EFFECT OF PENTOSANS ON THE RETROGRADATION OF WHEAT STARCH GELS[1]

S. K. KIM[2] and B. L. D'APPOLONIA[2], Department of Cereal Chemistry and Technology, North Dakota State University, Fargo, ND 58102

ABSTRACT Cereal Chem. 54(1): 150-160

The effect of wheat-flour pentosans (water-soluble and water-insoluble) on the process of aging of wheat starch, starch-amylose, and starch-amylopectin gels stored at 21° and 30°C was examined with an Instron Universal Testing Instrument. Kinetic studies indicated that the basic mechanism of retrogradation of starch is instantaneous nucleation followed by rod-like growth of crystals. Pentosans had a definite effect in retarding the retrogradation of starch gels upon aging, and the effect exerted by the water-insoluble pentosans was more pronounced than that exerted by water-soluble pentosans. The water-soluble pentosans slowed down the rate of retrogradation by affecting the amylopectin fraction of starch, while the water-insoluble pentosans retarded the extent of retrogradation by affecting both amylose and amylopectin. The rate of retrogradation of the starch-amylopectin gels containing water-soluble pentosans over the first day of storage was faster than the overall rate of retrogradation, indicating more crystallization of material over the first day of storage. The crystallization of starch gels was thus characterized by the retrogradation of amylose and amylopectin over the first day of storage; thereafter, the amylopectin alone controlled the retrogradation process. Neither storage temperature nor pentosans caused changes in the basic mechanism of the retrogradation of starch gels upon aging, suggesting that pentosans influence the extent of the retrogradation simply by reducing the amount of starch components available for crystallization. The pasting properties of starch, starch-amylose, and starch-amylopectin slurries in the presence and absence of pentosans indicated that pentosans did not exhibit any effect on starch gelatinization. However, pentosans decreased the rate of setback. The effect of pentosans on the rate of setback of starch, starch-amylose, and starch-amylopectin gels was in good agreement with the effect of pentosans on the firming rate of starch gels.

It is known (1) that pentosans, which represent a minor component of wheat and flour, influence the physical properties of dough and the baking performance of flour. However, the exact role of pentosans in baking is not fully understood.

Recent studies on the function of wheat-flour pentosans in breadmaking have suggested that pentosans may interact with other components in flour. Jelaca and Hlynka (2) reported that pentosans interact with gluten to increase the resistance of dough to extension and to decrease its extensibility. It has been postulated (3) that pentosans and glycoproteins are present as transitional compounds permitting physical association and chemical bonding between carbohydrates and proteins. A possible explanation of this concept was furnished by Patil *et al.* (4), who found that the hydrogen-bonding capacity of water-soluble pentosans was dramatically increased when flour constituents were mixed into dough. Mixing the flour into dough causes a conformational

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[2]Respectively: Graduate Research Assistant and Associate Professor.

change in pentosan molecules, which in turn intensifies the association between carbohydrate and protein constituents. Whether water-insoluble pentosans play a similar role is unknown.

If such interactions do occur, a possible association of pentosans with starch would influence the degree or rate of retrogradation of starch gels upon aging. This study was undertaken to examine this possibility and, if pentosans affected the rate of retrogradation of starch gels, to ascertain which component of starch was influenced by them.

MATERIALS AND METHODS

Flour Sample

The flour sample used for the isolation of starch and pentosans was a composite of hard red spring wheat flours, milled on a pilot mill (5). The flour was unbleached and unmalted.

Isolation and Fractionation of Starch

Starch was isolated by the hand-kneading procedure (6). The recovered starch was air-dried and sieved on a 70-mesh sieve. Prior to fractionation, the starch was defatted with methanol in a Soxhlet extractor for 24 hr. The starch was then air-dried and passed through a 70-mesh sieve.

The procedure used for starch fractionation was that of Montgomery and Senti (7).

Isolation and Purification of Pentosans

Crude water-soluble pentosans and the sludge fraction were obtained according to the procedure of Medcalf *et al.* (8). Water-insoluble pentosans were isolated from the sludge fraction by extraction with sodium hydroxide followed by ethanol precipitation (9).

Both crude pentosan preparations were subjected to treatment with crude papain (N F VIII, Difco Laboratories, Detroit, Mich.) as described in a recent communication (10).

Preparation of Gels

The starch gels were prepared by mixing 50 g of starch (moisture content 10.5%) with 39.5 ml water in a Waring Blendor. The slurry, which represented a 50% concentration of starch on a dry basis, was pipetted into petri dishes, covered, and gelatinized in an oven by increasing the temperature from 25° to 98°C. The time required to reach 98°C was 50 ± 1 min. The moisture loss of the sample during gelatinization was less than 1%. The gels were cooled at room temperature for 20 min before storage.

The starch gels containing amylose or amylopectin were prepared so that the effect of pentosans on such systems could be studied. Since it was impossible to mix slurries of 50% concentration (db) with the inclusion of amylose or amylopectin and pentosans, a concentration of 45% was used. The concentration of amylose and amylopectin employed was 2% (db), whereas that of pentosans was 1% (db).

Amylose was thoroughly mixed into the starch sample before any water was added, while the pentosans were predissolved in water before being incorporated into the starch.

Aging of Gels

The prepared gels were stored at 21° and 30° C in sealed containers to prevent moisture loss. Relative humidity of the storage cabinet was maintained between 90 and 95%. At 0, 1, 2, and 5 days, the gels were tested for firmness using an Instron Universal Testing Instrument (Instron Corporation, Canton, Mass.); 10–20 measurements were made on each gel and an average was taken. The limiting modulus was obtained from the gel which was stored at 2° C for 5 days.

The values of E_0, E_1, E_2, and E_5, which represent the firmness of the gels at 0, 1, 2, and 5 days, respectively, were subjected to Avrami analysis (11–13) to determine the rate constant and the Avrami exponent according to Cornford *et al.* (14) and McIver *et al.* (15):

$$\theta = \frac{E_L - E_t}{E_L - E_o} = \exp(-kt^n)$$

or

$$\log\left(-\log_e \frac{E_L - E_t}{E_L - E_o}\right) = \log k + n \log t$$

where θ is the fraction of uncrystallized material at time t, k is a rate constant, and n is the Avrami exponent. E_o and E_t are the measured values of elastic modulus at times o and t, respectively, and E_L is the limiting modulus. The reciprocal of the rate constant is termed time constant (1/k). A detailed explanation of these various terms has been given previously (14,15).

The Avrami exponent n was obtained by plotting $\log\left(-\log_e \frac{E_L - E_t}{E_L - E_o}\right)$ against log t, and the rate constant was determined from a graph of $\log_e (E_L - E_t)$ *vs.* t (15).

Starch-Pasting Properties

Pasting properties of starch, starch-amylose, and starch-amylopectin slurries in the presence or absence of pentosans were investigated with the Brabender Amylograph®. The conditions used for the amylograph have been given previously (6).

The pentosans (1%, db) were dissolved in 100 ml distilled water before they were added to the starch slurry. The concentration of amylose or amylopectin added to the starch slurry was 2% (db).

The information obtained from the amylograph curve included pasting temperature, 15-min height, and the rate of setback. The 15-min height is the viscosity of the sample after a 15-min holding period at 95° C. The rate of setback is defined as the increase in Brabender Units (BU) per min of the gelatinized starch slurry upon cooling from 95° to 50° C. Since a normal peak viscosity was not obtained, the 15-min height was used in its place.

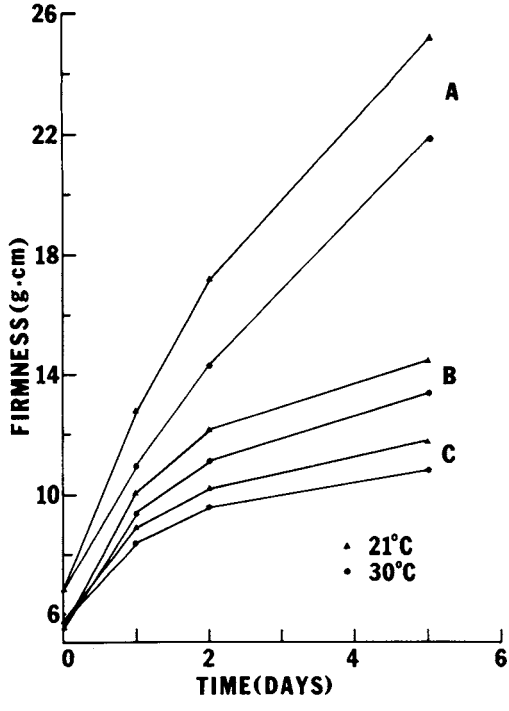

Fig. 1. Aging of A) starch gels and B) starch-soluble and C) starch-insoluble pentosan gels at 21° (▲) and 30° C (•).

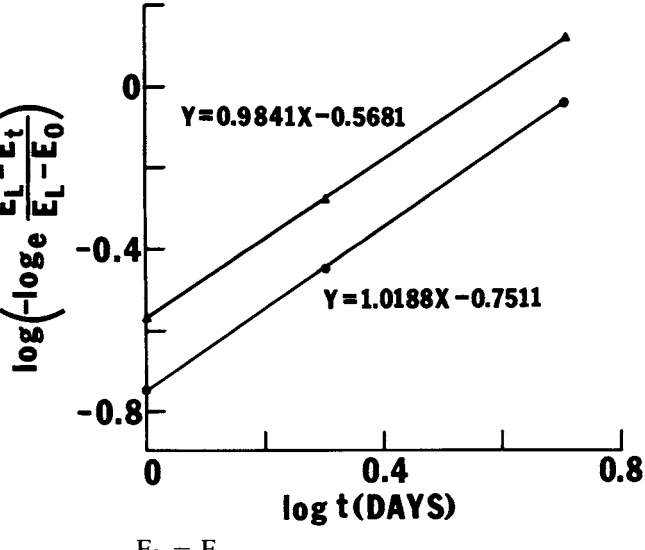

Fig. 2. Plot of log $\left(-\log_e \frac{E_L - E_t}{E_L - E_o}\right)$ against log t of 50% starch gels at 21° (▲) and 30° C (•).

RESULTS AND DISCUSSION

Aging of Starch Gels

The results of the firmness measurements on the aging of starch gels and starch-soluble and starch-insoluble pentosan gels at 21° and 30°C are shown in Fig. 1.

From Fig. 2, the Avrami exponents of the starch gels were found to be 0.98 and 1.02 at 21° and 30°C, respectively. The values of the rate constant at 21° and 30°C corresponded to 0.2627 and 0.1828 reciprocal days, giving time constants of 3.80 and 5.47 days (Fig. 3).

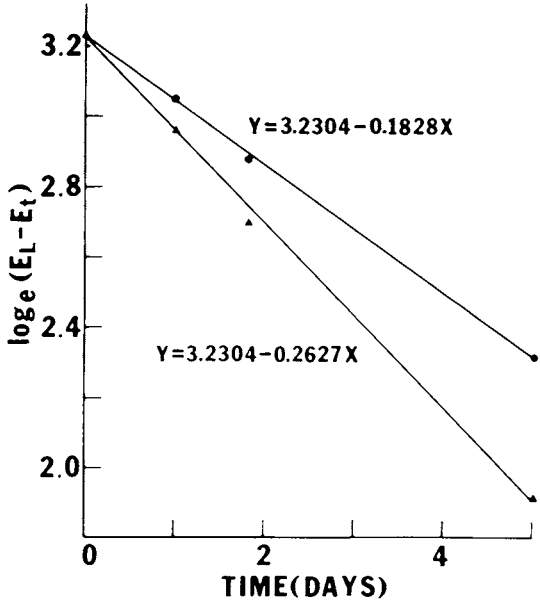

Fig. 3. Plot of $\log_e (E_L - E_t)$ against time of 50% starch gels at 21° (▲) and 30°C (•).

TABLE I
Comparison of the Avrami Exponents and the Time Constant (days) of Bread and 50% Starch Gels Stored at 21° and 30° or 32°C

	Avrami Exponent		Overall Time Constant		
	21°C	30° or 32°C	21°C	30° or 32°C	Reference
Starch gel	0.98	1.02[a]	3.80	5.47[a]	Present study
Starch gel	1.02	...	3.76	...	(15)
Starch gel	0.90	1.12	4.20	25.0	(16)
Bread[b]	1.00	...	3.68	5.47	(14)
Bread[c]	...	...	3.28	5.02	(17)

[a]30°C.
[b]Conventional baking process.
[c]Chorleywood bread process.

These values of the Avrami exponent and the time constant at 21° and 30°C were compared with those of earlier publications (Table I). As evident from this table, the Avrami exponent was unity (*i.e.*, n=1) at the two temperatures studied. This demonstrates that the basic mechanism of retrogradation of starch is instantaneous nucleation followed by rod-like growth of crystals (15).

Although the time constant in the present study agreed with that of bread at 32°C (Table I), it was significantly different from that of starch gels obtained by Colwell *et al.* (16). The different time constants obtained could be due to the use of different values of the limiting modulus in the two studies. Colwell *et al.* (16) found that the time constant was 25 days at 32°C when the limiting value was used as measured after 14 days' storage at 4°C (Table I). However, when the limiting value observed at the storage temperature of 32°C was used, the time constant was reduced to 17.9 days. The firming curves obtained by these workers show that starch gels stored at 21°C approached the limiting value after 6 days of storage; thereafter, the increase in firmness, determined by differential thermal analysis, was slow. Thus, the limiting modulus used in the present study and by Colwell *et al.* (16) would have no effect on the time constant at 21°C, as evident in

TABLE II
Effect of Pentosans on the Avrami Exponent and the Time Constant of 50% Starch Gels Stored at 21° and 30°C

Gels	Storage Temperature °C	Avrami Exponent	Overall Time Constant	Time Constant over the First Day of Storage
Starch (S)	21	0.98	3.80	3.70
	30	1.01	5.47	5.64
S-soluble pentosan	21	0.70	5.33	3.29
	30	0.75	6.80	3.49
S-insoluble pentosan	21	0.83	7.51	5.75
	30	0.89	9.65	8.14

TABLE III
Firmness and Limiting Modulus of 45% Starch-Amylose and Starch-Amylopectin Gels Stored at 21°C

Gels	Firmness (g cm)[a]			Limiting Modulus
	E_0	E_1	E_5	(g cm)
Starch-amylose (S-A)	7.91	14.20	23.40	26.77
S-A-soluble pentosan	7.88	12.40	19.32	21.77
S-A-insoluble pentosan	7.55	10.80	18.04	22.02
Starch-amylopectin (S-AP)	4.50	7.52	12.93	16.31
S-AP-soluble pentosan	4.33	6.50	10.35	16.15
S-AP-insoluble pentosan	4.03	5.35	8.00	15.03

[a] E_0, E_1, and E_5 represent the firmness of the gels at 0, 1, and 5 days' storage, respectively.

Table I. However, at 30°C the gels reached 90 and 40% of the firmness at 21°C after 5 days of storage in the present study and after 5–7 days in the study by Colwell *et al.* (16), respectively. The lower value obtained by Colwell *et al.* (16) will increase the time constant at a given value of the limiting modulus. For example, it could be shown in the present work that the time constant at 30°C was increased to 12.1 days if the firmness value of the gel after 5 days of storage was reduced by 30%.

Effect of Pentosans on the Retrogradation of Starch Gels

Pentosans had a definite effect on slowing down the firmness of starch gels upon aging (Fig. 1). Data on the Avrami exponent and the time constant of the starch and starch-pentosan gels are presented in Table II. Pentosans increased the time constant of the starch gels, and the effect exerted by the water-insoluble pentosans was more pronounced than that exerted by the water-soluble pentosans.

The rate of retrogradation of the starch gels over the first day of storage agreed

TABLE IV
Effect of Pentosans on the Avrami Exponent and the Time Constant of 45% Starch-Amylose Gels Stored at 21° and 30°C

Gels	Storage Temperature °C	Avrami Exponent	Overall Time Constant	Time Constant over the First Day of Storage
Starch-amylose (S-A)	21	0.92	2.90	2.56
	30	0.91	3.74	3.29
S-A-soluble pentosan	21	0.92	2.88	2.54
	30	0.91	3.74	2.88
S-A-insoluble pentosan	21	1.01	3.87	3.93
	30	1.01	4.27	4.30

TABLE V
Effect of Pentosans on the Avrami Exponent and the Time Constant of 45% Starch-Amylopectin Gels Stored at 21° and 30°C

Gels	Storage Temperature °C	Avrami Exponent	Overall Time Constant	Time Constant over the First Day of Storage
Starch-amylopectin (S-AP)	21	0.90	3.99	3.38
	30	0.87	4.89	3.99
S-AP-soluble pentosan	21	0.78	7.03	4.93
	30	0.77	8.18	5.61
S-AP-insoluble pentosan	21	0.78	11.17	7.82
	30	0.86	14.18	11.32

with that of the overall rate (Table II). In contrast, the time constant of the starch-pentosan gels over the first day of storage was considerably different from the overall time constant, even though the Avrami exponent of the starch-pentosan gels was close to unity. Thus, it appears that the starch gels containing pentosans retrograded at a faster rate over the first day. The time constant of the starch gels containing water-soluble pentosans over the first day of storage was similar to that of starch gels at 21°C. However, the water-insoluble pentosans at the same temperature increased the time constant over the first day of storage by 50%.

These results, together with the firming curves shown in Fig. 1, suggest that the water-insoluble pentosans are more effective than the water-soluble pentosans in retarding the extent of the retrogradation of starch gels. To increase the time constant, pentosans should interact with the starch component (amylose or amylopectin or both) or interfere in some way with the association of starch molecules during the aging of the starch gels.

Effect of Pentosans on the Aging of Starch-Amylose and Starch-Amylopectin Gels

To determine on which starch component pentosans exert their effect, the kinetic parameters of starch-amylose (S-A) and starch-amylopectin (S-AP) gels were investigated.

Table III shows the firmness and the limiting modulus values of the S-A and S-AP gels at 21°C. At 30°C, not shown in Table III, the firmness values were somewhat lower than at 21°C. The firmness of the S-A gels upon aging followed a pattern similar to that of the 50% starch gels (Fig. 1); this made it possible to use the Avrami concept in analyzing the kinetic parameters.

The Avrami exponent and the time constant of the S-A and S-AP gels in the presence and absence of pentosans are presented in Tables IV and V, respectively. These results may not be directly comparable to those obtained with the 50% starch or starch-pentosan gels (Table II). However, they did provide worthwhile information as to which starch component is affected by pentosans during the aging of starch gels.

As shown in Tables IV and V, the S-A gels retrograded at a faster rate than the S-AP gels, indicating that the amylose was principally responsible for the crystallization of starch gels upon aging. It is known (18) that in an artificial mixture of amylose and amylopectin the retrogradation of amylose is retarded by the amylopectin. The higher time constant values of the S-AP gels would therefore be due to dilution of the amylose in the starch gels.

Although the water-soluble pentosans did not cause any change in the time constant of the S-A gels (Table IV), they increased that of the S-AP gels (Table V). This suggests that the effectiveness of the water-soluble pentosans in retarding the retrogradation of starch gels (Fig. 1) is due to the prevention of or interference with the retrogradation of the amylopectin.

Unlike the water-soluble pentosans, the water-insoluble pentosans slightly increased the time constant of the S-A gels (Table IV), indicating an effect on the retrogradation of the amylose. However, the time constant of the S-AP gels was almost tripled in the presence of the water-insoluble pentosans (Table V), indicating that the water-insoluble pentosans are more effective than the water-soluble pentosans in retarding the retrogradation of the amylopectin. These results indicate that the effectiveness of the water-insoluble pentosans in

retarding the retrogradation of the starch gels is due to the prevention of or interference with the retrogradation of both amylose and amylopectin.

The time constant of the S-A gels containing water-soluble pentosans (Table IV) indicates that the rate of the retrogradation of these gels had a linear response as a function of storage time. However, the S-AP gels containing water-soluble pentosans did not show such a relationship (Table V); that is, the rate of the retrogradation over the first day was faster than the overall rate. The same trend was observed with the water-insoluble pentosans (Tables IV and V).

As indicated earlier, the water-soluble pentosans were effective in preventing the retrogradation of amylopectin (Table V). Thus, the increased value of the time constant of the S-AP gels in the presence of the water-soluble pentosans (Table V), compared to the time constant of the S-AP gels, was due to the effectiveness of the water-soluble pentosans in preventing the retrogradation of the amylopectin. However, the time constant of the S-AP gels containing the water-soluble pentosans over the first day of storage was smaller compared to the overall time constant (Table V), indicating more crystallization of the material over the first day of storage. These results, in turn, suggest that something other than the amylopectin was responsible for the faster rate of the retrogradation of the S-AP gels containing the water-soluble pentosans over the first day of storage since, if the amylopectin alone controlled the retrogradation, the time constant over the first day of storage should be comparable to the overall time constant. Since amylose retrogrades more readily than amylopectin (18), and the water-soluble pentosans had no effect on the rate in which the amylose retrograded (Table IV), the faster rate of retrogradation of the S-AP gels containing the water-soluble pentosans over the first day of storage (Table V) would be due in part to the retrogradation of the amylose. This also might imply that amylose retrogrades primarily over the first day of storage.

In differential thermal analysis studies, McIver *et al.* (15) suggested that the amylopectin was responsible for the retrogradation of the starch gels. However, the results in Tables IV and V suggest that the amylopectin retrograded together

TABLE VI
Effect of Pentosans on the Pasting Properties of Starch, Starch-Amylose, and Starch-Amylopectin Slurries

Slurries	Pasting Temperature °C	15-min Height BU	Rate of Setback[a] BU/min
Starch (S)	77.5	520	17.6
S-soluble pentosan	77.5	530	13.9
S-insoluble pentosan	77.5	540	11.1
S-amylose (S-A)	77.5	490	17.7
S-A-soluble pentosan	79.0	480	17.7
S-A-insoluble pentosan	77.5	500	17.4
S-amylopectin (S-AP)	77.5	500	15.8
S-AP-soluble pentosan	77.5	490	15.0
S-AP-insoluble pentosan	77.5	500	12.5

[a]The rate of increase in Brabender Units (BU) per min during cooling of the paste from 95° to 50°C.

with the amylose which would retrograde primarily over the first day of storage; thereafter, the amylopectin alone would undergo retrogradation. These results support the suggestion of Collins (18): "In a concentrated paste or gel the amylose and amylopectin are intermixed and will probably retrograde together in regions where amylose molecules and outer branches of amylopectin are suitably oriented for hydrogen-bond formation." Thus, pentosans may reduce the extent of the retrogradation of starch gels by forming hydrogen bonds with the amylopectin, thereby preventing the hydrogen-bond formation between amylose and amylopectin, and, in part, the retrogradation of the amylopectin itself.

Effect of Storage Temperature on the Aging of Starch Gels in the Presence of Pentosans

The Avrami exponents presented in Tables II, IV, and V were close to unity, and the deviations from unity were within the calculated 95% confidence limits. These results suggest that the basic mechansim of the crystallization of starch gels was not affected by pentosans. This implies that, to slow down the retrogradation of starch gels, pentosans simply reduce the availability of the starch fractions for crystallization upon aging.

The time constants at 30°C were higher than those at 21°C (Tables II, IV, and V). It is not known why starch gels retrograde at a slower rate at higher storage temperatures.

Effect of Pentosans on the Pasting Properties of Starch, Starch-Amylose, and Starch-Amylopectin Slurries

To investigate the effect of pentosans on the gelatinization of starch, S-A, and S-AP slurries, the pasting properties of such systems were examined. The concentrations of amylose, amylopectin, and pentosans used were the same as those employed in the gel studies.

Table VI shows the pasting temperature, 15-min height, and rate of setback of the various systems studied. Pentosans had no effect on the pasting temperature and only slightly increased the 15-min height. These results agree with those obtained by Tao and Pomeranz (19), who studied the pasting properties of a composite wheat flour in the presence of water-soluble pentosans at the 2% level.

Pentosans did influence the rate of setback of the gelatinized starch slurries. The water-soluble pentosans did not affect the rate of setback of the S-A system, but they decreased the rate of setback of the S-AP gel. The rate of setback of the S-AP gel was considerably lower in the presence of the water-insoluble pentosans. These findings are in good agreement with the effect of pentosans on the firming rate of the starch gels (Fig. 1 and Tables II, IV, and V).

The results of Table VI suggest that pentosans do not influence the gelatinization process of the starch, but exert their effect by retarding the retrogradation of starch gels upon cooling.

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