Effects of Temperature During Grain-Filling on Starches from Six Wheat Cultivars[1]

YONG-CHENG SHI,[2] PAUL A. SEIB,[2] and JOHN E. BERNARDIN[3]

ABSTRACT

Cereal Chem. 71(4):369-383

Six wheat cultivars were placed in growth chambers during the grain-filling period and were subjected to maximum daylight temperatures of 15, 25, or 40°C daily for 5 hr. The same wheats also were grown in the greenhouse at a mean temperature of 18°C. In addition to causing shriveled kernels and low test weight, elevated temperature reduced starch accumulation and size of starch granules, and resulted in deformed starch granules, especially in hard and durum wheats grown at 40°C. With increasing temperature during grain-filling, starch lipid levels were increased markedly, amylose level was increased slightly, gelatinization temperatures of the starches increased, and swelling of the starches in hot water decreased. Annealing the starches for 48 hr at 6°C below the onset temperature (T_o) of gelatinization increased the T_o, but the differences in T_o values among the starches remained. Starches from most wheats grown at 40°C had increased proportions of unit chains with degrees of polymerization (DP) of 10-16 and reduced proportions of unit chains with DP 17-21. Starches from wheats grown at 25°C had the highest proportions of unit chains with DP 16-24, which appeared to explain the increased extent of retrogradation.

Cereals grown at various locations and in different crop years can vary significantly in grain yield, kernel size, and kernel composition, including starch composition. Environmental temperature is one of the principal factors causing those variations. In rice, elevated ambient temperature generally reduced amylose (AM) content, reduced the size of AM molecules, increased the chain length (CL) in amylopectin (AP), and increased the gelatinization temperature (Asaoka et al 1984, 1985a,b, 1989; Sano et al 1985). Starches from six nonwaxy rice cultivars grown at 10 sites in Southern Europe and Morocco showed considerable variations in AM content, lipid content, and gelatinization temperature. Those variations were attributed primarily to environmental temperature differences (Morrison and Nasir Azudin 1987).

Jones et al (1985) reported that kernel mass was reduced when corn kernels were grown at unfavorable temperatures during endosperm cell division. They suggested that the reduced mass of mature kernels was due to a decreased number of endosperm cells, starch granules, or both. Using differential scanning calorimetry (DSC), White et al (1991) found that starch from corn grown under tropical conditions gave an elevated and narrow gelatinization temperature range; after retrogradation, it gave an elevated melting temperature of the crystallites.

In barley, MacLeod and Duffus (1988a,b) reported that environmental temperature affects starch content as well as the size and number of starch granules in the developing grain. Recently, Tester et al (1991) found that barley grown at elevated temperature gave reduced starch accumulation, reduced size of starch granules, and a reduced number of B-type granules. AM and AP levels were little affected by temperature during grain-filling. No differences in the fine structure of AP were detected by high-performance liquid chromatography (HPLC) with gel-permeation columns. Starch lipid level and onset gelatinization temperature of the starches increased with increasing temperature. The swelling of starch granules in water decreased in response to elevated growth temperature. That decrease was attributed to high levels of starch lipid with formation of AM-lipid complexes.

In wheat, it is well documented (Kolderup 1975; Sofield et al 1977; Spiertz 1977; Al-Khatib and Paulsen 1984; Nicolas et al 1984; Bhullar and Jenner 1985; Tashiro and Wardlaw 1989, 1990) that temperature during grain-filling affects wheat grain yield, protein content, and the size and number of starch granules. Morrison (1989) reported variation in the starches of wheat cultivars grown at various sites in the United Kingdom, the United States, and Canada. For two British cultivars, he found significant positive correlations between starch phosphorus (P), which is a measure of lysophospholipids (LPL) content, and accumulated temperature, solar radiation, and hours of sunshine over the grain-filling period. AM and P (or LPL) contents also were positively correlated.

Wheat plants grown in many areas of the world are subjected routinely to temperatures substantially higher than the optimal growth temperature of 15-21°C. In the central plains area of

---

[1] Contribution 94-215-J from the Kansas Agricultural Experiment Station.

[2] Graduate research assistant and professor, respectively, Department of Grain Science, Shellenberger Hall, Kansas State University, Manhattan.

[3] USDA, ARS, Western Regional Research Center, Albany, CA.

the United States, wheat is often subjected to temperatures higher than 35° C for several hours a day during a normal growing season. Because protein and starch are the major constituents of the mature wheat kernels, their levels and properties determine, in large measure, the quality of food products derived from wheat. It is important to understand how grain-filling temperature affects protein and starch in mature wheat kernels. Such information would be useful to scientists concerned with the quality of wheat-based foods and to wet-millers producing vital wheat gluten and starch. This investigation focuses on the effects of temperature during grain-filling on starch from wheat. The effects of growth temperature on protein are being investigated by John Bernardin at the USDA Western Regional Research Center, Albany, CA.

MATERIALS AND METHODS

Materials

Three hard red winter (HRW) wheats (Century, Cheyenne, and Karl); one hard red spring (HRS) wheat (Butte 86); one soft red winter (SRW) wheat (Caldwell); and one durum wheat (Monroe) were grown in a greenhouse at 15–21° C until anthesis. The plants were then transferred to growth chambers, wherein light intensity, relative humidity, and temperature were controlled until grain-filling was complete. The same conditions were used in the growth chambers, except for the maximum daylight temperature, which was set at 15, 25, or 40° C for 5 hr daily. Figure 1 illustrates the growth chamber conditions with the maximum daylight temperature set at 40° C. The same wheats also were grown to maturity in the greenhouse.

Moisture, protein, and starch levels in the wheat samples were determined by AACC methods (1983) 44-18, 46-13, and 76-11, respectively. Average kernel weight was determined by dividing the dry weight of kernels by the number of kernels. The maximum length and width of a kernel were measured with dial calipers. The mean values of five kernels from each sample were reported.

Maltose monohydrate (grade HHH) was obtained from Hayashibara Biochemical Laboratories (Okayama, Japan). Malto-oligosaccharides of DP 3–7 (pure grade) were gifts from T. Nakakuki (Nihon Shokuhin Kako Co., Tokyo), and those of DP 8–15 were purchased from Nakano Vinegar Co. (Tokyo). Other chemicals were reagent-grade unless otherwise indicated.

Scanning Electron Microscopy

A wheat kernel was cut transversely with a razor blade to cause the endosperm to fracture. The fractured surface was coated with gold-palladium alloy and viewed with a scanning electron microscope (Etec-autoscan U-I, Perkin-Elmer, Norwalk, CT). For starch isolated from wheat kernels, the sample was sprinkled on double-sided adhesive tape attached to specimen stubs, coated with gold-palladium, and viewed.

Isolation of Starch

Starch was isolated from wheat kernels by the method of Karlsson et al (1983), except that steeped wheat kernels were ground with a pestle by hand to minimize starch damage. Cell-wall fragments and membranes were wet-ground a total of five times to avoid the loss of small granules.

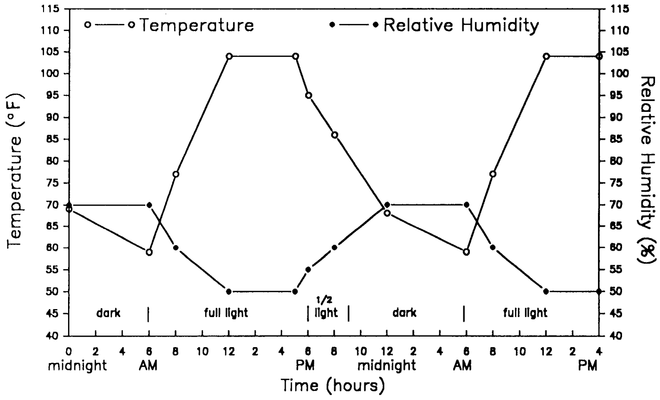

Fig. 1. Growth chamber conditions with the maximum daylight temperature set at 40° C (104° F).

General Properties of Isolated Starch

Granular size distribution of starch was determined on a Coulter Counter with 16 channels (Model PCA I, Coulter Corp., Hialeah, FL). Apparent AM content (AM_a) was determined by the intensity of the iodine-blue color of wheat starch before removal of starch lipids. Total AM content (AM_t) was measured on lipid-free starch (Morrison and Laignelet 1983). Starch lipids were removed by dissolving the starch in a urea-dimethyl sulfoxide mixture followed by precipitation of the starch with ethanol. Phosphorus in starch was quantitated by the method of Morrison (1964). Swelling power and solubility of starches were determined with a 1% starch slurry heated at a rate of ~10° C/min to the desired temperature (Leach et al 1959).

DSC studies were performed in a Perkin-Elmer DSC-2 as described by Shi and Seib (1992). Annealing of starch was done by heating (10 K/min) a mixture of starch and water (1:2, w/w) in a DSC pan from 300 K to a specific temperature, and holding it at that temperature for 0.5–48 hr. The DSC pan then was cooled rapidly (320 K/min) and scanned from 280 K to 400 K.

After a mixture of starch and water (1:1 or 1:2, w/w) was heated in a DSC pan, the starch gel was stored at 4° C for one day, then at room temperature (23±1° C) for periods of one to four weeks. The extent of retrogradation of the starch gel was estimated from the enthalpy of melting of recrystallized starch measured by DSC.

Size-Exclusion Chromatography

Wheat starch (20 mg, db) was stirred in 10 ml of a dimethyl sulfoxide and water mixture (9:1, v/v) at 25° C. The mixture was heated in a boiling water bath for 15 min. The solution was cooled to room temperature, and an aliquot (5 ml, 10 mg of starch) was applied to a column (2.6 × 60 cm) of Sepharose CL-2B (Pharmacia Fine Chemicals, Piscatway, NJ) at room temperature. The components were eluted with 1% sodium hydroxide at a flow rate of 30 ml/hr, and fractions (6 ml) were collected every 12 min. The carbohydrate concentration in each fraction was determined by phenol-sulfuric acid (Dubois et al 1956) using maltose as a standard.

CL Distribution of Debranched Starch

Wheat starch was debranched as described by Yuan et al (1993) with slight modification. Wheat starch (10 mg, db) was dissolved in 2 ml of 90% (v/v) dimethyl sulfoxide (DMSO, spectrophotometric grade, Aldrich, Milwaukee, WI) by heating in a boiling water bath for 20 min and cooling to 25° C. Ethanol (8 ml) was added, and the mixture was shaken vigorously and immersed in an ice bath for 30 min. The precipitated starch was collected by centrifugation at 1,000 × *g* for 10 min, and the supernatant was discarded. The sediment was washed with acetone twice and vacuum-dried in a desiccator for 10 min. To the starch molecules was added 5 ml of sodium acetate buffer (pH 3.8, 50 m*M*), and the mixture was heated in a boiling water bath for 5 min and cooled to room temperature. Isoamylase (15 μl; EC 3.2.1.68; Hayashibara) was added, and the solution was incubated at 35° C for 24 hr. The mixture was boiled for 10 min, cooled, filtered through a syringe filter, and analyzed by high-performance anion-exchange chromatography (Shi and Seib 1992).

Statistical Analysis

All experiments were replicated at least twice. Means and standard errors were obtained using the general linear model (GLM) on the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Kernel Weight and Size

Increasing temperature during grain-filling from 15 to 40° C reduced kernel weight and kernel size as illustrated for samples of HRW Century wheat (Fig. 2). For SRW Caldwell and the three HRW samples, the weight of a seed grown at 40°C was 38–48% less than the weight of a seed produced at 15°C (Table I). The reduction of kernel weight was 60–65% for seeds from HRS Butte 86 and durum Monroe grown at 40°C. The average weight of a kernel produced at 15 and 18°C increased in the order of SRW, HRW, HRS, and durum wheat (Table I). The HRS and durum cultivars should be more tolerant to cool temperatures, when compared to the SRW and HRW cultivars.

Scanning Electron Micrographs of Wheat Kernels

In addition to the reduction of kernel weight and size, physiological stress at elevated temperature was also evident in the appearance of a kernel's surface (Fig. 2). Generally, wheat kernels filled at 40°C showed a wrinkled surface because of shriveling. It is known that cell walls are formed in the endosperm four days after anthesis, and that a period of rapid cell division, with cells filling the embryo sac, occurs six days after anthesis. High temperature soon after fertilization may result in abnormal nuclear cell division, which would explain the production of shrunken kernels (Tashiro and Wardlaw 1990).

Changes in the internal structure induced by increased temperature during grain-filling are illustrated again in the scanning electron micrograph of HRW Century kernels (Fig. 3). When the wheats were grown at high temperature, the pericarp and the adjacent tissues in a kernel appeared to be less tightly associated. Scanning electron micrographs of starchy endosperms are shown in Figures 4–6. In SRW Caldwell (Fig. 4), wheat grown at 25 or 40°C appeared to contain less proteinaceous matrix between the starch granules when compared to wheat filled at 18°C. In addition, the size of starch granules was reduced at high temperature. In HRW Century, wheat grown in the greenhouse (18°C) showed tight adherence of the protein to starch (Fig. 5a). At 25°C, the protein did not seem to coat the starch surface as well. Some starch granules appeared free of protein (Fig. 5b). Moreover, a few deformed starch granules were observed in HRW Century grown at 40°C during grain-filling (Fig. 5c). Figure 6a shows the endosperm of durum Monroe grown in the greenhouse. A number of starch granules were sheared in two when the kernel was split, which is indicative of a strong bond between protein and starch (Hoseney and Seib 1973). Sheared starch granules also were noticed for durum Monroe grown at 25°C (Fig. 6b), but a few intact starch granules were exposed. At 40°C, a large number of deformed starch granules occurred

TABLE I
Temperature During Grain-Filling and Kernel Weight (KW) and Kernel Size of Six Wheat Cultivars

Class[a]	Cultivar	°C of Growth	KW mg	Length mm	Width mm
SRW	Caldwell	15	30.2	5.69	3.24
		18	29.1	5.62	3.20
		25	19.7	4.80	2.61
		40	18.7	5.84	2.22
HRW	Century	15	...	...	...
		18	34.3	6.45	3.16
		25	33.2	6.05	3.04
		40	23.5	5.89	2.34
	Cheyenne	15	39.9	5.88	3.31
		18	37.9	6.22	3.29
		25	23.9	5.44	2.68
		40	21.1	5.45	2.60
	Karl	15	29.8	6.23	2.63
		18	33.6	6.47	3.10
		25	24.5	5.45	2.64
		40	23.0	6.16	2.43
HRS	Butte 86	15	38.7	6.15	3.39
		18	44.2	6.69	3.45
		25	...	...	...
		40	16.3	6.15	2.41
Durum	Monroe	15	43.0	7.89	3.20
		18	50.3	8.65	3.50
		25	31.3	7.35	2.85
		40	15.5	7.52	2.38

[a] SRW = soft red spring; HRW = hard red winter; HRS = hard red spring.

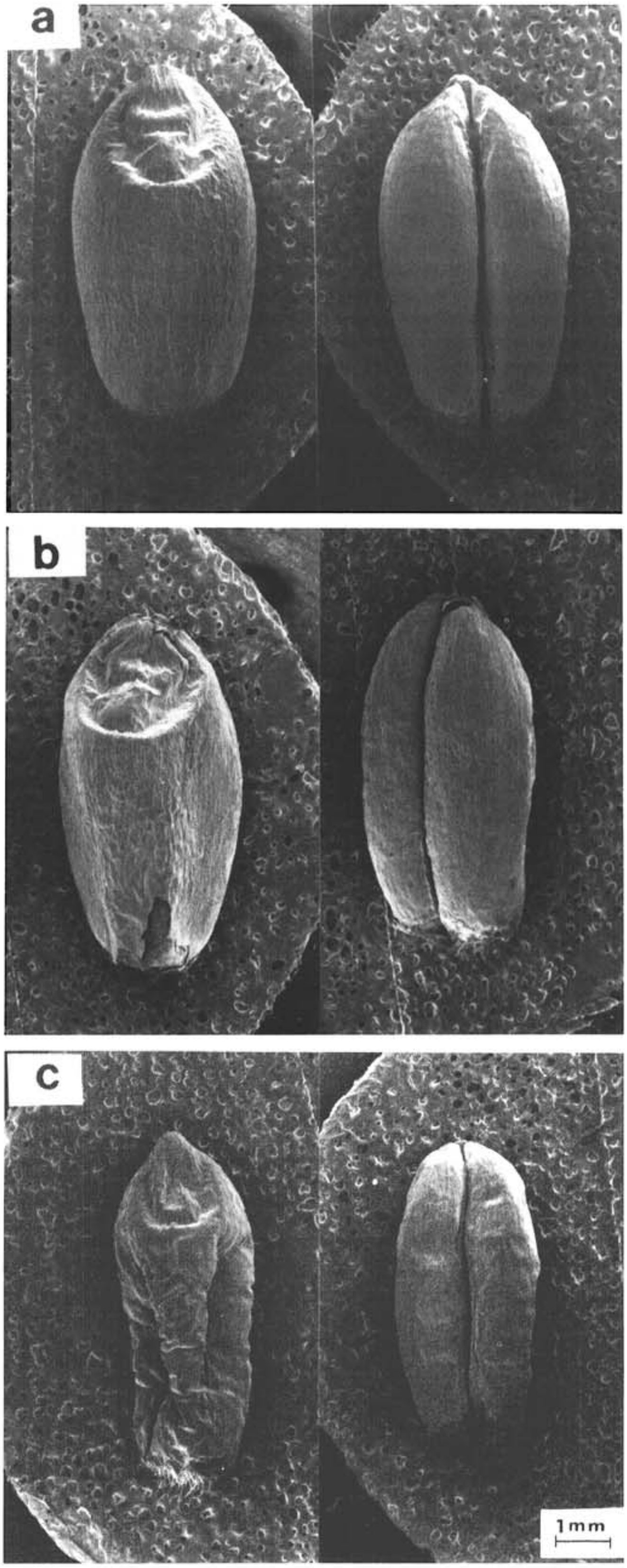

Fig. 2. Scanning electron micrographs of the dorsal and crease sides of kernels from the hard red winter wheat cultivar Century grown at 18°C (**a**), 25°C (**b**), and 40°C (**c**) during the grain-filling period.

during grain-filling in the endosperm of Monroe wheat (Fig. 6c).

Preliminary results (B. W. Seabourn and O. K. Chung, *personal communication*) from a 1993 investigation on wheat hardness at the USDA Grain Marketing Research Laboratory, Manhattan, KS, indicated that hardness varied inversely with increasing growing temperature. If wheat hardness depends upon the strength of the starch-protein interaction (Barlow et al 1973, Hoseney and Seib 1973), elevated temperature during grain-filling may somehow weaken the starch-protein interaction, and thereby decrease kernel hardness.

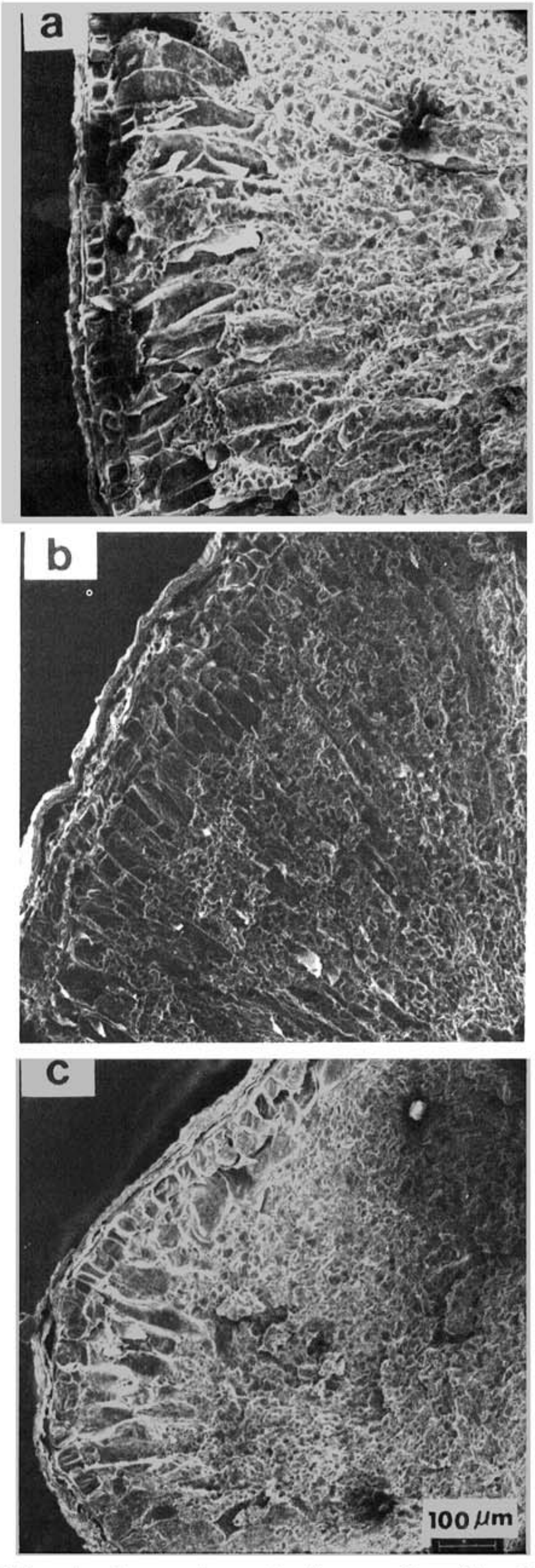

Fig. 3. Scanning electron micrographs of cross sections of kernels from the hard red winter wheat cultivar Century grown at 18°C (**a**), 25°C (**b**), and 40°C (**c**) during the grain-filling period.

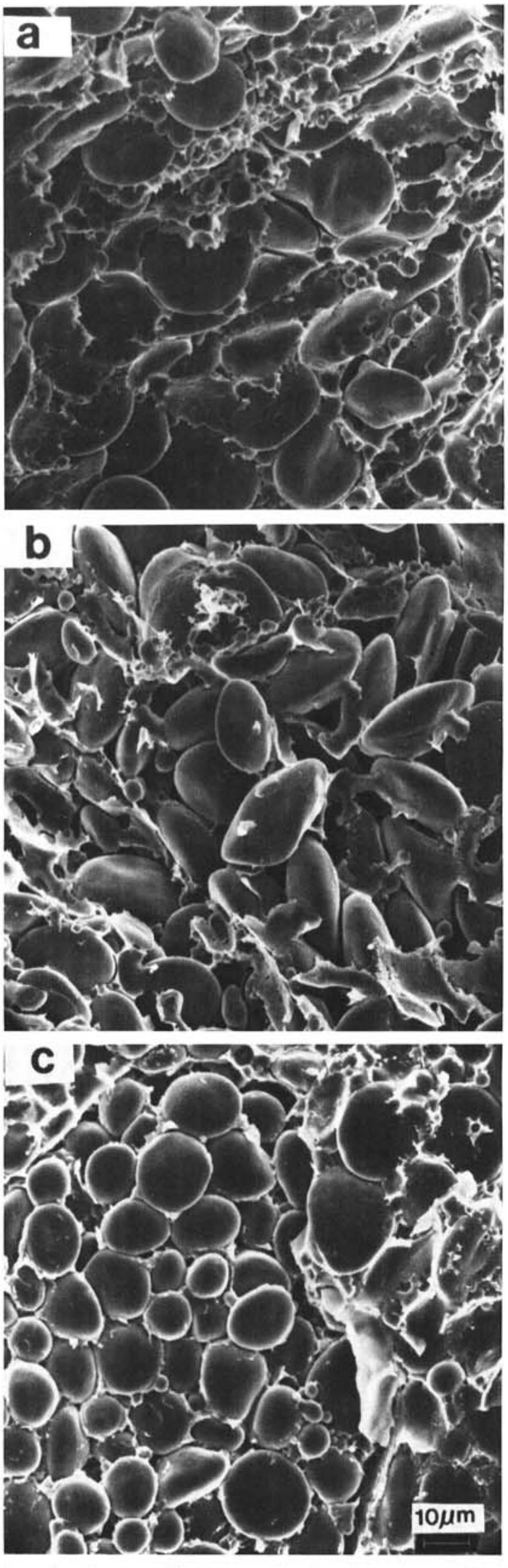

Fig. 4. Scanning electron micrographs showing the contents of endosperm cells of kernels from the soft red winter wheat cultivar Caldwell grown at 18°C (**a**), 25°C (**b**), and 40°C (**c**) during the grain-filling period.

Starch and Protein Levels of Wheat

For wheats grown at 15°C, starch levels ranged from 61 to 72% based on kernel weight, whereas at 40°C, the range was 38–50% (Table II). Increasing temperature during grain-filling from 15 to 40°C reduced the starch level in wheat kernels by 12–25%.

Consistent with the reduction of kernel weight and size (Table I), the quantity of starch per kernel also decreased in response to elevated temperature during grain-filling (Table II). For wheat plants grown at 40°C, the reduction of starch per kernel ranged

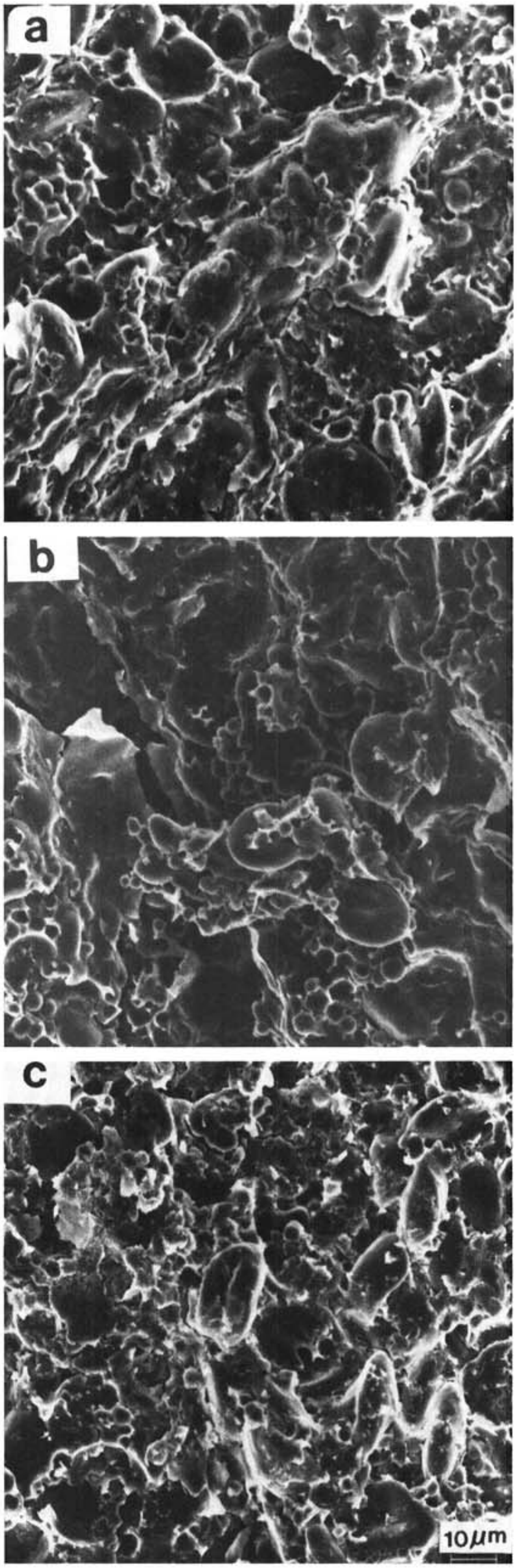

Fig. 5. Scanning electron micrographs showing the contents of endosperm cells of kernels from the hard red winter wheat cultivar Century grown at 18°C (**a**), 25°C (**b**), and 40°C (**c**) during the grain-filling period.

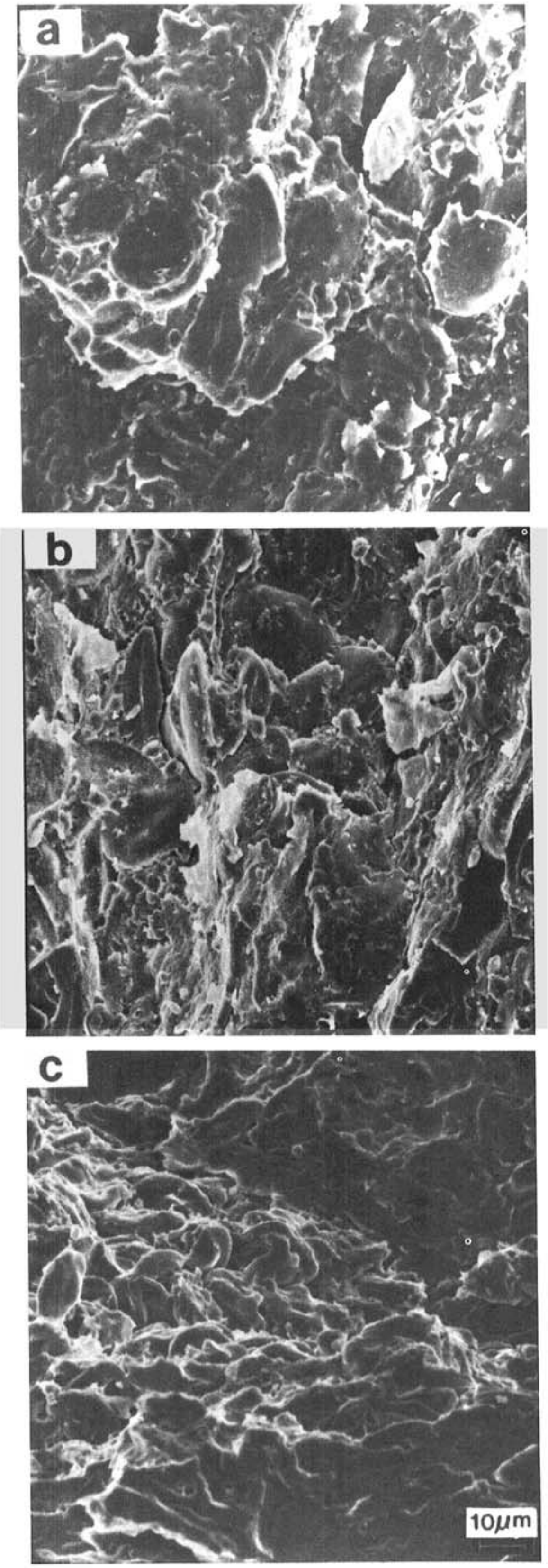

Fig. 6. Scanning electron micrographs showing the contents of endosperm cells of kernels from the durum wheat cultivar Monroe grown at 18°C (**a**), 25°C (**b**), and 40°C (**c**) during the grain-filling period.

from ~50% in HRW Century to as high as ~75% in HRS Butte 86 and durum Monroe.

Isolation of Starch

Although the isolation procedure was time-consuming and tedious, 92–95% of the starch in wheat kernels was isolated. Protein was effectively removed by shaking aqueous starch slurries with toluene (Adkins and Greenwood 1966). Starch lipid, which is the lipid inside starch granules, was not affected by the washing procedure (Cauvain et al 1977).

Figure 7 shows the scanning electron micrographs of starches isolated from SRW Caldwell, HRW Century, and durum Monroe wheats grown at 18 and 40° C. As observed in the scanning electron micrographs of the endosperm sections (Figs. 4–6), distorted starch granules occurred in the wheats grown at 40° C, and the size of the granules was reduced.

Size Distribution of Starch Granules

Starch in the wheat grain is deposited in two distinct types of granules: large, lenticular A-type granules; and small, spherical B-type granules. The A-type granules are initiated in an amyloplast about four to five days after anthesis (Parker 1985, Bechtel et al 1990). The final number of A-type amyloplasts is achieved about seven days later when cell division ceases (Briarty et al 1979). The size of A-type granules increases throughout kernel growth to a final diameter ranging from ~10 μm to 50 μm (Evers 1971, Simmonds and O'Brien 1981, Dengate and Meredith 1984, Bechtel et al 1990). Final granule size depends upon the cultivar (Dengate and Meredith 1984), season (Baruch et al 1979), and environmental temperature (the present study).

The diameter of B-type starch granules ranges from 1 to 10 μm (Buttrose 1963, Evers 1971). The small granules are initiated about 10–14 days after anthesis and grow during the phase of endosperm cell enlargement (Parker 1985, Bechtel et al 1990). The number of B-type granules per endosperm increases throughout most stages of grain development, whereas the number of A-type granules per endosperm remains constant while granule size increases (Morrison and Gadan 1987). In addition to A- and B-type starch granules, Bechtel et al (1990) defined small C-type granules (<5.3 μm), initiated at 21 days after flowering, that constituted 3–4% of the total weight of starch at maturity.

TABLE II
Temperature During Grain-Filling and Levels of Starch and Protein in Wheat Kernels from Six Cultivars

Class[a] and Cultivar	°C of Growth	Starch (%)	Starch (mg/kernel)	Protein (%)	Protein (mg/kernel)
SRW					
Caldwell	15	72.2	21.7	10.1	3.1
	18	72.2	21.0	10.7	3.1
	25	55.5	10.9	9.5	1.9
	40	49.9	9.3	16.2	3.0
HRW					
Century	15	...	...	...	...
	18	62.0	21.3	10.6	3.6
	25	58.7	19.5	14.8	4.9
	40	42.4	10.0	16.1	3.8
Cheyenne	15	61.9	24.7	11.4	4.5
	18	55.4	21.0	17.0	6.4
	25	45.9	11.0	9.2	2.2
	40	41.2	8.7	18.2	3.8
Karl	15	63.2	18.8	13.4	4.0
	18	62.6	21.0	17.1	5.7
	25	56.1	13.7	12.2	3.0
	40	37.9	8.7	22.6	5.2
HRS					
Butte 86	15	61.4	23.8	10.2	4.3
	18	58.1	25.7	16.3	7.2
	25	...	...	...	...
	40	39.4	6.4	18.9	3.1
Durum					
Monroe	15	70.0	10.1	10.1	4.3
	18	61.6	31.0	13.0	6.5
	25	51.2	16.0	15.4	4.8
	40	45.1	7.0	21.3	3.3

[a] SRW = soft red winter; HRW = hard red winter; HRS = hard red spring.

Granular size distributions of the starches from the six wheat cultivars in the present investigation are shown in Figure 8. In agreement with the scanning electron micrographs of starchy endosperms (Figs. 4–6) and isolated starches (Fig. 7), the general trend was a reduction in starch granule size with elevated temperature during grain-filling. The proportion of large A-type granules was reduced with an increase in temperature during grain-filling (Fig. 8). Increasing temperature from 15 to 40° C decreased the volume percentage of A-type granules (>16 μm) from 44 to 26% for SRW Caldwell, 37 to 22% for HRW Cheyenne, 35 to 15% for HRS Butte 86, and 25 to 2% for durum Monroe (Fig. 9). In addition, the proportion of granules <10 μm also decreased with increasing temperature during grain-filling (Fig. 8).

The effect of environmental temperature on the size of wheat starch granules also was reported by Batey et al (1989). Distinct differences were found in the proportion of B-type starch granules isolated from five wheat cultivars grown at four sites in Australia. The weight percentage of B-type granules was much smaller from the same cultivars grown at one location than it was at other sites. A hot spell occurred in that location in the last weeks before harvest that resulted in a rapid finish to grain-filling. The number of B-type granules, which normally increases rapidly towards the end of grain-filling, were not synthesized during that time period.

Tester et al (1991) previously showed that barley grown at elevated temperatures also gave starch with reduced sizes of A- and B-type granules and a decreased number of B-type granules.

AM and Phosphorus Contents of Starch

The effects of temperature during grain-filling on starch components are shown in Table III. Except for starches from HRS wheat grown at 40° C and durum wheat grown at 15° C, AM_a content was not affected by temperature during grain-filling. Most starches had AM_a contents of 22–23%. AM_t contents of the 21 starch samples ranged from 27 to 31%, except for the starch from Butte 86 grown at 40° C, which contained 33.8%. Starches from wheats grown at 15° C had 3–6% less AM_t content than did starches from wheat grown at 40° C. Except for Butte 86 grown at 40° C, AM_t of starches from the wheats grown at the same temperature varied within 2% among the 21 samples.

The differences between AM_t and AM_a contents is ΔAM (Table III). Morrison (1993) distinguished AM in nonwaxy cereal starches in two forms: lipid-free amylose (F·AM) and lipid-complex amylose (L·AM). He further suggested that apparent AM is equal to F·AM, so that $\Delta\text{AM} = \text{AM}_\text{t} - \text{AM}_\text{a} = \text{L·AM}$. Morrison et al (1993) calculated that seven parts of AM bind one part of LPL, which is identical to the saturating value determined by DSC (Kugimiya and Donovan 1981). Thus, L·AM also can be calculated more accurately from $7 \times \text{LPL}$ ($7 \times 16.5 \times \%\text{P}$ in wheat starch) and $\text{F·AM} = \text{AM}_\text{t} - \text{L·AM} = \text{AM}_\text{t} - 7 \times 16.5 \times \%\text{P}$.

In this investigation, starch lipid and ΔAM (L·AM) increased with increasing temperature during grain-filling (Table III). This increase of lipid in the form of lipid-AM complex had a considerable effect on the swelling of wheat starch in hot water.

Differences in AM content between A- and B-type granules have been reported by Duffus and Murdoch (1979); however, Bathgate and Palmer (1972) and Evers et al (1974) have reported none. Using an improved colorimetric procedure to determine AM content (Morrison and Laignelet 1983), Morrison and his coworkers reported that A-type granules have a greater AM content than do B-type granules (Morrison and Laignelet 1983, Morrison and Gadan 1987, Morrison 1989), although the differences are generally within 4%. Moreover, those authors proposed an asymmetric composition model for an A-type granule in which there is a low-AM, low-LPL zone in the central region and high-AM, high-LPL zone in the peripheral region. The changes in AM_t in wheat starch with changing temperature observed in this study during grain-filling (Table III) might be explained by a reduced proportion of B-type starch granules (Fig. 8).

The changes of wheat starch compositions with temperature during grain-filling are different from those found for barley (Tester et al 1991) and rice (Asaoka et al 1984, 1985a,b, 1989). In normal barley, AM and AP contents were little affected by increasing temperature during grain-filling, but the lipid contents of starches from four barley cultivars increased markedly (Tester et al 1991). In rice, AM content decreased with increasing growing temperature (Asaoka et al 1984, 1985a,b, 1989). In wheat, we found that the AM contents of the starches decreased slightly

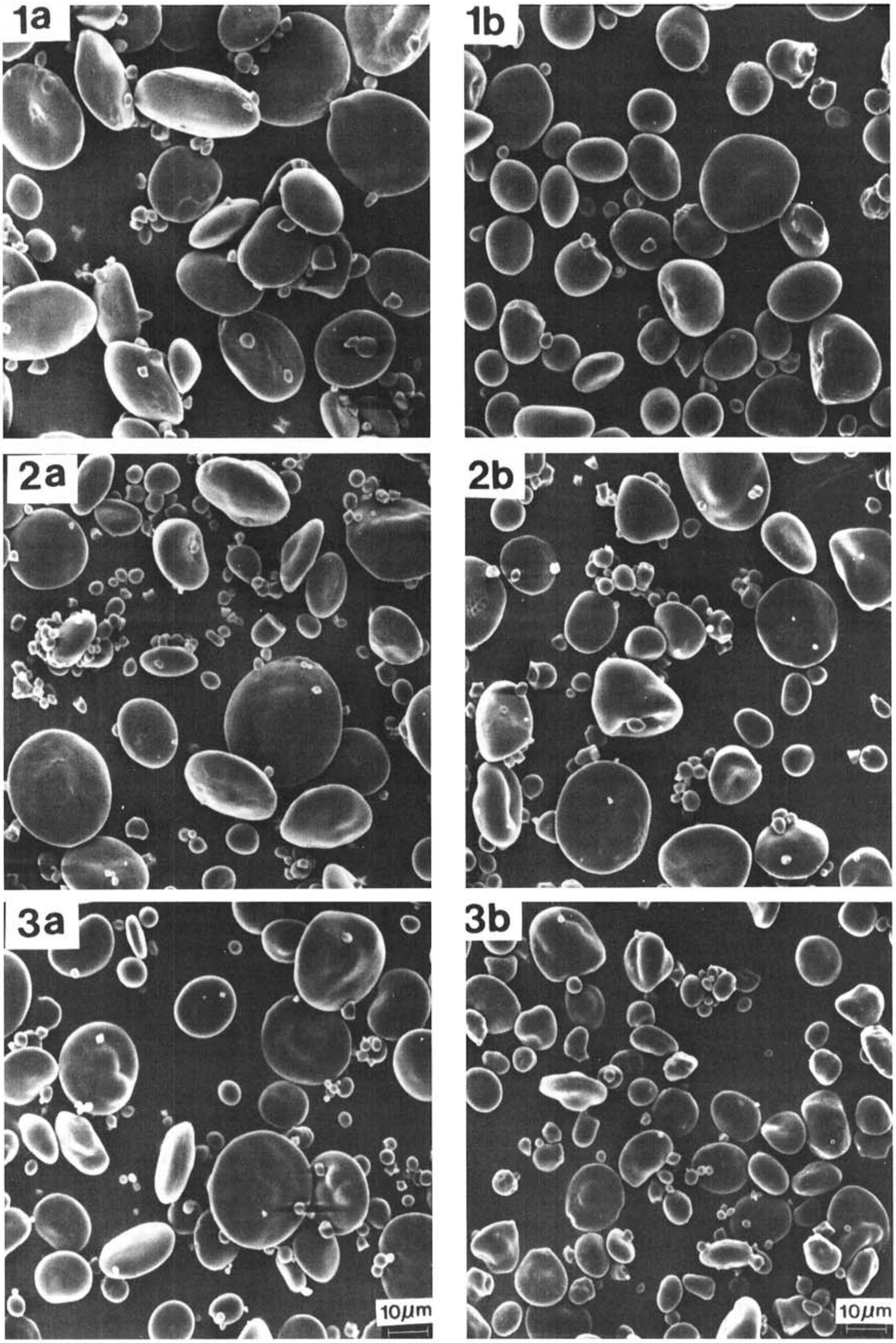

Fig. 7. Scanning electron micrographs of starches isolated from the soft red winter wheat cultivar Caldwell (**1**), the hard red winter wheat cultivar Century (**2**), and the durum wheat cultivar Monroe (**3**) grown at 18°C (**a**) and 40°C (**b**) during the grain-filling period.

when wheat heads were filled at 15°C, compared to wheat heads filled at 40°C. Furthermore, the lipid level of wheat starch increased significantly in response to elevated temperature; the same was observed in barley.

To test whether the molecular size of starch was affected by temperature during grain-filling, starches from HRW Century grown at 18 and 40°C were separated by size-exclusion chromatography. The differences between the two starches, if any, were not detected by size-exclusion chromatography (data not shown). Nor were the ratios of AM and AP in the HRW Century starches different when determined from area ratios of size-exclusion chromatography, probably because the change of AM content was only ~1% in this cultivar (Table III).

CL Distribution of Debranched Starch

Unit chains of the debranched starches were separated by high-performance anion-exchange chromatography with pulsed-amperometric detection. Because molar responses for malto-oligosaccharides above DP 17 are not known (Koizumi et al 1989, 1991; Shi and Seib 1992), we injected the same level of

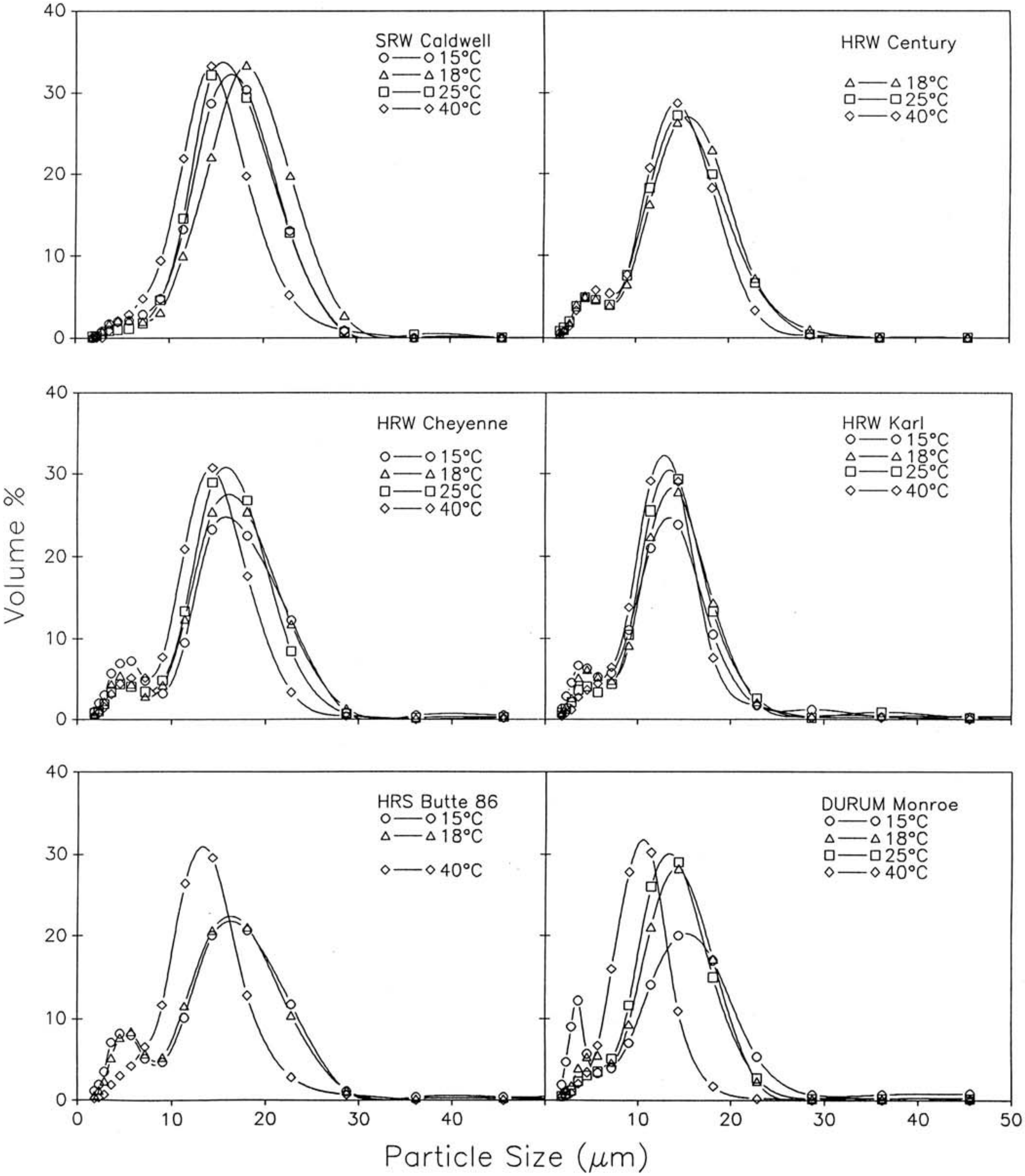

Fig. 8. Size distributions of starch granules from the six wheats grown at 15, 18, 25, and 40°C during the grain-filling period.

carbohydrate for each debranched starch and compared peak areas between the samples.

The CL distribution of wheat AP was altered in response to elevated temperature during grain-filling. The proportion of unit chains with DP 16–24 increased for starches from the wheats grown at 25°C when compared to starches from wheats grown at 15°C (Fig. 10). All hard wheats grown at 40°C showed an increase in the proportion of unit chains between DP 10–15, although the magnitude of increase was different among the starch samples (Fig. 11). Changes in the fine structure of wheat AP caused by grain-filling temperature altered the physical properties of the starches.

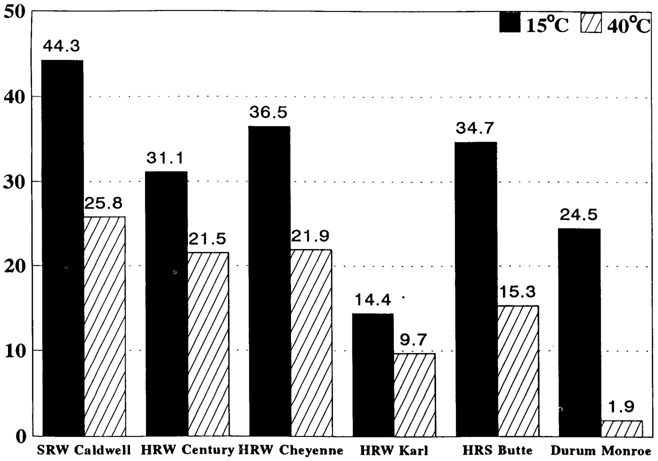

Fig. 9. Volume (%) of granules >16 μm in starches isolated from six wheat cultivars grown at 15 and 40°C during the grain-filling period. SRW = soft red winter; HRW = hard red winter; HRS = hard red spring.

Gelatinization Properties

Gelatinization properties of the wheat starches in 50% water (wb) are given in Table IV. The endothermic peak caused by the melting of the AM-lipid complex increased with increasing temperature during grain-filling. This confirmed that LPL, and to some extent AM, increased in response to elevated grain-filling temperature (Table III).

The T_0 for starches from the wheats grown at 15°C were 50–52°C (Table IV). For wheats grown at different temperatures, T_0 of starches varied from 50.4 to 63.3°C. Generally, T_0 increased with increasing grain-filling temperature. The increase in T_0 was ~12°C for the starches from HRW Cheyenne grown at 40°C versus 15°C, whereas the increase was 6–8°C for the other starches.

The differences in T_0 values between starches could be due to different degrees of crystallite perfection or to structural differ-

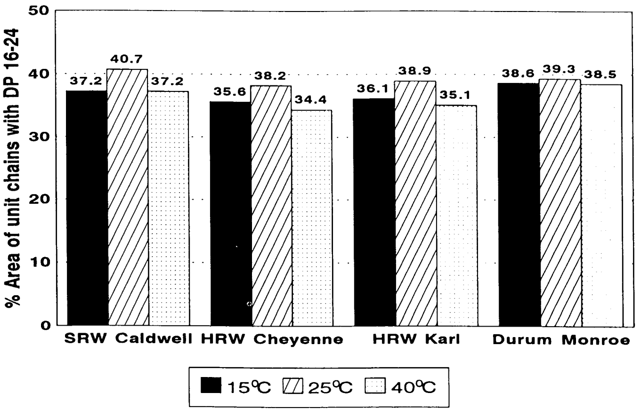

Fig. 10. Area (%) of groups of unit chains with DP 16–24 in wheat starches from four wheat cultivars grown at 15, 25, and 40°C during the grain-filling period. SRW = soft red winter; HRW = hard red winter.

TABLE III

Temperature During Grain-Filling and Levels of Amylose[a] and Lipid[b] Levels in Starches from Six Wheat Cultivars[c]

Class[c] and Cultivar	°C of Growth	AM_a (%)	AM_t (%)	ΔAM (%)	Phosphorus (mg/100 g of sample)	LPL (mg/100 g of sample)
SRW						
Caldwell	15	22.1 e–h[d]	27.2 k–m	5.1	47.2 m	779
	18	23.8 ab	28.1 h–k	5.3	47.9 m	791
	25	23.6 bc	27.6 j–m	6.0	50.0 l	825
	40	23.5 b–d	30.8 bc	7.3	57.9 h	955
HRW						
Century	15	...	...	...	...	...
	18	21.8 f–h	27.7 j–m	5.9	55.5 i	915
	25	22.4 d–g	28.2 g–j	5.8	60.6 g	1,000
	40	21.8 f–h	28.1 h–k	6.3	71.5 a	1,180
Cheyenne	15	22.5 c–g	28.0 i–l	5.5	52.1 k	859
	18	23.1 b–e	28.7 gh	5.6	57.9 h	955
	25	22.5 c–g	28.4 ghi	5.9	61.6 fg	1,017
	40	22.2 e–h	31.3 b	9.1	69.2 b	1,142
Karl	15	21.8 f–h	27.5 klm	5.7	51.7 k	853
	18	22.2 e–h	28.8 fg	6.6	54.1 j	892
	25	22.5 c–g	29.4 ef	6.6	57.9 h	955
	40	22.1 ef–h	30.4 cd	8.3	70.8 a	1,169
HRS						
Butte 86	15	21.5 g–i	27.9 i–l	6.4	54.8 ij	904
	18	22.2 e–h	29.9 de	7.7	64.7 ge	1,068
	25	...	...	...	...	...
	40	24.9 a	33.8 a	8.9	66.1 c	1,090
Durum						
Monroe	15	20.5 e	27.4 lm	6.9	54.1 j	892
	18	22.5 c–g	29.8 ef	7.3	62.2 f	1,027
	25	22.7 b–f	30.4 cd	7.7	64.3 e	1,061
	40	22.2 e–h	30.1 de	7.9	65.7 cd	1,084

[a] AM_t = total amylose; AM_a = apparent amylose; $\Delta AM = AM_t - AM_a$.

[b] LPL = lysophospholipid calculated as starch Phosphorus × 16.5.

[c] SRW = soft red winter; HRW = hard red winter; HRS = hard red spring.

[d] Values with the same letter within a column are not significantly different ($P < 0.05$).

ences in the starch granules. The question arose whether the difference in T_0 could be eliminated if starch crystallites were perfected in vitro. To answer this question, we annealed each starch in 65% water at 6°C below its T_0. Figure 12 shows the increase of T_0 upon annealing the starches from HRW Century grown at 18 and 40°C. The T_0 of the starches increased rapidly during the first 3 hr and then leveled off after 12 hr. Although T_0 increased with annealing time, the differences in T_0 between the two samples remained after annealing for 48 hr. Gelatinization enthalpy (ΔH) was little affected when the two starches were annealed at 6°C below the T_0 of the native starches.

The results of annealing the starches from SRW Caldwell are listed in Table V. Compared to the starch from wheat grown at 18°C, T_0 of the starch from Caldwell grown at 40°C was 4°C higher. After annealing for 48 hr at 6°C below the T_0 of the native starches, the new T_0 of the two starches increased almost equally. However, annealing the starch from the wheat grown at 18°C caused a significant reduction in ΔH, whereas much less change was noted in ΔH of the starch from Caldwell grown at 40°C. The melting of starch crystallites (gelatinization) is thought to be affected by the surrounding amorphous regions. Perhaps the hydration and swelling of the amorphous phase were higher in Caldwell grown at 18°C than in the same cultivar grown at 40°C. The added hydration and swelling during annealing would cause more melting of imperfect crystallites and thereby reduce ΔH.

In another study on commercial wheat starch (Y.-C. Shi and P. A. Seib, *unpublished data*), we found that annealing wheat starch at a temperature too close to T_0 decreased ΔH. For example, one wheat starch sample in 65% water had a T_0 of 58°C and a ΔH of 2.5 cal/g. Annealing that starch at 56°C for 30 min

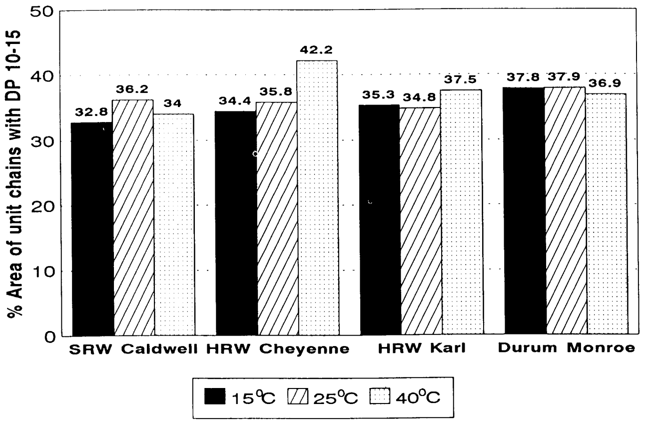

Fig. 11. Area (%) of groups of unit chains with DP 10–15 in wheat starches from four wheat cultivars grown at 15, 25, and 40°C during the grain-filling period. SRW = soft red winter; HRW = hard red winter.

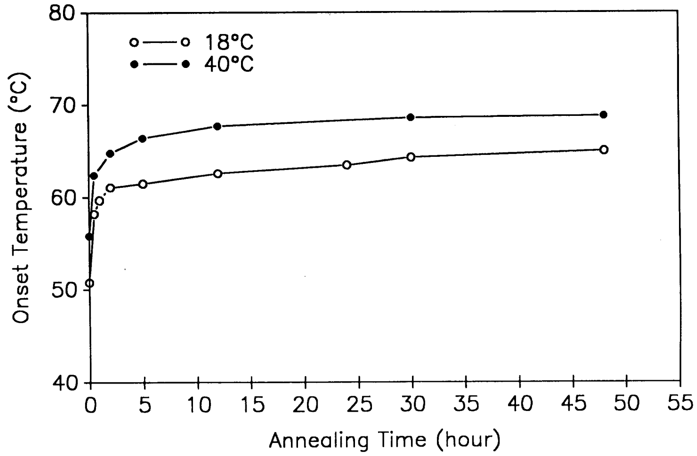

Fig. 12. Increase of melting temperature by annealing the starches from the hard red winter wheat cultivar Century grown at 18 and 40°C during the grain-filling period.

TABLE IV
Temperature During Grain-Filling and Gelatinization of Starches from Six Wheat Cultivars in 50% Water

Class[a] and Cultivar	°C of of Growth	Transition Temperature, °C			Enthalpy, cal/g	
		Onset	Peak	Conclusion	ΔH	$\Delta H_{\text{AM-Lipid}}$
SRW						
Caldwell	15	50.4 m[b]	54.3	84.9	2.60 f–i	0.20 k
	18	53.6 j	56.2	87.1	2.50 ij	0.24 j
	25	57.1 cd	61.2	87.6	2.71 b–e	0.29 i
	40	58.2 b	63.5	92.6	2.64 de–h	0.42 ef
HRW						
Century	15	. . .	. . .	. . .	. . .	. . .
	18	51.4 kl	86.7	86.7	2.43 jk	0.34 gh
	25	54.8 hi	86.2	86.2	2.58 g–i	0.46 d
	40	55.9 fg	91.8	91.8	2.35 k	0.63 a
Cheyenne	15	51.6 k	86.0	86.0	2.66 c–g	0.21 jk
	18	55.1 gh	87.1	87.1	2.55 hi	0.30 i
	25	56.2 ef	87.0	87.0	2.75 bc	0.47 cd
	40	63.3 a	97.0	97.0	2.56 hi	0.65 a
Karl	15	51.9 k	86.2	86.2	2.80 ab	0.22 jk
	18	54.2 ij	85.4	85.4	2.66 c–g	0.31 hi
	25	57.2 cd	87.8	87.8	2.73 b–e	0.35 g
	40	57.9 bc	93.4	93.4	2.43 jk	0.47 cd
HRS						
Butte 86	15	50.6 lm	82.9	82.9	2.63 e–h	0.32 g–i
	18	51.4 kl	86.7	86.7	2.70 b–f	0.42 ef
	25	. . .	. . .	. . .	. . .	. . .
	40	56.8 de	90.2	90.2	2.35 k	0.50 c
Durum						
Monroe	15	52.0 k	87.0	87.0	2.88 a	0.35 g
	18	54.0 ij	86.0	86.0	2.74 b–d	0.40 f
	25	56.0 ef	86.9	86.9	2.87 a	0.44 de
	40	58.2 b	90.9	90.9	2.66 c–g	0.59 b

[a] SRW = soft red winter; HRW = hard red winter; HRS = hard red spring.
[b] Values with the same letter within a column are not significantly different ($P < 0.05$).

TABLE V
Gelatinizatin of Starches from the Soft Red Winter Cultivar Caldwell Before and After Annealing in 65% Water

°C of Growth	Annealing Conditions[a] °C	ΔT, °C	Time, hr	Transition, °C Before	After[b]	Enthalpy, cal/g Before	After[b]
18	47	6	48	53.1	66.2 (+13.1)	2.45	1.70 (−0.75)
40	51	6	48	57.3	71.3 (+14.0)	2.49	2.26 (−0.23)

[a] Onset temperature minus annealing temperature.

[b] Data in parentheses are differences between transition temperature or enthalpy values before and after annealing.

decreased ΔH to 1.6 cal/g, while increasing T_0 to 66°C. The partial melting of crystallites while a starch was held at a temperature 2-6°C below its T_0 may explain the deformation of granules in wheat filled at 40°C. A large number of deformed starch granules was observed in durum Monroe grown at 40°C (Fig. 6c). Some low-perfection crystallites might have been melted at 40°C during grain development.

In studying four waxy cereal starches, we found differences in the T_0 values between the starches (Shi and Seib 1992). Annealing increased T_0 for all four starches without affecting ΔH, but the differences in T_0 values between the starches remained after annealing for 48 hr. The cold-acid resistant crystallites from the four waxy starches had the same T_0. It was concluded that the waxy starches contained the similar structure of crystallites and that the amorphous phase controlled T_0.

In barley, Tester et al (1991) also found that the gelatinization temperature of starches increased with an increase in temperature during grain-filling. Annealing all the starches at the same temperature (55°C) eliminated the differences in gelatinization temperatures between starches. However, annealing at 55°C caused partial or total gelatinization of starches with low T_0.

Present investigation indicates that the starches from wheats grown at different temperatures contained crystallites with a distribution of perfection, because all T_0 values increased upon annealing. However, the differences in T_0 values, which persisted after annealing (Fig. 12 and Table V), indicated a fundamental difference in granular structure.

Retrogradation Properties

After gelatinization, the starch pastes (50% H_2O) in DSC pans were retrograded by storing at 4°C for one day and by storing at room temperature for periods up to four weeks. Figure 13 shows that the onset melting temperatures (T_0) of retrograded starches from HRW Century grown at 18, 25, and 40°C were 40.6, 41.2, and 43.9°C, respectively, after retrogradation at 4°C for one day. Another day of retrogradation at room temperature increased the T_0 for all starches to ~46°C. The T_0 then slowly increased to ~50°C over the next four weeks.

Most recrystallization of starch appeared to occur within the first week of storage, because enthalpy values increased only slightly after the first week of storage at 23°C (Fig. 14). Starch from HRW Century grown at 25°C retrograded to a greater extent than did starches from the same cultivar grown at 18 and 40°C.

Interestingly, recrystallization of the AM-lipid complex in all samples was apparently completed within one day at 4°C, because its enthalpy value remained the same upon further storage at 23°C (Fig. 15). The level of AM-lipid complex increased in starches with increasing temperature during grain-filling, in agreement with the LPL level (Table III) and the ΔH values

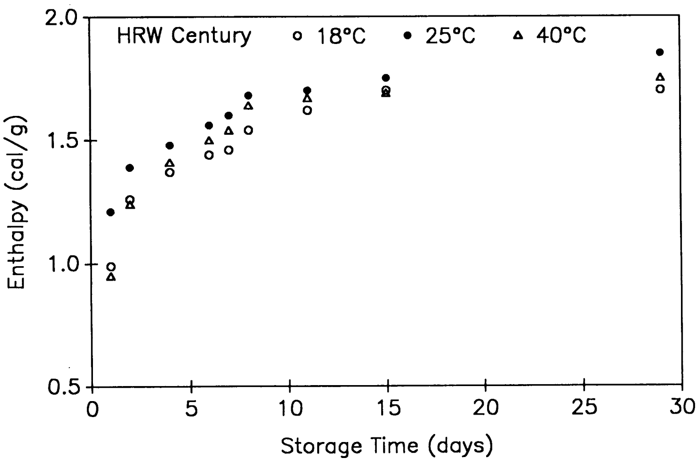

Fig. 14. Enthalpy values of melting amylopectin in retrograded gels of starches from the hard red winter (HRW) wheat cultivar Century grown at 18, 25, and 40°C during the grain-filling period. The gels (50% water) were stored at 4°C for one day and then stored at 23°C for four weeks. Standard deviation was ±0.06 cal/g.

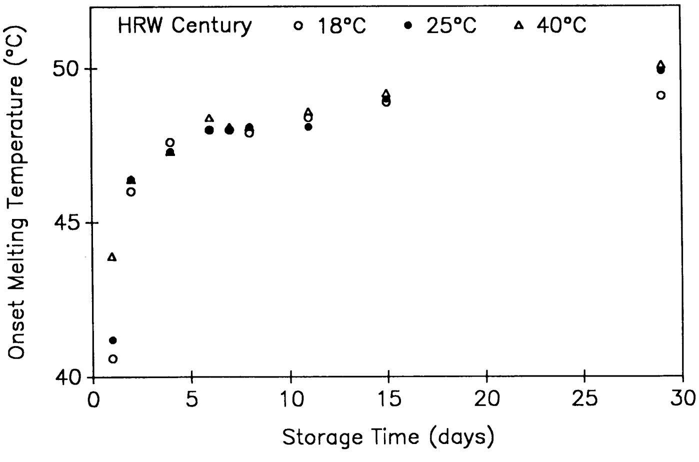

Fig. 13. Onset temperatures of melting amylopectin in retrograded gels of starches from the hard red winter (HRW) wheat cultivar Century grown at 18, 25, and 40°C during the grain-filling period. The gels (50% water) were stored at 4°C for one day and then stored at 23°C for four weeks. Standard deviation was ±0.4°C.

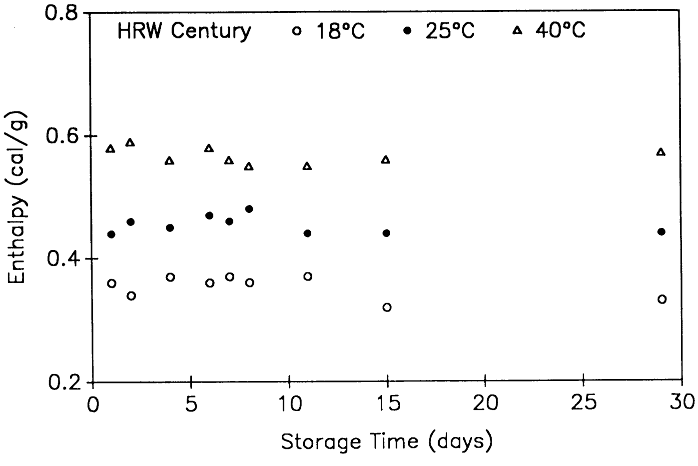

Fig. 15. Enthalpy values of melting amylopectin-lipid complexes in retrograded gels of starches from the hard red winter (HRW) wheat cultivar Century grown at 18, 25, and 40°C during the grain-filling period. The gels (50% water) were stored at 4°C for one day and then stored at 23°C for four weeks. Standard deviation was ±0.08 cal/g.

for melting of the AM-lipid complex during gelatinization (Table IV).

Table VI shows the retrogradation properties of the starches from all wheat samples after four weeks of storage at room temperature. T_0 values were nearly equal for all retrograded starches. Within a cultivar series, starch from wheat grown at 25°C gave the highest ΔH value, which indicated the greatest extent of retrogradation. Starches from HRW Cheyenne, Karl, and HRS Butte 86 grown at 40°C retrograded to the least extent.

The differences in retrogradation between the wheat starch

TABLE VI
Temperature During Grain-Filling and Retrogradation Properties of Starches from Six Wheat Cultivars

Class[a] and Cultivar	°C of of Growth	Transition Temperature, °C			Enthalpy, cal/g	
		Onset	Peak	Conclusion	ΔH	$\Delta H_{\text{AM-Lipid}}$
SRW						
Caldwell	15	49.2 bc[b]	58.0	65.2	1.85 de	0.22 j
	18	49.5 a–c	57.1	64.8	1.77 ef	0.22 j
	25	49.9 ab	58.0	66.2	2.08 a	0.27 hi
	40	49.6 a–c	57.9	66.9	1.77 ef	0.37 e
HRW						
Century	15	...	...	...	...	...
	18	49.1 bc	58.0	65.4	1.70 fg	0.33 fg
	25	49.9 ab	58.9	66.0	1.85 de	0.44 bc
	40	50.1 a	58.2	66.0	1.75 ef	0.47 ab
Cheyenne	15	49.2 bc	58.0	65.7	1.86 c–e	0.25 ij
	18	49.5 a–c	58.6	66.1	1.86 c–e	0.32 g
	25	49.5 a–c	58.4	66.6	1.98 bc	0.30 gh
	40	49.6 a–c	58.9	67.0	1.62 gh	0.51 a
Karl	15	49.4 a–c	57.6	65.0	1.98 bc	0.25 ij
	18	49.4 a–c	57.1	65.0	1.86 c–e	0.27 hi
	25	48.9 c	57.9	66.5	2.14 a	0.32 g
	40	49.4 a–c	57.4	65.0	1.62 gh	0.41 cd
HRS						
Butte 86	15	49.0 c	57.0	64.3	1.92 cd	0.25 ij
	18	49.1 bc	57.0	65.5	1.91 cd	0.36 ef
	25	...	...	...	...	...
	40	49.6 a–c	58.0	65.0	1.57 h	0.38 de
Durum						
Monroe	15	49.1 bc	57.2	64.0	1.78 ef	0.28 hi
	18	49.3 a–c	57.0	64.6	1.77 ef	0.28 hi
	25	49.4 a–c	57.8	66.0	1.86 c–e	0.33 fg
	40	49.5 a–c	58.0	66.8	1.80 d–f	0.42 c

[a] SRW = soft red winter, HRW = hard red winter, HRS = hard red spring.

[b] Values with the same letter within a column are not significantly different ($P < 0.05$).

TABLE VII
Temperature During Grain-Filling and Solubility and Swelling Power of Starches from Six Wheat Cultivars

Class[a] and Cultivar	°C of of Growth	Solubility (%) at				Swelling Power (g/g) at			
		65°C	75°C	85°C	95°C	65°C	75°C	85°C	95°C
SRW									
Caldwell	15	5.4	6.5	13.3	29.5	10.3	12.6	15.2	27.8
	18	5.4	6.4	12.9	28.8	10.0	12.1	14.8	27.2
	25	5.2	6.0	11.5	26.2	9.3	11.5	14.0	24.5
	40	4.0	5.0	10.2	23.9	7.3	8.7	11.5	21.6
HRW									
Century	...	...	...	...	...	...	...	...	...
	18	3.2	15.2	15.2	30.2	8.5	11.0	12.3	24.4
	25	2.4	12.8	12.8	28.0	7.2	10.6	11.5	22.8
	40	2.3	11.4	11.4	26.1	6.2	8.1	10.5	18.4
Cheyenne	15	5.3	16.0	16.0	29.4	8.0	11.5	13.1	24.4
	18	4.6	15.0	15.0	30.1	7.2	10.6	11.5	22.8
	25	3.4	12.2	12.2	26.0	6.8	9.7	11.0	20.8
	40	2.0	10.6	10.6	24.3	6.0	7.9	9.1	17.5
Karl	15	4.4	15.7	15.7	28.0	8.1	11.3	12.8	24.3
	18	4.4	15.1	15.1	28.8	7.0	9.9	11.2	21.7
	25	3.8	13.0	13.0	27.5	6.3	9.0	10.4	20.0
	40	2.5	10.2	10.2	24.1	5.5	7.1	8.8	17.5
HRS									
Butte 86	15	4.6	15.1	15.1	28.0	8.8	11.0	12.2	24.1
	18	3.0	11.7	11.7	29.0	6.5	9.0	10.1	19.9
	...	...	...	...	...	...	...	...	...
	40	2.4	9.0	9.0	27.5	5.3	6.1	8.5	17.7
Durum									
Monroe	15	4.2	14.5	14.5	29.0	8.2	10.6	11.0	23.6
	18	3.6	11.7	11.7	28.2	6.7	9.0	10.3	20.4
	25	2.7	9.4	9.4	27.0	6.0	7.2	9.5	18.8
	40	3.0	8.9	8.9	26.0	5.8	6.7	9.0	18.0

[a] SRW = soft red winter; HRW = hard red winter; HRS = hard red spring.

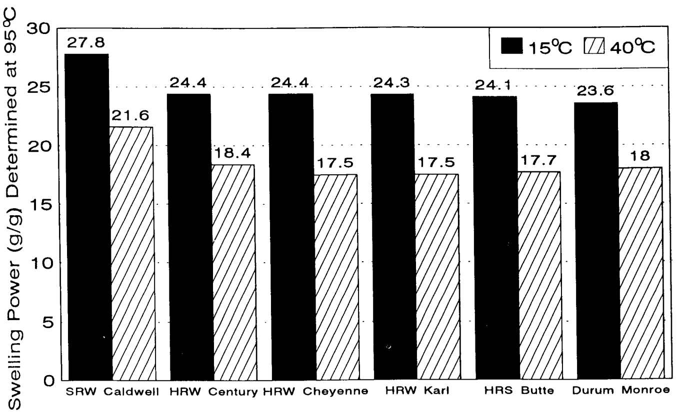

Fig. 16. Swelling power (g/g) determined at 95°C for starches from the six wheat cultivars grown at 15 and 40°C during the grain-filling period. SRW = soft red winter; HRW = hard red winter; HRS = hard red spring.

samples may be explained by differences in the AP CL distributions. The starches from wheats grown at 25°C had a high proportion of unit chains with DP 16–24 (Fig. 10), which led those starches to retrograde to a relatively greater extent (Table VI). In contrast, the starches from HRW Cheyenne, Karl, and HRS Butte 86 grown at 40°C had reduced levels of unit chains with DP 16–24, and they retrograded to a lesser extent. These results are in accord with previous data (Shi and Seib 1992) showing the retrogradation of four waxy cereal starches to be directly proportional to the mole fraction of unit chains with DP 14–24 and inversely proportional to the mole fraction of unit chains with DP 6–9. Ward (1991) also found that corn AP had a higher proportion of unit chains with DP 14–24 than did wheat AP, and corn AP retrograded to a greater extent.

Solubility and Swelling of the Starches

The solubility and swelling of the starches were measured over a temperature range of 65–95°C (Table VII). Generally, starches from wheat kernels grown at elevated temperature resulted in reduced solubility and swelling power. When temperature increased from 15 to 40°C during grain-filling, starch swelling power at 95°C was reduced by 6–7%. For wheat plants grown at the same temperature (15°C), the starch from SRW Caldwell gave the highest swelling power at 95°C (Fig. 16). Those results agree with previous work on starches from four classes of wheats, in which the swelling power decreased in the order SRW, HRW, HRS, and durum wheats (X. S. Liu, Y. T. Liang, and P. A. Seib, *unpublished data*).

The swelling behaviors of the starches reflected the differences in composition (Table III) and in the fine structure of their AP. Starch lipids are known to inhibit swelling of starch in hot water, presumably through AM-lipid complexes (Takahashi and Seib 1988, Tester and Morrison 1990, Tester et al 1991). In this study, the AM-lipid complex (L·AM) content of wheat starch increased with increasing temperature during grain-filling (Table III), which caused reduced swelling of starch from wheats grown at elevated temperature.

Recently, Morrison et al (1993) found that the swelling of starch in water is correlated positively with both the AP fraction and the unleached F·AM, but it correlated negatively with AM-lipid complex. The swelling factor (SF) at 80°C, defined as swollen starch volume per initial volume of air-dried starch, was given as:

$$SF = 41.1\ (AP + 0.8\ F{\cdot}AM)(1 - 2.7\ L{\cdot}AM^{0.485})$$

where AP, F·AM, and L·AM are fractions of unity instead of percentages.

Using the above equation, we calculated the SF values of the starches from SRW Caldwell, HRW Karl, and durum Monroe (Fig. 17). L·AM was calculated from 7 × LPL (Table III), and F·AM was calculated from total AM − L·AM. In general, the calculated SF values (Fig. 17) were in the same order as the experimental values of swelling power (Table VII and Fig. 16).

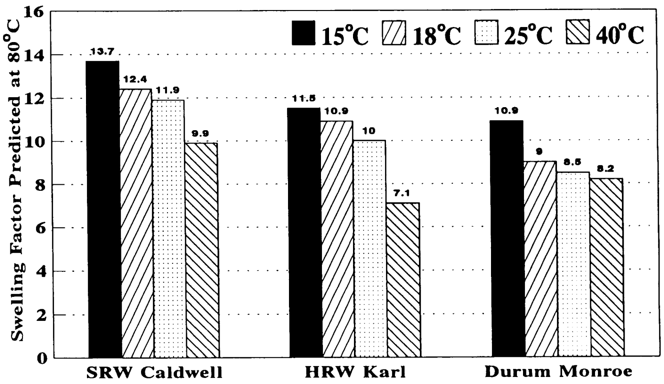

Fig. 17. Calculated swelling factors (v/v) at 80°C for starches from three wheat cultivars grown at 15, 18, 25, and 40°C during the grain-filling period. SRW = soft red winter; HRW = hard red winter.

It is worth noting that the swelling factor defined by Tester and Morrison (1990) is different from swelling power used by many researchers. SF measures only intragranular water based on the observation that blue dextran (MW 2×10^6) diffuses freely throughout the supernatant and interstitial water, but it does not penetrate swollen granules. SF usually measures starch swelling in water below 85°C. Above 85°C, SF values cannot be obtained because starch granules begin to disintegrate. The swelling power method developed by Leach et al (1959) includes both intragranular and intergranular or interstitial water.

Recently, Konik et al (1993) reported that starch swelling power was dependent upon the cultivar, growth location, and growth season of wheats in Australia. Furthermore, those workers found a significant positive correlation between starch swelling power and eating quality of Japanese white salted noodles. Many researchers (Miskelly and Moss 1985; Toyokawa et al 1989a,b; Konik et al 1992) have suggested that starch swelling power and pasting properties play important roles in determining the quality of salt noodles. Kim and Seib (1993) proposed that restricted swelling of starch in the flour was desirable in cooked instant fried noodles.

CONCLUSIONS

Elevated temperature during grain-filling resulted in shriveled kernels with reduced kernel weight, starch accumulation, starch granule size, and number of B-type granules. Heat during grain-filling changed the composition of wheat starch and elevated the temperature required to gelatinize and swell starch. The altered swelling power of starch indicates that environmental temperature would affect the quality of foods derived from wheat.

ACKNOWLEDGMENT

We thank Clodualdo C. Maningat and Earlene D. Hegarty at Midwest Grain Products, Inc., for their assistance in determining size distribution of starch granules; and Ping Yang for determination of protein level in three wheat cultivars.

LITERATURE CITED

ADKINS, G. K., and GREENWOOD, C. T. 1966. The isolation of cereal starches in the laboratory. Staerke 18:213-218.

AL-KHATIB, K., and PAULSEN, G. M. 1984. Mode of high temperature injury to wheat during grain development. Physiol. Plant 61:363-368.

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC, 8th ed. Method 44-18, approved April 1961, reviewed October 1976 and October 1982; Method 46-13, approved October 1976, reviewed October 1982, revised October 1986; Method 76-11, approved October 1976, reviewed October 1982. The Association: St. Paul, MN.

ASAOKA, M., OKUNO, K., SUGIMOTO, Y., KAWAKAMI, J., and

FUWA, H. 1984. Effect of environmental temperature during development of rice plants on some properties of endosperm starch. Starch/Staerke 36:189-193.

ASAOKA, M., OKUNO, K., and FUWA, H. 1985a. Genetic and environmental control of starch properties in rice seeds. In: New Approaches to Research on Cereal Carbohydrates. R. D. Hill and L. Munck, eds. Elsevier: Amsterdam.

ASAOKA, M., OKUNO, K., HARA, K., and FUWA, H. 1985b. Effect of environmental temperature at the milky stage on amylose content and fine structure of amylopectin of waxy and nonwaxy endosperm starches of rice (*Oryza sativa* L.). Agric. Biol. Chem. 49:373.

ASAOKA, M., OKUNO, K., HARA, K., OBA, M., and FUWA, H. 1989. Effects of environmental temperature at the early developmental stage of seeds on the characteristic of endosperm starch of rice (*Oryza sativa* L.). J. Jpn. Soc. Starch Sci. (Denpun Kagaku) 36:1-8.

BARLOW, K. K., BUTTROSE, M. S., SIMMONDS, D. H., and VESK, M. 1973. The nature of the starch-protein interface in wheat endosperm. Cereal Chem. 50:443.

BARUCH, D. W., MEREDITH, P., JENKINS, L. D., and SIMMONDS, L. D. 1979. Starch granules of developing wheat kernels. Cereal Chem. 56:554-558.

BATEY, I. L., GRAS, P. W., and KONIK, C. M. 1989. Structural differences in starches from wheats of different quality. In: Proc. Aust. Cereal Chem. Conf., 39th. T. Westcott, Y. Williams, and R. Ryker, eds. Royal Australian Chemical Institute: Victoria.

BATHGATE, G. N., and PALMER, G. H. 1972. A reassessment of the chemical structure of barley and wheat starch granules. Staerke 24:336-341.

BECHTEL, D. B., ZAYAS, I., KALEIKAU, L., and POMERANZ, Y. 1990. Size-distribution of wheat starch granules during endosperm development. Cereal Chem. 67:59.

BHULLAR, S. S., and JENNER, C. F. 1985. Differential responses to high temperatures of starch and nitrogen accumulation in the grain of four cultivars of wheat. Aust. J. Plant Physiol. 12:363-75.

BRIARTY, L. G., HUGHES, C. E., and EVERS, A. D. 1979. The developing endosperm of wheat—A stereological study. Ann. Bot. (Lond.) 44:641-658.

BUTTROSE, M. S. 1963. Ultrastructure of the developing wheat endosperm. Aust. J. Biol. Sci. 16:305-317.

CAUVAIN, S. P., GOUGH, B. M., and WHITEHOUSE, M. E. 1977. The role of starch in baked goods. 2. The influence of purification procedure on the surface properties of the granule. Staerke 29:91-95.

DENGATE, H., and MEREDITH, P. 1984. Variation in size distribution of starch granules from wheat grain. J. Cereal Sci. 2:83-90.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBER, P. A., and SMITH, F. 1956. Colorimetric method for determination of sugars and related substance. Anal. Chem. 28:350.

DUFFUS, C. M., and MURDOCH, S. M. 1979. Variation in starch granule size distribution and amylose content during wheat endosperm development. Cereal Chem. 56:427-429.

EVERS, A. D. 1971. Scanning electron microscopy of wheat starch. III. Granule development in the endosperm. Staerke 23:157-162.

EVERS, A. D., GREENWOOD, C. T., MUIR, D. D., and VENABLES, C. 1974. Studies on biosynthesis of starch granules. 8. A comparison of the properties of the small and the large granules in mature cereal starches. Staerke 26:42-46.

HOSENEY, R. C., and SEIB, P. A. 1973. Structural differences in hard and soft wheats. Baker's Dig. 47(6):26.

JONES, R. J., ROESSLER, J., and QUATTAR, S. 1985. Thermal environment during endosperm cell division in maize: Effects on number of endosperm cells and starch granules. Crop Sci. 25:830-834.

KARLSSON, R., OLERED, R., and ELIASSON, A.-C. 1983. Changes in starch granule size distribution and starch gelatinization properties during development and maturation of wheat, barley and rye. Starch/Staerke 35:335.

KIM, W. S., and SEIB, P. A. 1993. Apparent restriction of starch swelling in cooked noodles by lipids in some commercial wheat flours. Cereal Chem. 70:367-372.

KOIZUMI, K., KUBOTA, Y., TANIMOTO, T., and OKADA, Y. 1989. High-performance anion-exchange chromatography of homogeneous D-gluco-oligosaccarides and polysaccharides (polymerization degree ≥ 50) with pulsed amperometric detection. J. Chromatogr. 464:365-374.

KOIZUMI, K., FUKUDA, M., and HIZUKURI, S. 1991. Estimation of the distribution of chain length of amylopectins by high-performance liquid chromatography with pulsed amperometric detection. J. Chromatogr. 582:233-238.

KOLDERUP, F. 1975. Effects of temperature, photoperiod, and light quantity on protein production in wheat grains. J. Sci. Food Agric. 26:583-592.

KONIK, C. M., MISKELLY, D. M., and GRAS, P. W. 1992. Contribution of starch and non-starch parameters to the eating quality of Japanese white salted noodles. J. Sci. Food Agric. 58:403.

KONIK, C. M., MISKELLY, D. M., and GRAS, P. W. 1993. Starch swelling power, grain hardness and protein: Relationship to sensory properties of Japanese noodles. Starch/Staerke 45:139-144.

KUGIMIYA, M., and DONOVAN, J. W. 1981. Calorimetric determination of the amylose content of starches based on formation and melting of the amylose-lysolecithin complex. J. Food Sci. 46:765-770, 777.

LEACH, H. W., MacCOWEN, L. D., and SCHOCH, T. J. 1959. Structure of the starch granule. I. Swelling and solubility patterns of various starches. Cereal Chem. 36:534.

MacLEOD, L. C., and DUFFUS, C. M. 1988a. Temperature effects on starch granules in developing barley grain. J. Cereal Sci. 8:29-37.

MacLEOD, L. C., and DUFFUS, C. M. 1988b. Reduced starch content and sucrose synthase activity in developing endosperm of barley plants grown at elevated temperatures. Aust. J. Plant Physiol. 15:367-375.

MISKELLY, D. M., and MOSS, H. J. 1985. Flour quality requirements for Chinese noodle manufacture. J. Cereal Sci. 3:379.

MORRISON, W. R. 1964. A fast, simple and reliable method for the microdetermination of phosphorus in biological materials. Anal. Biochem. 7:218.

MORRISON, W. R. 1989. Uniqueness of wheat starch. Page 193 in: Wheat Is Unique. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

MORRISON, W. R. 1993. Cereal starch granule development and composition. In: Seed Storage Compounds: Biosynthesis, Interactions and Manipulation. P. R. Shewry and A. K. Stobart, eds. Oxford University Press: Oxford.

MORRISON, W. R., and GADAN, H. 1987. The amylose and lipid contents of starch granules in developing wheat endosperm. J. Cereal Sci. 5:263.

MORRISON, W. R., and LAIGNELET, B. 1983. An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. J. Cereal Sci. 1:9.

MORRISON, W. R., and NASIR AZUDIN, M. 1987. Variation in the amylose and lipid contents and some physical properties of rice starches. J. Cereal Sci. 5:35.

MORRISON, W. R., TESTER, R. F., SNAPE, C. E., LAW, R., and GIDLEY, M. J. 1993. Swelling and gelatinization of cereal starches. IV. Some effects of lipid-complexed amylose and free amylose in waxy and normal barley starches. Cereal Chem. 70:385-391.

NICOLAS, M. E., GLEADOW, R. M., and DALLING, M. J. 1984. Effects of drought and high temperature on grain growth in wheat. Aust. J. Plant Physiol. 11:553-66.

PARKER, M. 1985. The relationship between A-type and B-type starch granules in the developing endosperm of wheat. J. Cereal Sci. 3:271-278.

SANO, Y., MAEKAWA, M., and KIKUCHI, H. 1985. Temperature effects on the *wx* protein level and amylose content in the endosperm of rice. J. Hered. 76:221.

SHI, Y. C., and SEIB, P. A. 1992. The structure of four waxy starches related to gelatinization and retrogradation. Carbohydr. Res. 227:131-145.

SIMMONDS, D. H., and O'BRIEN, T. P. 1981. Morphological and biochemical development of the wheat endosperm. In: Advances in Cereal Science and Technology. IV. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St Paul, MN.

SOFIELD, I., EVANS, L. T., COOK, M. G., WARDLAW, I. F. 1977. Factors influencing the rate and duration of grain filling in wheat. Aust. J. Plant Physiol. 4:785-797.

SPIERTZ, J. H. J. 1977. The influence of temperature and light intensity on grain growth in relation to carbohydrate and nitrogen economy of wheat plant. Neth. J. Agric. Sci. 25:182-97.

TAKAHASHI, S., and SEIB, P. A. 1988. Paste and gel properties of prime corn and wheat starches with and without native lipids. Cereal Chem. 65:474-483.

TASHIRO, T., and WARDLAW, I. F. 1989. A comparison of the effect of high temperature on grain development in wheat and rice. Ann. Bot. (Lond.) 64:59-65.

TASHIRO, T., and WARDLAW, I. F. 1990. The response to high temperature shock and humidity changes prior to and during the early stages of grain development in wheat. Aust. J. Plant Physiol. 17:551-61.

TESTER, R. F., and MORRISON, W. R. 1990. Swelling and gelatinization of cereal starches. II. Waxy rice starches. Cereal Chem. 67:558-563.

TESTER, R. F., SOUTH, J. B., MORRISON, W. R., and ELLIS, R. P. 1991. The effects of ambient temperature during the grain-filling period on the composition and properties of starch from barley genotypes. J. Cereal Sci. 13:113-127.

TOYOKAWA, H., RUBENTHALER, G. L., POWERS, J. R., and SCHANUS, E. G. 1989a. Japanese noodle qualities. I. Flour components. Cereal Chem. 66:382-386.

TOYOKAWA, H., RUBENTHALER, G. L., POWERS, J. R., and SCHANUS, E. G. 1989b. Japanese noodle qualities. II. Starch components. Cereal Chem. 66:387-391.

WARD, K. H. 1991. Retrogradation of amylopectin from maize and wheat starches. PhD dissertation. Kansas State University: Manhattan, KS.

WHITE, P., POLLAK, L., and BURKHART, S. 1991. Thermal properties of starches from corn grown in temperate and tropical environments. (Abstr.) Cereal Foods World 36:704.

YUAN, R. C., THOMPSON, D. B., and BOYER, C. D. 1993. Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three *wx*-containing genotypes in two inbred lines. Cereal Chem. 70:81-89.

[Received November 19, 1993. Accepted April 20, 1994.]