87.7\pm0.16\mathrm{bc}$	$84.7\pm0.12\mathrm{c}$	$-0.9\pm0.35\mathrm{a}$	
HS 420	$98.1\pm0.12\mathrm{ab}$	$103.6\pm0.30\text{b}$	$106.2\pm0.15b$	$0.8\pm0.21\text{b}$	$89.2\pm0.23\mathrm{b}$	$88.7\pm0.19\mathrm{c}$	$85.8\pm0.20\text{d}$	$-0.8\pm0.36\mathrm{a}$	
VL 616	$98.7\pm0.20\mathrm{b}$	$103.7\pm0.22b$	$106.6\pm0.24\mathrm{c}$	$0.8\pm0.16\text{b}$	$89.3\pm0.33\mathrm{bc}$	$87.6\pm0.16\text{bc}$	$84.6\pm0.25\mathrm{c}$	$-0.8\pm0.35\mathrm{a}$	
PBW 34	$98.0 \pm 0.10 a$	$103.5 \pm 0.13 \text{b}$	$106.4\pm0.03\text{bc}$	$0.9\pm0.03\mathrm{b}$	$88.7\pm0.46\text{ab}$	$87.4\pm0.26\mathrm{b}$	$84.0\pm0.05\text{bc}$	$-0.9\pm0.26\mathrm{a}$	
PDW 215	$98.7\pm0.40\text{b}$	$103.0 \pm 0.09 \mathrm{a}$	$105.5 \pm 0.17 a$	$0.6\pm0.06\text{ab}$	$88.5 \pm 0.10 a$	$86.9\pm0.11\mathrm{ab}$	$83.5\pm0.16\text{bc}$	$-1.4\pm0.15\mathrm{b}$	
PDW 233	$99.1\pm0.13\mathrm{c}$	$103.6\pm0.25b$	$105.3 \pm 0.31 a$	$0.7\pm0.16\mathrm{ab}$	$88.8\pm0.03\mathrm{ab}$	$87.0\pm0.16\mathrm{b}$	$84.0\pm0.17\text{bc}$	-1.1 ± 0.01 ab	
PDW 274	$98.6\pm0.30\text{b}$	$103.6\pm0.26b$	$106.3\pm0.16\text{bc}$	$0.8\pm0.30\text{b}$	$88.8\pm0.16ab$	$86.1\pm0.16\mathrm{a}$	$82.2\pm0.03\text{ab}$	$-1.2 \pm 0.23{ m ab}$	
PDW 291	$97.8\pm0.24a$	$103.4\pm0.31ab$	$106.0\pm0.05b$	$0.9\pm0.03b$	$88.2\pm0.25a$	$87.0\pm0.19b$	$81.2\pm0.26a$	$-1.4\pm0.22\mathrm{b}$	

^a Values are means \pm SD. Means with similar letters in a column do not differ significantly (p > 0.05).



Figure 8. DSC exotherms showing smaller fraction of complexes reassociated during the successive cooling cycles (1, 2, and 3).

relationship with starch gelatinization temperatures and enthalpy while medium and long chain of amylopectin showed weak positive relations. Pasting properties showed significant positive correlations with A-granules and negative with B- and C-granules. Pasting results indicated that the starches with higher A-type granules were less stable toward the shearing compared to those with the higher B- and C-type granules.

LITERATURE CITED

- Bechtel, D. B.; Zayas, I.; Dempster, R.; Wilson, J. D. Size distribution of starch granules isolated from hard red winter wheat and soft red winter wheat. *Cereal Chem.* **1993**, *70*, 238–240.
- (2) Raeker, M. O.; Gaines, C. S.; Finney, P. L.; Donelson, T. Granule size distribution and chemical composition of starches from soft wheat cultivars. *Cereal Chem.* **1998**, *75*, 721–728.
- (3) Peng, M.; Gao, M; Abdel-Aal, E. S. M.; Hucl, P.; Chibbar, R. N. Separation and characterization of A- and B-type starch granules in wheat endosperm. *Cereal Chem.* **1999**, *76*, 375–379.
- (4) Soulaka, A. B.; Morrison, W. R. The amylose and lipid contents, dimensions, and gelatinization characteristics of some wheat starches and their A- and B-granule fractions. J. Sci. Food Agric. 1985, 36, 709–718.
- (5) Geera, B. P.; Nelson, J. E.; Souza, E.; Huber, K. C. Composition and properties of A- and B-type starch granules of wild-type,

partial waxy, and waxy soft wheat. Cereal Chem. 2006, 83, 551–557.

- (6) Jane, J. L. Structure of starch granules. J. Appl. Glycosci. 2007, 54, 31–36.
- (7) Jane, J.; Chen, Y. Y.; Lee, L. F.; McPherson, A. E.; Wong, K. S.; Radosavljevic, M.; Kasemsuwan, T. Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chem.* **1999**, *76*, 629–637.
- (8) Singh, N.; Isono, N.; Srichuwong, S.; Noda, T.; Nishinari, K. Structural and viscoelastic properties of potato starches. *Food Hydrocolloid*. 2008, 22, 979–988.
- (9) Lin, Y. P.; Czuchajowska, Z. Starch properties and stability of club and soft white winter wheats from the Pacific Northwest of the United States. *Cereal Chem.* **1997**, *74*, 639–646.
- (10) Singh, N.; Singh, J.; Kaur, L.; Sodhi, N. S.; Gill; B. S. Morphological, thermal and rheological properties of starches from different botanical sources – a review. *Food Chem.* 2003, *81*, 219–231.
- (11) Singh, N.; Kaur, L. Morphological, thermal, rheological and retrogradation properties of starch fractions varying in granule size. *J. Sci. Food Agric*. 2004, 84, 241–252.
- (12) Hizukuri, S.; Takeda, Y.; Abe, J.; Hanashiro, I.; Matsunobu, G.; Kiyota, H. In *Starch. Structure and Functionality*; Frazier, P. J., Richmond, P., Donald, A. M., Eds.; Royal Society of Chemistry: London, 1997; pp 121–128.
- (13) Tester, R. F.; Morrison, W. R.; Gidley, M. J.; Kirkland, M.; Karkalas, J. Properties of damaged starch granules III. Microscopy and particle size analysis of undamaged granules and remnants. *J. Cereal Sci.* **1994**, *20*, 59–67.
- (14) Tester, R. F.; Morrison, W. R. Swelling and amylosization of cereal starches. I. Effects of amylopectin, amylose and lipids. *Cereal Chem.* 1990, 67, 551–557.
- (15) Jane, J. L.; Chen, Y. Y. Effects of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chem.* 1992, 69, 60–65.
- (16) Wolf, M. J. Wheat starch isolation. In *Methods in Carbohydrate Chemistry IV*; Whistler, R. L., Ed.; Academic Press: New York, 1964; pp 6–9.
- (17) Williams, P. C.; Kuzina, F. D.; Hlynka, I. A rapid colorimeteric procedure for estimating the amylose content of starches and flours. *Cereal Chem.* **1970**, *47*, 411–420.
- (18) Srichuwong, S.; Sunarti, T. C.; Mishima, T.; Isono, N.; Hisamatsu, M. Starches from different botanical sources I. Contribution of amylopectin fine structure to thermal properties and enzyme digestibility. *Carbohydr. Polym.* **2005**, *60*, 529–538.
- (19) Edwards, A.; Fulton, D. C.; Hylton, C. M.; Jobling, S. A.; Gidley, M.; Rossner, U.; et al. A combined reduction in activity of starch

synthases II and III of potato has novel effects on the starch of tubers. *Plant J.* **1999**, *17*, 251–261.

- (20) Komiya, T.; Nara, S. Changes in crystallinity and gelatinization phenomena of potato starch by acid treatment. *Starch/Staerke* 1986, 38, 9–13.
- (21) Leach, H. W.; Mccowen, L. D.; Schoch, T. J. Structure of the starch granule. I. Swelling and solubility patterns of various starches. *Cereal Chem.* **1959**, *36*, 534–537.
- (22) Hung, P. V.; Maeda, T.; Miskelly, D.; Tsumori, R.; Morita, N. Physicochemical characteristics and fine structure of high-amylose wheat starches isolated from Australian wheat cultivars. *Carbohydr. Polym.* **2008**, *71*, 656–663.
- (23) Bertolini, A. C.; Souza, E.; Nelson, J. E.; Huber, K. C. Composition and reactivity of A- and B-type starch granules of normal, partial waxy and waxy wheat. *Cereal Chem.* 2003, *80*, 544–549.
- (24) McDonald, A. M. L.; Stark, J. R.; Morrison, W. R.; Ellis, R. P. The composition of starch granules from developing barley genotypes. *J. Cereal Sci.* 1991, *13*, 93–112.
- (25) Morrison, W. R.; Gadan, H. J. The amylose and lipid contents of starch granules in developing wheat endosperm. *J. Cereal Sci.* 1987, 5, 263–275.
- (26) Singh, N.; Singh, S.; Isono, N.; Noda, T.; Singh, A. M. Diversity in amylopectin structure, thermal and pasting properties of starches from wheat varieties/ lines. *Int. J. Biol. Macromol.* 2009, 45, 298–304.
- (27) Ao, Z. H.; Jane, J. L. Characterization and modeling of the A- and Bgranule starches of wheat, triticale, and barley. *Carbohydr. Polym.* 2007, 67, 46–55.
- (28) Zobel, H. F. Starch crystal transformations and their industrial importance. *Starch/Staerke* 1988, 40, 1–7.
- (29) Morrision, W. R.; Tester, R. F.; Snape, C. E.; Law, R.; Gidley, M. J. Swelling and gelatinization of cereal starches. 4. Some effects of lipidcomplexed amylose and free amylose in waxy and normal barley starches. *Cereal Chem.* **1993**, *70*, 385–391.
- (30) Tang, H.; Ando, H.; Watanabe, K.; Takeda, Y.; Mitsunaga, T. Physicochemical properties of large, medium and small granule starches in fractions of normal barley endosperm. *Carbohydr. Res.* 2001, 330, 241–248.
- (31) Hoover, R. Composition, molecular structure, and physicochemical properties of tuber and root starches. a review. *Carbohydr. Polym.* 2001, 45, 253–267.
- (32) Sandhyarani, M. R.; Bhattacharaya, K. R. Rheology of rice-flour pastes. Effect of variety, concentration and temperature and time of cooking. J. Texture Stud. 1989, 20, 127–137.
- (33) Morris, V. J. Starch gelation and retrogradation. *Trends Food Sci. Technol.* 1990, 1, 2–6.
- (34) Wong, R. B. K.; Lelievre, J. Rheological characteristics of wheatstarch measured under steady shear conditions. J. Appl. Polym. Sci. 1982, 27, 1433–1440.
- (35) Shinde, S. V.; Nelson, J. E.; Huber, K. C. Soft wheat starch pasting behaviour in relation to A- and B-type granule content and composition. *Cereal Chem.* 2008, 80, 91–98.
- (36) Blazek, J.; Copeland, L. Pasting and swelling properties of wheat flour and starch in relation to amylose content. *Carbohydr. Polym.* 2008, 71, 380–387.
- (37) Takeda, Y.; Takeda, C.; Suzuki, A.; Hizukuri, S. Structure and properties of sago starches with low and high viscosities on amylograph. J. Food Sci. 1989, 54, 177–182.
- (38) Yuryev, V. P.; Krivandin, A. V.; Kiseleva, V. I.; Wasserman, L. A.; Genkina, N. K.; Fornal, J.; Blaszczak, W.; Schiraldi, A. Structural parameters of amylopectin clusters and semicrystalline growth rings

in wheat starches with different amylose content. *Carbohydr. Res.* 2004, *339*, 2683–2691.

- (39) Loh, J. The effect of shear rate and strain on the pasting behaviour of food starches. J. Food Eng. 1992, 16, 75–89.
- (40) Miles, M. J; Morris, V. J; Orford, P. D.; Ring, S. G. The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydr. Res.* **1985**, *135*, 271–281.
- (41) Wilson, J. D.; Bechtel, D. B.; Wilson, G. W.T.; Sieb, P. A. Bread quality of spelt and its starch. *Cereal Chem.* 2008, 85, 629–638.
- (42) Barichello, V.; Yada, R. Y.; Coffin, R. H.; Stanley, D. W. Low temperature sweetening in susceptible and resistant potatoes. Starch structure and composition. J. Food Sci. 1990, 54, 1054–1059.
- (43) Banks, W.; Greenwood, C. T.; Muir, D. D. The characterization of starch and its components. Part 3. The techniques of semi-micro, differential, potentiometric iodine titration and the factors affecting it. *Starch/Staerke* 1971, 23, 118–124.
- (44) Nakamura, Y.; Sakurai, A.; Inaba, Y.; Kimura, K.; Iwasawa, N.; Nagamine, T. The fine structure of amylopectin in endosperm from Asian cultivated rice can be largely classified into two classes. *Starch/ Staerke* 2002, 54, 117–131.
- (45) Noda, T.; Nishiba, Y.; Sato, T.; Suda, I. Properties of starches from several low-amylose rice cultivars. *Cereal Chem.* 2003, 80, 193–197.
- (46) Hizukuri, S. Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydr. Res.* **1986**, *147*, 342–347.
- (47) Cooke, D.; Gidley, M. J. Loss of crystalline and molecular order during starch gelatinization—origin of the enthalpic transition. *Carbohydr. Res.* **1992**, 227, 103–112.
- (48) Gidley, M. J.; Bulpin, P. V. Crystallisation of malto-oligosaccharides as models of the crystalline forms of starch: minimum chain length requirement for the formation of double helices. *Carbohydr. Res.* **1987**, *161*, 291–300.
- (49) Yamin, F. F.; Lee, M.; Pollak, L. M.; White, P. L. Thermal properties of starch in corn variants isolated after chemical mutagenesis of inbred line B73. *Cereal Chem.* 1999, 76, 175–181.
- (50) Elliasson, A. C. Interaction between starch and lipids studied by DSC. *Thermochim. Acta* 1994, 246, 343–356.
- (51) Inouchi, N.; Glover, D. V.; Sugimoto, Y.; Fuwa, H. Developmental changes in starch properties of several endosperm mutants of maize. *Starch/Staerke* 1984, 38, 8–12.
- (52) Ottenhof, M. A.; Hill, S. E.; Farhat, I. A. Comparative study of the retrogradation of intermediate water content waxy maize, wheat, and potato starches. J. Agric. Food Chem. 2005, 53, 631–638.
- (53) Singh, S.; Singh, G.; Singh, P.; Singh, N. Effect of water stress at different stages of grain development on the characteristics of starch and protein of different wheat varieties. *Food Chem.* 2008, 108, 130–139.
- (54) Shamekh, S.; Mustranta, A.; Poutanen, K.; Forsell, P. Enzymatic hydrolysis of barley starch lipids. *Cereal Chem.* 1998, 75, 624–628.
- (55) Karim, A. A.; Norziah, M. H.; Seow, C. C. Methods for the study of starch retrogradation. *Food Chem.* 2000, 71, 9–36.
- (56) Shi, Y. C.; Seib, P. A. The structure of four waxy starches related to gelatinization and retrogradation. *Carbohydr. Res.* 1992, 227, 131–145.
- (57) Morikawa, K.; Nishinari, K. Effects of concentration dependence of retrogradation behaviour of dispersions for native and chemically modified potato starch. *Food Hydrocolloids* **2000**, *14*, 395–401.

Received for review August 7, 2009. Revised manuscript received November 12, 2009. Accepted November 25, 2009. The financial assistance from UGC, New Delhi, to N.S. is acknowledged.