

Effects of Environmental Temperature on Structure and Gelatinization Properties of Wheat Starch

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ABSTRACT

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The effects of environmental temperature on gelatinization properties and amylopectin structures of wheat endosperm starch were examined by isolating starches from four wheat cultivars matured in growth chambers at daytime temperatures of 15, 20, 25, or 30°C. Kernel weight and starch content per kernel were reduced by high maturation temperature. Amylose content showed no significant change at high maturation temperature in some cultivars; in other cultivars, there was a slight increase. Principal component analysis of data on relative peak areas of debranched amylopectin showed that amylopectin from wheat grown at a

lower temperature had a greater proportion of shorter chains. Amylopectin branch chains were classified into three groups based on the correlation coefficients between the data of branch chain length distribution and principal component scores, degree of polymerization (DP) of 6–12, DP 13–34, and DP \geq 35. The gelatinization temperature of starches increased markedly at a higher maturation temperature, with increases exceeding 10°C at high maturation temperatures. Gelatinization properties correlated significantly with amylopectin chain length distribution.

Starch is one of the major components of the wheat (*Triticum aestivum* L.) kernel and has great influence on the physicochemical properties of flour dough and products. Amylose content has strong influence on the eating quality of white salted noodles (Oda et al 1980), and Shibamura et al (1994) have shown that the size and branching of amylose and average chain length of amylopectin may also determine noodle quality. α -Amylase digestion studies showed that starch for good noodle quality has a more open branching structure (Batey and Mueller 1991). Synergistic effects on paste viscosities and gel strength were observed when the amyloses and amylopectins of different size were mixed, longer branch chains of the amylopectin entangle with amylose to a greater extent (Jane and Chen 1992).

Environmental temperatures affect the amylose content, structural features and gelatinization properties of starches in several cereal species. In rice, higher ambient temperatures reduce amylose content, increase amylopectin chain length, and increase gelatinization temperature (Asaoka et al 1984, 1985, 1989). Starch accumulation and starch granule size were reduced at high temperatures in barley, but amylose contents were little affected, and no change in amylopectin fine structures was detected (MacLeod and Duffus 1988; Tester et al 1991). Gelatinization properties measured by differential scanning calorimetry (DSC) showed increasing onset, peak, and conclusion temperatures of gelatinization with increased growing temperature. Maize grown at high temperature during endosperm cell division had reduced kernel mass due to reduced number of endosperm cells, starch granules, or both (Jones et al 1985). Experiments with maize ear showed that as developmental temperature increased, starch granule size decreased, gelatinization temperature increased, and amylose content decreased. Genetically unrelated inbreds responded differently to developmental temperature change (Lu et al 1996). In one line, higher temperatures resulted in increases in the medium branch-chain fraction of amylopectin and decreases in the long and short branch-chain fraction, whereas in another line, amylopectin had increased long and medium branch-chain fraction and decreased short branch-chain fraction. In wheat, environmental temperature affects grain yield, protein content, and the size and number of starch granules (Sofield et al 1977; Bhullar and Jenner

1985; Tester et al 1995). Shi et al (1994) reported that with increasing temperature during grain-filling, amylose content was slightly increased and starch gelatinization temperatures increased. Starches from most wheats grown at 40°C had increased proportions of unit chains with DP 10–16 and reduced proportions of unit chains with DP 17–21. Two of the key enzymes in starch biosynthesis, the starch synthase and branching enzyme, have been proposed to play a significant role in limiting starch deposition when temperatures exceed 25°C (Keeling et al 1994).

To examine the effects of environmental temperature on starch properties, we exposed four wheat cultivars to four different maturation temperatures. Starch content, amylose content, amylopectin chain length distribution, and gelatinization properties of starches were determined.

MATERIALS AND METHODS

Materials

Four wheat cultivars, Norin 3, Norin 29, Haruhikari, and Haruyutaka were grown in pots in a greenhouse at 20–25°C until anthesis. Plants were then transferred to growth chambers and grown to maturity. Daytime temperatures were set at 15, 20, 25, and 30°C for 14 hr, and nighttime temperatures at 10, 15, 20, and 25°C, for 10 hr, relative humidity set to 60%, and light intensity set to 16,000 m²•cd•sr (lx) at daytime.

Starch Content

Wheat kernels were ground using an agate mortar and pestle. Total starch content was determined by the amyloglucosidase/ α -amylase method using a total starch assay kit (Megazyme International, Ireland).

Starch Isolation

Starch was isolated from degermed wheat kernels by modification of the method of Sulaiman and Morrison (1990) to avoid the loss of small granules and to minimize the adsorption of lipids onto the surface of starch granules. Isolated starch was freeze-dried.

Amylose Contents

Starch lipid was extracted with water-saturated *n*-butanol containing 0.01% (w/v) butylated hydroxytoluene (Morrison et al 1980). Iodine affinity was determined using the iodine amperometric titration by the method of Fukuba and Kainuma (1977). The apparent amylose content of starch was calculated assuming the iodine affinity value of pure amylose to be 20 g/100 g (Banks and Greenwood 1975).

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Amylopectin Fine Structure Analysis

Starch samples were treated with heated methanol to avoid α -amylase activation. Methanol-treated starches were dispersed in water (1 mg/mL) and heated at 100°C for 1 hr for gelatinization. For digestion, 50 μ L of 50 mM sodium acetate buffer, pH 4.5, and 10 μ L of 2% sodium azide solution were added to aliquots of 930 μ L of gelatinized starch. After 10 μ L of isoamylase (700 units, Hayashibara Biochemical Laboratories, Okayama, Japan) was added, the solution was incubated at 40°C for 8 hr. Another 10 μ L of isoamylase was added and incubated for 16 hr, and then the solution was boiled for 5 min to terminate digestion and freeze-dried for further analysis.

Chain length distribution was determined as described by Koizumi et al (1989, 1991) with slight modifications. Isoamylase-debranched samples were dissolved in 0.4M NaOH at 1 mg/mL and kept at room temperature for 10 min, then diluted to 0.1M NaOH and filtered through a 0.2- μ m polytetrafluoroethylene (PTFE) membrane. Samples (20 μ L) were analyzed by high-performance anion exchange chromatography (HPAEC). HPAEC analyses were performed with a Dionex DX300 system (Dionex, CA) equipped with a pulsed-amperometric detector (PAD). A Dionex Carbopac PA1 column (4 \times 250 mm) was used with a Carbopac PA guard column (3 \times 25 mm). Samples were eluted at 1 mL/min with a linear gradient of 0.1–0.4M sodium acetate in 0.1M NaOH in 50 min. Peak area ratios (%) were calculated using an AI-450 chromatography workstation (Dionex).

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measurement was performed on a Seiko Electronics SSC580 with DSC10 module as described by Kohyama and Nishinari (1991). The instrument was calibrated with indium. Starch samples were suspended in water to 10% (w/w) and heated from 30 to 120°C at the rate of 1°C/min.

Statistics

All experiments were replicated at least twice. Starch properties were evaluated by analysis of variance (ANOVA) using the general linear model of the Statistical Analysis System (SAS Institute, Cary, NC). Multiple comparisons were made by least significant difference (LSD). Principal component analysis was performed with a princomp procedure in SAS.

RESULTS AND DISCUSSION

Kernel Weight and Starch Content

Increasing the maturation temperature from 15 to 30°C significantly reduced kernel weight in all cultivars, as shown in Table I. The reduction in kernel weight in different cultivars was 28–49%. Norin 3 was most susceptible to high temperatures in respect to grain filling. Starch content per kernel was also reduced in response to increased maturation temperature. The reduction of starch content was 32–49%. Starch deposition in Norin 3 was most susceptible to higher temperature, consistent with the effect on kernel weight.

Amylose Content

The effects of maturation temperature on the amylose content of starches are shown in Table II. Amylose contents of starches from wheat grown at 15°C were 28.0–30.5%, and those grown at 30°C were 30.2–31.7%. Amylose contents were not significantly affected by elevated maturation temperature in the cultivars Norin 3, Norin 29, and Haruhikari. However, in Haruyutaka, amylose content increased slightly with increasing temperature. Shi et al (1994) showed that there is a slight increase in the total amylose content overall when grown at 40°C compared with 15°C, with differences as large as 6% observed depending on the cultivar. This difference in range of increase compared with our results is probably due to different temperature regimes.

Also, responses to maturation temperature may be different among cultivars with different genetic backgrounds. As for starch content, synthesis of both amylose and amylopectin were affected by higher temperature, but amylopectin synthesis to a slightly greater extent leading to relatively higher value in amylose content.

The amylose content of starch from rice endosperms decreased dramatically as the maturation temperature rose (Asaoka et al 1984, 1985, 1989). In barley, amylose content was little affected by ambient temperature (Tester et al 1991). Responses in maize were consistent with that of rice (Lu et al 1996). The present study showed that wheat behaved similarly to barley in response to maturation temperature, which is different from rice and maize.

Amylopectin Chain Length Distribution

Amylopectin chain length distribution obtained by HPAEC is compared in Fig. 1. Because authentic standard materials for malto-oligosaccharides DP >15 were not available, and relative detector response reduces with increase in DP (Koizumi et al 1991), relative peak areas were used for comparison. Shorter chains appeared to decrease with increasing temperature. Principal component analysis on relative peak areas for samples matured at different temperatures, including data from four cultivars each in duplicate, was performed to confirm this observation. It showed that samples matured at a lower temperature are distributed on the negative side of the first principal component axis and those matured at a higher temperature on the positive side (Fig. 2). The first and second principal components contributed 47.9 and 23.5% of the total variation, respectively, which means that 71.4% of the total variation was explained by the first two principal components. This result indicated that the chain length distribution of amylopectin changed with the maturation temperature.

A plot of correlation coefficients between the data on amylopectin branch chain length distribution and the principal component scores (Fig. 3) showed that amylopectin branch chains with DP 6–12 correlated negatively with the first principal component, and branch chains with DP 13–34 correlated positively. These results indicated that branch chains could be classified into three groups based on the correlation coefficient with the values of principal components, and these groups behaved differently according to the maturation temperature. The proportion of amylopectin branch chains with DP 6–12 decreased as the maturation

TABLE I
Kernel Weight and Starch Content of Four Cultivars
Matured at Different Temperatures

Cultivar and Temperature (°C)	Kernel Wt	Starch Content	
	(mg as-is)	(% dry basis)	(mg/kernel)
Norin 3			
15	63.8	65.0a ^a	38.0a
20	44.1	65.9a	26.8b
25	41.5	64.2a	24.5bc
30	32.8	64.0a	19.3c
Norin 29			
15	57.8	73.9a	39.2a
20	58.5	71.1a	38.0a
25	51.7	69.1ab	32.7a
30	36.0	64.7b	21.9b
Haruhikari			
15	53.9	73.3a	36.0a
20	49.4	72.7a	32.9a
25	52.5	71.5a	34.2a
30	38.8	68.5a	24.4b
Haruyutaka			
15	44.6	69.2a	29.0a
20	39.1	70.2ab	24.5a
25	37.9	64.4ab	23.5a
30	25.3	65.3b	14.9b

^a Values followed by the same letter within a cultivar and column show no significant difference ($P < 0.05$, LSD analysis).

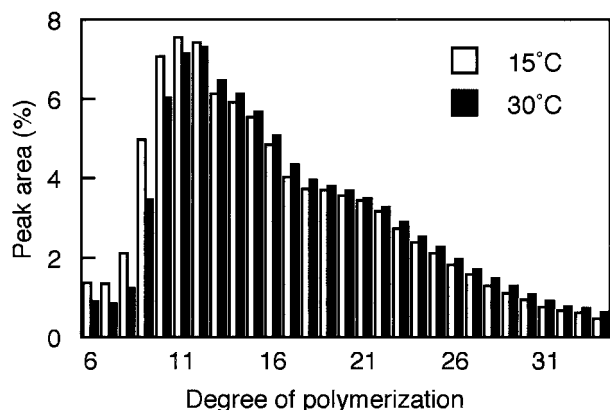


Fig. 1. Comparison of amylopectin chain length distribution of Haruyutaka matured at 15 and 30°C by HPAEC-PAD.

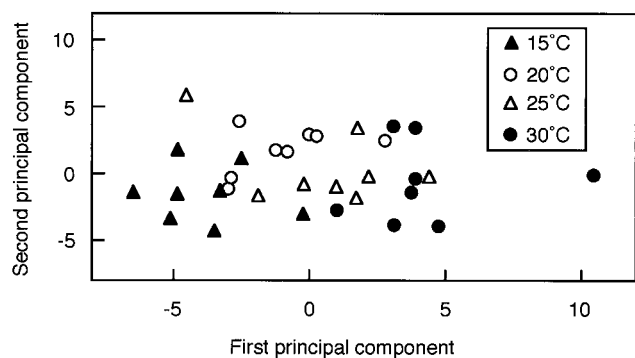


Fig. 2. Principal component scores of samples matured at different environmental temperatures.

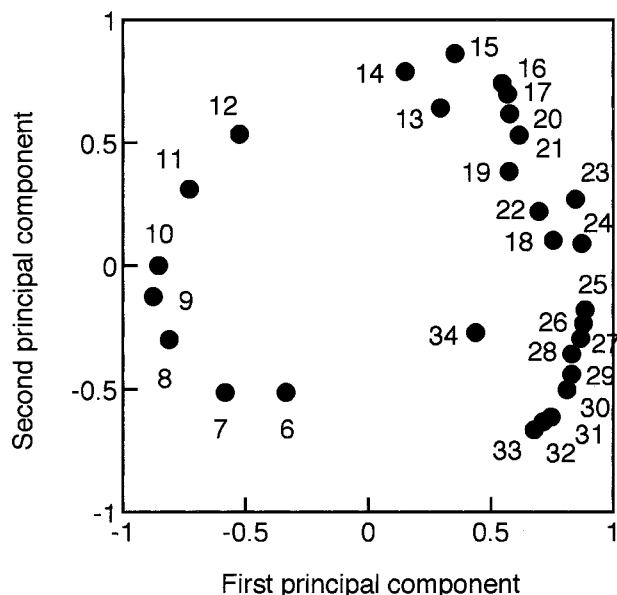


Fig. 3. Correlation coefficients between amylopectin branch chain length and first and second principal components. Numbers indicate degree of polymerization of amylopectin branch chains.

temperature increased, from 30.2–32.6% for starches from wheat grown at 15°C to 26.7–27.5% for starches from wheats matured at 30°C (Table II). On the other hand, the proportion of DP 13–34 increased as the maturation temperature increased from 61.2–62.6% at 15°C to 64.3–66.1% at 30°C. No significant difference was observed for the proportion of amylopectin chains with DP \geq 35. Significant correlations between DP 6–12 and maturation temperature, and between DP 13–34 and maturation temperature were observed

(Table III). These results conflict partially with results presented previously by Shi et al (1994). In their experiment, the proportion of short chains (DP 10–15) was increased in most, but not all, wheats grown at 40°C compared with those grown at 15°C. This was opposite from our results. Because not all cultivars gave the same results in their experiments, this difference could be due to differences in genetic background. Our results are consistent with results from maize (Lu et al 1996), rice (Umamoto et al 1999; Inouchi et al 2000), and sweet potatoes (Noda et al 2001). In barley, there was little influence of growth temperature on the amylopectin structure using gel-permeation chromatography (Tester et al 1991). Shi et al (1994) had shown that the proportion of DP 16–24 was highest when grown at 25°C. In our results, DP 13–34 increased when maturation temperature was raised from 15 to 30°C. This is probably due to different temperature regimes.

Enzyme activities in the pathway of starch biosynthesis in developing wheat grains were affected by elevated temperature (Keeling et al 1994). Two of the key enzymes involved in starch biosynthesis, soluble starch synthases, and branching enzymes, are heat-sensitive, and their activity decreases quickly when temperatures exceeded 25°C. Because both enzymes are responsible for the elongation and branching of amylopectin chains, reduced activity in higher temperature is likely to affect amylopectin structure. Multiple forms of both enzymes are known, and temperature optimum and substrate specificity differs among the isoforms (Takeda et al 1993; Keeling et al 1994; Guan et al 1997). Affinity of the starch synthase for its substrate changes according to temperature (Jenner et al 1995). These factors are likely to cause differences in amylopectin structure of starches matured at different temperature (Lu et al 1996). Four isoforms of starch synthase and three isoforms of branching enzyme are known for wheat. It is of great interest to investigate which isoforms are affected by high temperature. This may allow definition of the mechanism through which high temperature causes changes in amylopectin structure.

Gelatinization Properties

Gelatinization properties determined by DSC measurements are shown in Table II.

The peak temperature (T_p) increased as the maturation temperature increased. The T_p for starches from wheat grown at 15°C was 49.8–53.5°C, and those grown at 30°C were 59.7–60.2°C. Gelatinization enthalpy (ΔH) increased slightly with increasing maturation temperature, averaging 8.2 J/g of starch for that grown at 15°C and 9.8 J/g for that grown at 30°C.

These properties positively correlated with maturation temperature (Table III).

The gelatinization enthalpy data suggests that the crystallite structure of starches from wheat grown at a higher temperature is more stable. Starch granules are thought to have alternating layers of crystalline and amorphous regions, and the crystalline region consists mainly of amylopectin packed in a double helical manner (French 1984). Because gelatinization enthalpy reflects the loss of double-helical structure of the amylopectin molecule (Cooke and Gidley 1992), high ΔH of starch from wheat grown at higher temperature was probably due to more stable crystalline structure, formed from longer helices with high proportion of longer branch chains of amylopectin. Correlation between the amount of long or short chains and gelatinization temperature within the same botanical origin is observed in other species such as sweet potatoes and buckwheat (Noda et al 1998) and maize (Shi and Seib 1995). These results also support the idea that starch gelatinization is influenced by the amylopectin chain length distribution.

Annealing is often defined as a physical treatment that involves incubation of starch granules in excess water or intermediate water content for a certain period of time at a temperature above the glass transition temperature but below the gelatinization temperature (Jacobs and Delcour 1998). Increase in the gelatinization temperature and decrease in the gelatinization temperature

TABLE II
Amylose Content, Amylopectin Chain Length Distributions, and Gelatinization Properties of Starches Matured at Different Temperatures

Cultivar and Temperature (°C)	Chain Length Distribution (%) ^a			Gelatinization Properties ^b		
	Amylose (%)	DP 6–12	DP 13–34	DP ≥ 35	T _p (°C)	ΔH (J/g)
Norin 3						
15	29.6a ^c	32.6a	61.3b	6.1b	49.8d	7.8b
20	29.4a	30.0b	63.2a	6.8ab	55.4c	9.5a
25	30.5a	28.1c	64.3a	7.6ab	57.1b	9.8a
30	30.5a	27.2c	64.6a	8.3a	60.2a	10.2a
Norin 29						
15	30.5a	31.6a	61.2c	7.2a	51.6d	7.9a
20	31.4a	29.5b	62.3bc	8.2a	54.3c	8.9a
25	32.0a	29.1b	63.5ab	7.4a	57.1b	9.0a
30	31.7a	27.1c	64.5a	8.3a	59.7a	9.2a
Haruhikari						
15	29.9b	30.2ab	62.6b	7.2a	53.5c	8.4b
20	30.0b	29.0bc	62.9ab	8.1a	56.5b	8.9ab
25	32.2a	28.8bc	62.6b	8.6a	56.6b	10.1a
30	30.5ab	27.5c	64.3a	8.2a	59.8a	9.6ab
Haruyutaka						
15	28.0b	30.9a	62.2c	6.9a	51.6c	8.5b
20	28.1b	28.6b	63.4bc	8.0a	55.7b	9.9ab
25	30.2a	28.1c	63.8b	8.1a	56.5b	10.0a
30	30.2a	26.7c	66.1a	7.3a	60.0a	10.2a

^a DP 6–12, DP 13–34, and DP ≥ 35, sum of proportion of oligosaccharide chains with degree of polymerization of 6 to 12, 13 to 34, and 35 and above, respectively.

^b T_p, peak temperature; ΔH, gelatinization enthalpy.

^c Values followed by the same letter within a cultivar and column show no significant difference (*P* < 0.05, LSD analysis).

TABLE III
Correlations Among Starch Properties^a

	Weight	Starch	Amylose	DP 6–12	DP 13–34	DP ≥ 35	T _p	ΔH (J/g)
Maturation temp	-0.625**	-0.550**	0.499**	-0.919**	0.871**	0.614**	0.952**	0.779**
Kernel weight		0.529**	0.259**	0.732**	-0.818**	-0.267**	-0.665**	-0.679**
Starch content			0.009**	0.409**	-0.583**	0.088**	-0.406**	-0.447**
Amylose content				-0.322**	0.159**	0.473**	0.408**	0.153**
DP 6–12					-0.923**	-0.715**	-0.967**	-0.851**
DP 13–34						0.391**	0.905**	0.787**
DP ≥ 35							0.667**	0.616**
T _p								0.804**

^a ** and *, significant at *P* < 0.01 and *P* < 0.05.

range are observed when the starch granule is annealed. Although annealing occurs most rapidly and to the largest extent just below the onset of gelatinization, there is evidence that it occurs at temperatures as low as 25°C (Lorenz and Kulp 1984; Tester et al 1998). It is also possible to interpret the increase in the T_p as a result of increase in the degree of order of the crystallinity of the starch granule, which is enhanced by annealing. The moisture content in the kernel is ≈70% during grain filling, which can be considered to be in the state of excess water or intermediate water content, meeting the condition for annealing to occur. This interpretation is consistent with the hypothesis that crystallization of starch granule in vivo is inferred to be mainly a thermodynamic process, and that elevated growth temperature enhances crystallite formation (Hizukuri 1969; Tester 1997). Because annealing causes no effect on ΔH, the observed correlation between maturation temperature and ΔH is most likely the effect of chain length distribution.

CONCLUSIONS

Environmental temperature had a great effect on starch content, amylopectin structure and gelatinization properties of wheat starch. Starch content per kernel was greatly reduced by elevated maturation temperature, following the same trend as other plant species. Amylose content was not markedly affected by increased maturation temperature, although there was some variation in response between cultivars. This observation is similar to that made previously in wheat and barley, but differs from the situation reported for rice and maize. The amylopectin chain length distribution

changed in groups of DP 6–12, DP 13–34, and DP ≥ 35 in response to environmental temperature. Higher maturation temperature lead to a decrease in the proportion of amylopectin short chains and an increase in long chains, which was also consistent with other species. Increase in T_p and ΔH were observed at higher temperatures, also in agreement with previous findings in wheat and other species. The crystallite structure of starches matured at a higher temperature appeared to be more ordered and therefore more stable.

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