

Role of Phosphorus in Viscosity, Gelatinization, and Retrogradation of Starch

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ABSTRACT

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The effect of starch crystallinity and phosphorus on starch gelatinization and retrogradation were studied using wide-angle X-ray powder diffraction, cross polarization/magic angle spinning (CP/MAS) ¹³C nuclear magnetic resonance (NMR) spectroscopy, ³¹P NMR spectroscopy, Rapid Visco Analyzer (RVA) and differential scanning calorimetry (DSC). Two starches differing significantly in peak viscosity (cv. Stephens, 283 BU; cv. Crew, 560 BU) were comparable in amylose content and starch crystallinity, while differing significantly in phospholipids content. Starch of lower peak viscosity had a higher phospholipids content and showed a slower rate of retro-

gradation. Starch from Stephens (0.098% phosphorus) had an enthalpy value of retrograded starch of 2.2 J/g after 14 days of storage, while starch from Crew (0.062% phosphorus) had an enthalpy value as high as 4.4 J/g. Defatting with a hot *n*-propanol and water (3:1) mixture caused substantial changes in peak viscosity. Peak viscosity for starch from Crew decreased by 75 RVU due to defatting, while starch from Stephens decreased by as much as 125 RVU. After defatting with the hot *n*-propanol water mixture, the rate and extent of starch retrogradation were comparable between the prime starches, which differed significantly in peak viscosity.

Starch is the major component of the wheat kernel, comprising ≈80% in soft wheat flour (Lin and Czuchajowska 1997). Starch gelatinization and retrogradation are two important starch functional properties. Starch functional properties may vary due to the ratio of amylose to amylopectin, starch crystallinity, granule size distribution, and the amount of minor constituents (e.g., phosphorus, lipids, proteins, and enzymes). Both dough rheology and end-use of flour are greatly affected by the starch functional properties (Eliasson 1989, Baik et al 1994, Lin and Czuchajowska 1997).

Amylose content in a starch is mainly dependent on its origin. In wheat starch, amylose content is ≈28% (Morrison and Laigneul 1983). Besides amylose-to-amylopectin ratio, starch crystallinity also plays an important role in starch functional properties. The starch granule crystallinity mainly resides in the amylopectin component (Zobel and Stephen 1995). Miles et al (1985) studied retrogradation of starch gel using X-ray diffraction and concluded that amylose was responsible for short-term irreversible development of gel structure and crystallinity in a starch gel, while amylopectin was involved in development of long-term reversible crystallization during storage.

X-ray diffraction is a widely used nondestructive technique for studying starch granule organization (Zobel 1988a,b). X-ray diffraction reports the regularly repeating nature of double helical molecular structures, but it does not detect irregularly packed structures. Muhrbeck et al (1991) studied potato starches differing in degree of phosphorylation and concluded that crystallinity decreased linearly with an increased degree of phosphorylation.

High-resolution solid-state nuclear magnetic resonance (NMR) spectroscopy is sensitive to structural organization at the molecular level and should therefore complement information obtained from X-ray diffraction (Gidley and Bociek 1985). ¹³C cross polarization/magic angle spinning (CP/MAS) NMR spectroscopy of native and acid modified starches from different origins have been studied by Vasudeva et al (1993). They found that cereal starches exhibit a triplet signal for anomeric carbons, while pulse, tuber, and root starches show doublets. Veregin et al (1987), using ¹³C CP/MAS NMR, confirmed that A and B starches consist of right-handed helices, while V-amylose complexes and starch in noncrystalline regions consist of left-handed helices.

A number of minor noncarbohydrate constituents (lipids, proteins, phosphorus, and endogenous enzymes) may also be present in wheat starch. Although present in small quantities, they can affect the properties and behavior of the starch (Lineback and Rasper 1988, Deffenbaugh and Walker 1989). Among these minor starch constituents, phosphorus has gained a great deal of attention. Phosphorus, although existing at a very low concentration, plays an important role in starch functional properties (Jane et al 1996). Phosphorus in starch is found in three major forms: phosphate monoester, phospholipids, and inorganic phosphate. Lim et al (1994) reported that phosphorus content in normal cereal starches is 0.02–0.06%, mainly in the form of phospholipids. Phosphate monoester increases paste clarity and viscosity, whereas phospholipids make starch paste opaque and decrease viscosity (Kasemsuwan and Jane 1996, Jane et al 1996). ³¹P nuclear magnetic resonance (NMR) spectroscopy can determine phosphorus chemical structures and individual components (Kasemsuwan and Jane 1996).

Differential thermal analysis techniques provide quantitative measurement of heat flow during phase transition, a very useful technique for following starch gelatinization and retrogradation. Roulet et al (1988) studied the retrogradation kinetics of gelatinized wheat starch in gel and powder form using X-ray and differential scanning calorimetry (DSC) and concluded that X-ray and DSC appear to respond to the amylopectin crystallization at a molecular level. Yuan et al (1993) studied the fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starch. They observed that a higher proportion of longer chains in amylopectins is associated with higher temperature and enthalpy for both gelatinization and retrogradation. Vasanathan and Hoover (1992) found that wheat starch defatted with hot *n*-propanol and water (3:1, v/v) results in almost complete removal of lipids. The relative crystallinity of wheat starch remains unchanged. They also reported that defatting eliminates the pasting peak of cereal starches, increases thermal stability, and reduces the hot paste consistency. The gelatinization temperatures of native and defatted wheat starches were similar.

Zeng et al (1997) reported that higher peak, greater breakdown, lower final viscosity, negative setback, and less total setback are associated with lower amylose content. In our recent study (Lin and Czuchajowska 1997), we found that several soft wheat starches had similar amylose content and amylopectin fine structure, while differing significantly in viscosity. These results indicate that minor components might be responsible for the large differences in viscosity, while information related to the effects of minor starch components on starch viscosity is still very limited. Therefore, the objective of this study was to investigate the effect of starch crystallinity and phosphorus content on gelatinization and retrogradation of soft wheat starches.

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MATERIALS AND METHODS

Two soft wheat prime starches were used in this study. Amylose content and amylograph peak viscosity of these prime starches are listed in Table I. The prime starches were isolated from flour according to the method of Czuchajowska and Pomeranz (1993) and purified two times with deionized water. Protein content ($N \times 5.7$) in the prime starches was $<0.5\%$, indicating highly purified starches. Amylose content of the prime starch was determined by iodometric method as described by Knutson and Grove (1994). Fine structure of isoamylase debranched amylopectin was described by Lin and Czuchajowska (1997). Pasting property of prime starch was evaluated using a Brabender Visco/amylograph (Shuey and Tipples 1980). Prime starch (40 g, dwb) was suspended in 450 mL of distilled water, and peak viscosity was obtained from the amylogram.

Wide-Angle X-Ray Powder Diffraction

Starch crystallinity of the prime starch was evaluated using wide-angle X-ray powder diffraction (Sievert et al 1991). X-ray powder diffraction was performed at three moisture levels (2, 12, and 16%) for each prime starch. Starch samples were packed in an aluminum frame. X-ray diffraction patterns of the prime starches were recorded on a diffractometer (D 500, Siemens, Madison, WI) operating at 35 kV, 30 mA. Diffractograms were obtained from 4° 2θ to 30° 2θ with a step size of 0.05° 2θ , with 4 sec on each step.

CP/MAS ^{13}C NMR

^{13}C spectra of prime starches were obtained using the method of Li et al (1996). Spectra were acquired at ambient temperature with

TABLE I
Amylose Content and Amylograph Peak Viscosity of Prime Starches Isolated from Cultivars Crew and Stephens^a

Cultivar	Amylose Content (%)	Amylograph Peak Viscosity (BU)
Crew	31.04a	560a
Stephens	31.93a	283b

^a Values are means of at least two replicates. Means followed by the same letter in the same column are not significantly different ($P < 0.05$).

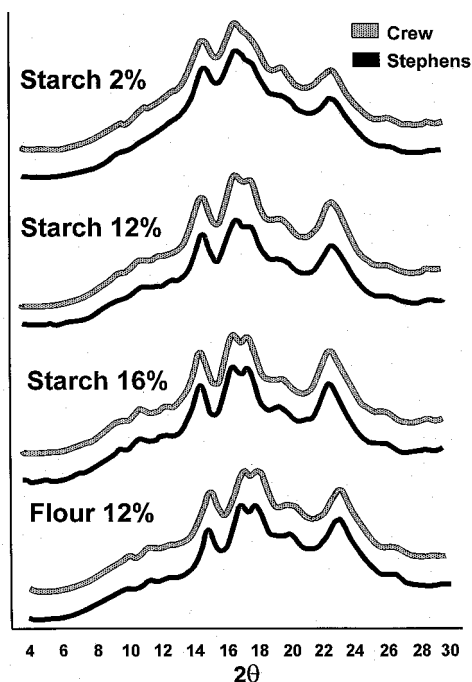


Fig. 1. Wide-angle X-ray powder diffraction patterns of prime starches at three moisture levels (2, 12, and 16%) and flours (12%) from cvs. Crew and Stephens.

an NMR spectrometer (ASX 300, USA Bruker Instruments, Billerica, MA). Prime starch samples were packed into 7-mm ceramic rotors and spun at about 3,500 Hz at MAS to eliminate chemical shift anisotropy.

^{31}P NMR

The phosphorus content and its form in prime starches were determined by using ^{31}P NMR following the procedure of Kasemsuwan and Jane (1996), with modification as described below. Prime starch (100 mg, dwb) was solubilized in 5 mL of deuterium dimethyl sulfoxide (DMSO) without enzyme treatment. Nicotinamide adenine dinucleotide (NAD) was used as an internal standard at a concentration proportional to the phosphorus content in the starch analyzed.

Starch Paste Viscosity Analysis

The prime starches were used as-is or defatted with hot *n*-propanol and water (3:1) mixture (Sievert and Wüsch 1993). Wheat starch was defatted with hot *n*-propanol and water mixture, resulting in almost complete removal of lipids (Vasanthan and Hoover 1992). The pasting properties of starches and defatted starches were analyzed using a Rapid ViscoAnalyser (RVA) (Newport Scientific, Narrabeen, Australia). Starch sample (3 g, dwb) and 25 mL of water were heated in an RVA (held 2 min at 60°C , heated to 93°C in 6

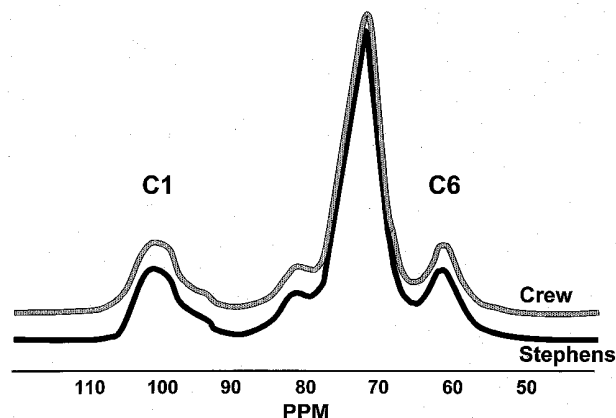


Fig. 2. Cross polarization/magic angle spinning ^{13}C nuclear magnetic resonance spectra of starches isolated from cvs. Crew and Stephens.

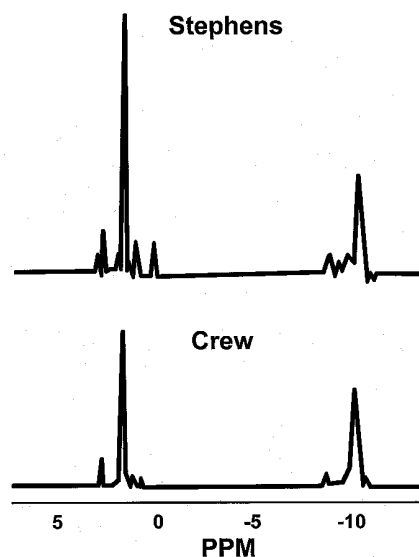


Fig. 3. ^{31}P nuclear magnetic resonance spectra of starches isolated from cvs. Crew and Stephens. Signal between 0 to 5 ppm indicated phospholipids. Signal at -10.5 ppm represented internal reference standard (Nicotinamide adenine dinucleotide, 1 mg).

min, held at 93°C for 20 min, cooled to 50°C in 6 min, and finally held 6 min at 50°C). Peak viscosity, breakdown, and setback were calculated.

DSC

DSC (Pyris 1, Perkin-Elmer Corp., Norwalk, CT) was used to follow gelatinization and retrogradation of starches and defatted starches. The method for DSC determination was described by Czuchajowska and Pomeranz (1989). Thirty samples of 10 mg of starch or defatted starch and 20 µL of water were sealed in a DSC pan and scanned from 20 to 180°C at a heating rate of 10°C/min. Next, the samples were divided into 10 sets of three and scanned after storage. Each set was rescanned only one time after storing at 4°C for 2, 4, 6, 8, 10, 12, 14, 18, 22, and 30 days. Onset (T_o) and peak (T_p) temperatures, enthalpies of starch gelatinization (ΔH_g) and melting of the amylose-lipid complexes (ΔH_{cx}) were calculated from DSC thermogram.

RESULTS AND DISCUSSION

Starches have comparable amylose content (Table I), while differing significantly in peak viscosity, ranging from 283 to 560 BU. There was no detectable difference in amylopectin fine structure between starch samples (Lin and Czuchajowska 1997). These results indicate that other factors (e.g., starch crystallinity, phosphorus content and form, protein, and enzyme content), rather than amylose content and amylopectin fine structure, are responsible for the large differences in starch viscosity.

Starch Crystallinity

Starch crystallinity of the prime starch samples was evaluated by wide-angle X-ray powder diffraction at 2, 12, and 16% moisture levels. Both prime starches exhibited typical A pattern, as shown in Fig. 1. Both prime starches showed a similar X-ray pattern at each moisture level, indicating no difference in starch crystallinity between prime starches, although there were large differences in starch peak viscosity (283 to 560 BU). An increase in peak sharpness was observed with increased moisture content (Fig. 1). Similar phenomena have been reported by Nara et al (1978) with potato starches.

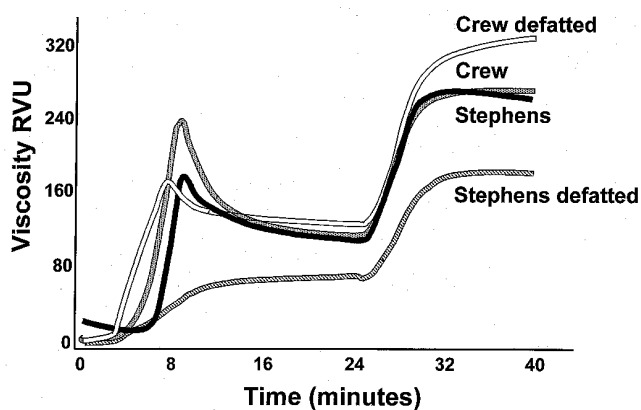


Fig. 4. Rapid ViscoAnalyzer pasting curve of starches and defatted starches isolated from cvs. Crew and Stephens.

X-ray diffraction patterns of prime starches (12% moisture) exhibited a pattern similar to that of original flours (12% moisture), indicating that isolation of starch is not necessary for X-ray diffraction measurement, especially for soft wheat flour, which contains ~80% starch.

The results of X-ray diffraction were further confirmed by using a relatively new technique, CP/MAS ^{13}C NMR. The CP/MAS ^{13}C spectra from prime starch isolated from cvs. Stephens and Crew are shown in Fig. 2. The resonance at 95–103 ppm represented the C1 position, the resonance at 60 ppm corresponded to the C6 position, and the strong signals at around 71 ppm represented the resonance from C2–C5 positions in starch. The amorphous C4 resonance appeared as a weak peak at 79 ppm (Veregin et al 1986). A triplet splitting in the C1 position after resolution enhancement (data not shown) indicated an A-type crystalline structure (Gidley and Bociek 1985, 1988; Vasudeva et al 1993; Li et al 1996). Prime starch isolated from Crew (peak viscosity 560 BU) exhibited a similar ^{13}C NMR spectrum to prime starch isolated from Stephens (peak viscosity 283 BU), indicating no detectable difference in starch crystallinity between prime starch samples.

Phosphorus Content and Form

From ^{31}P NMR, wheat starch showed the signals mainly at chemical shifts between 0.0 and 1.5 ppm, indicating phospholipids (Kasemsuwan and Jane 1994, 1996; Lim et al 1994). The ^{31}P NMR spectra for starch isolated from Stephens and Crew are shown in Fig. 3. Phosphorus exists in the form of phospholipids in these prime starch samples, which is consistent with findings of Lim et al (1994). Phosphorus content in Stephens was ~0.097%, while Crew had ~0.062% phosphorus content. Starch isolated from Stephens had significantly higher phospholipids content than starch isolated from Crew. This result can explain the large differences in starch peak viscosity.

Effect of Phosphorus Lipids on Starch Gelatinization and Retrogradation

Results from RVA for starches and defatted starches are shown in Fig. 4. Defatting with hot *n*-propanol and water mixture resulted in a decrease in peak temperature and peak viscosity. The peak viscosity for Crew, which is low in phospholipids, decreased from 240 to 165 RVU after defatting. A substantial decrease in viscosity was observed in Stephens, which is high in phospholipids. The viscosity at 93°C dropped from 175 to 50 RVU, and no peak could be seen in Stephens after defatting. Vasanthan and Hoover (1992) reported that defatting with hot *n*-propanol and water mixture eliminated the pasting peak of cereal starches. This was found to be true for Stephens; however, a clear peak was visible in defatted Crew starch. An increase in setback was observed in both starches after defatting. These results confirm that phospholipids have a strong influence on starch viscosity and retrogradation.

The gelatinization and retrogradation of starches and defatted starches were evaluated by DSC. The results for starches and defatted starches subjected to first heating in DSC are summarized in Table II. Defatting caused a significant increase in starch gelatinization onset and peak temperatures and a significant decrease in gelatinization enthalpies in both cultivars. Melting of amylose-lipid complexes disappeared completely after starches were defatted

TABLE II
Differential Scanning Calorimetry of Native and Defatted Starches^a

	Starch Gelatinization			Melting of Amylose-Lipid Complexes		
	T_o (°C)	T_p (°C)	ΔH_g (J/g)	T_o (°C)	T_p (°C)	ΔH_{cx} (J/g)
Crew	59.2b	63.7c	12.32a	98.2a	103.5a	1.35a
Crew (defatted)	61.5a	65.8b	11.04c	0.00b
Stephens	57.2c	62.5d	11.56b	97.6b	103.3a	1.15a
Stephens (defatted)	61.6a	66.7a	9.04d	0.00b

^a Values are means of at least three replicates. Means followed by the same letter in the same column are not significantly different ($P < 0.05$).

with hot *n*-propanol and water mixture. The DSC thermograms representing starch retrogradation after the first heating from 20 to 180°C and storage at 4°C from 0 to 30 days are shown in Fig. 5. Prime starch isolated from Crew (high in viscosity and low in phospholipids) retrograded at a much faster rate than prime starch isolated from Stephens (low in viscosity and high in phospholipids). The rate of starch retrogradation for starches and defatted starches is shown in Fig. 6. Starch from Crew retrograded approximately two times faster than starch from Stephens to day 14. Both starches showed a plateau at ≈10 days stored at 4°C. Similar results were observed by Roulet et al (1988) when they stored starch gel at 20°C for seven days. Roulet et al reported that the degree of crystallinity in a completely retrograded state of starch gel, stored 14 days at 4°C, was <15% of that found in native starch. According to our results, at the end of storage at 4°C for 30 days, starch isolated from Stephens restored ≈37.6% crystallinity, while starch isolated from Crew restored ≈45.5% crystallinity. Defatting did not cause a significant change in the rate of starch retrogradation in Crew (low in phospholipids), while defatting resulted in a significant increase in rate of retrogradation in Stephens (high in phospholipids). This result indicates that phospholipids play an important role in starch retrogradation, especially in starch with high phospholipids content. Defatting caused a significant increase in restoration of crystallinity in Stephens, from 37.6 to 55.8%, while Crew only increased from 45.5 to 52.5%. Starches isolated from Crew and Stephens had a comparable rate and extent of retrogradation after being defatted with hot *n*-propanol and water mixture.

In conclusion, it should be pointed out that this research has focused on the phenomenology of the effects of phosphorus and

starch crystallinity on starch gelatinization and retrogradation. The results indicate that phospholipids bound to starch slow retrogradation of amylopectin. Removal of phospholipids by defatting makes the hydroxyl group of glucose units available for the formation of new hydrogen bonds, which results in the increase of retrogradation.

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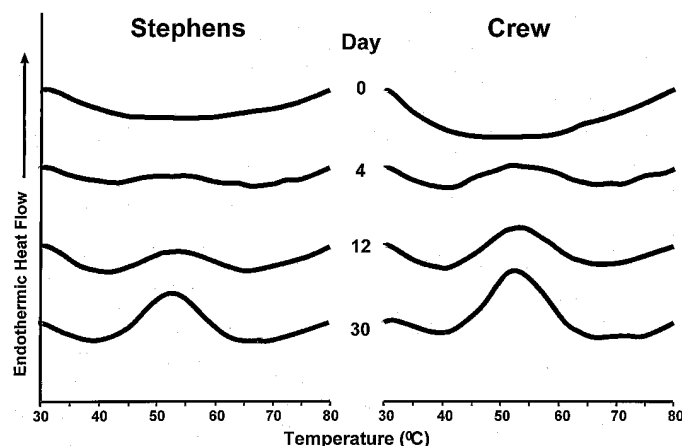


Fig. 5. Differential scanning calorimetry thermograms of prime starches isolated from cvs. Crew and Stephens after first heating and storage at 4°C for 0-30 days.

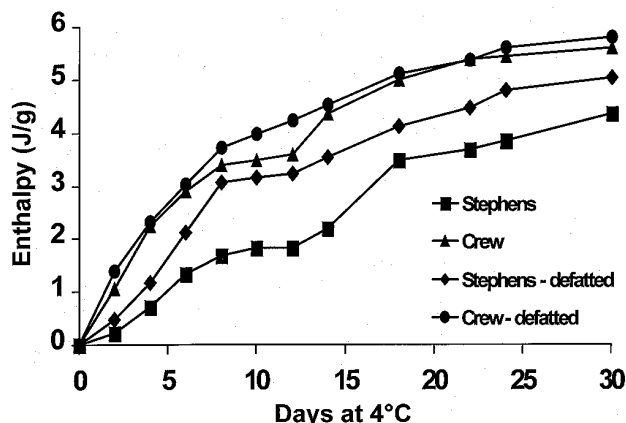


Fig. 6. Gelatinization enthalpy of starches and defatted starches isolated from cvs. Crew and Stephens after first heating and storage at 4°C for 0-30 days.

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