

# Some Physicochemical Properties of Small-, Medium-, and Large-Granule Starches in Fractions of Waxy Barley Grain

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## ABSTRACT

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Barley grain was divided into eight fractions from the surface layer to the center with a machine used to polish brewers' rice. Small-, medium-, and large-granule starches were isolated from classified barley flour, and their physicochemical properties were investigated. The starch granules were oval to round with a median size of 2  $\mu\text{m}$  for small, 10  $\mu\text{m}$  for medium, and 12–19  $\mu\text{m}$  for large granules. From the surface layer to the center, both the median sizes and the ratio of large granules decreased,

and the ratio of medium- and small-granules increased. The starches had A-type X-ray diffraction patterns typical of cereal starches. The moisture sorption showed a negative correlation to the granule size. The gelatinization temperatures of starch granules in each layer were approximately the same, but the enthalpies decreased in the order of large, medium, and small granules.

Barley is the world's fourth most important cereal after wheat, rice, and corn. It is the most widely cultivated but for the most part, it is used for feed and brewing material rather than as foodstuff for human consumption (Bhatty 1993, Mitsunaga et al 1994). In Japan, a small amount of barley is used for food in the forms of rolled barley and roasted barley flour. One barrier to the use of barley is the complex processing method, which involves heat and pressure, resulting in undesired changes of barley components. Therefore, to promote the use of barley in food, we milled barley grain with a machine used to polish brewers' rice. Barley grain was milled to flour with particle diameters of 10–40  $\mu\text{m}$  from the surface layer to the center successively (Mitsunaga et al 1994).

Starch is the major component of barley grain and the dominant constituent of barley flour. Barley starches have bimodal granules containing large (A-type) and small (B-type) size particles. The physicochemical properties and structural characteristics of the A- and B-type or total starch granules have been reported (MacGregor et al 1971; MacGregor and Morgan 1984; Kang et al 1985; Naka et al 1985; Morrison et al 1986; Wu and Seib 1990; Tester and Morrison 1990, 1992; Inagaki and Seib 1992; Morrison et al 1993; Jenkins and Donald 1995; Schulman et al 1995; Vasanthan and Bhatta 1996; Bhatta and Rosnagel 1997; Czuchajowska et al 1998; Zheng et al 1998). However, the result obtained recently with a particle-size analyzer showed that the distribution curves of barley starch had two peaks and an intermediate curve between the peaks. The intermediate size particle had diameters of 3.5–7.0  $\mu\text{m}$  (volume basis, waxy 17.0%, normal 9.0%) (Tang et al 1998). Takeda et al (1999) reported large (79%), medium (11%), and small (10%) starch granules from normal barley grain and characterized the structure of their amylose and amylopectin. But physicochemical properties and structural characteristics of the starch granules in the different layers of barley grain have not been examined.

This article describes the isolation and physicochemical properties of large, medium, and small starch granules from the surface layer to the center in waxy barley grain.

## MATERIALS AND METHODS

### Materials

Mature barley grain (*Hordeum vulgare* L. emed. 'Yonezawa No. 2' six-rowed, waxy, TKW = 30.5 g) grown in Okayama, Japan, in 1996 was used. The grains were polished and milled from the surface layer to the center with a modification of a machine used for polishing brewers' rice (Fig. 1) and classified into eight fractions (A–H) (Mitsunaga et al 1994). The size class of the grains was calculated as: (polished grain weight/whole-grain weight)  $\times$  100 (%). Flour fractions are described as the product obtained during preparation of grains of a certain size class from grains of a larger size class. Sample fractions were A (100–90), B (90–80), C (80–70), D (70–60), E (60–50), F (50–40), G (40–30), and H (30–0). Numbers show the starting and ending sizes of the grains from which the flour fraction was obtained. All chemicals were purchased from commercial suppliers.

### Preparation and Fractionation of Starch Granules

Starch granules were prepared from each barley flour by the modified alkali method (Tang et al 1998). Large, medium, and small granules were fractionated by decantation (Takeda et al 1988). Barley flours were steeped at 5°C for 48 hr in a 0.1% NaOH solution (weight sixfold that of the flour), then the solution was decanted. The residue was thoroughly washed with 0.1% NaOH solution until it became colorless. The suspension was adjusted to pH 7.0 with 0.5M HCl solution and screened through a 125-mesh metal screen. After three to four treatments with 1-pentanol to remove protein, the suspension was screened through a 400-mesh metal screen, diluted to  $\approx$ 1L with water, and vigorously stirred. The suspension was allowed to stand for 2 hr in a 1L glass cylinder and the supernatant collected by decantation to another 1L glass cylinder was allowed to stand for 20 hr. By this process, barley starches were separated into large (sediment within 2 hr), medium (sediment 2–22 hr), and small (sediment at 22 hr) granules, respectively. Each fraction was collected on glass-filter, washed with ethanol and diethyl ether, and dried in a vacuum desiccator. The yields of large, medium, and small granules in the fractions were 82–71, 15–17, and 3–12% (w/w), respectively.

### Properties of Starch Granules

The granule size of each fraction was examined by a particle-size analyzer (Horiba, Ltd. LA-700 type). The shape and size of the starch granules was observed by scanning electron microscopy (Nippon Datam, JSM-5400 LV) at an accelerating voltage of 10–20 kV. X-ray diffraction was performed on barley starches (10% moisture) with an X-ray diffractometer (Rigaku, Ltd., Rint-2000 type) operating at 40 kV and 80 mA. Diffractograms were obtained from 4°C 2 $\theta$  to 40°C 2 $\theta$  with a scanning speed of 8°C/min and

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scanning step of 0.02°C 2θ. Relative crystallinity was calculated as the ratio of the areas of crystalline and amorphous regions of X-ray diffractograms by Hermans' method (Nara et al 1978). The starch granules were dried in a vacuum desiccator at 10<sup>-3</sup> mmHg absolute pressure for one week at room temperature (Boki et al 1989, 1990). The dried granules were analyzed as of the amount of equilibrium moisture sorption at 20°C for two weeks under 20, 42, 66, 81 and 98% relative humidity, respectively. Differential scanning calorimetry (DSC) was performed with a starch-to-water ratio of 5 mg to 20 μL. The samples were heated from 25 to 100°C at a heating rate of 5°C/min (Rigaku, Ltd., DSC-8240D).

## RESULTS AND DISCUSSION

### Distribution of Starch Particles

The distribution curves of whole starch granules in each fraction showed two peaks and an intermediate curve between the peaks, as well as a tendency of decreasing particle size from the surface layer to the center (Fig. 2). The curve shape in each fraction was similar to that for whole grain starch (Tang et al 1998). The large granule (>15 μm) ratio decreased, and the medium (5–15 μm) and small granule (<5 μm) ratios increased from 72.0, 24.0, and 4.0% in the surface layer to 19.0, 70.0, and 11.0% in the center (Fig. 3). Figure 4 shows the median sizes of whole, large, medium, and

small granule starches isolated by the precipitation method in each fraction. The median sizes of whole and large granules decreased from 17.5 and 19.0 μm in the surface layer to 10.0 and 11.2 μm in the center, respectively. But median sizes of small and medium granules were the same in all fractions, 2.0 and 9.9 μm, respectively. And it was observed that the percentage of small granules increased with the barley grain filling (May et al 1959, Merritt and Walker 1969, MacGergor et al 1971). Consequently, uneven distribution of the granule sizes in barley grain may be a hereditary characteristic.

### Scanning Electron Microscopy

The large, medium, and small granule starches from each fraction were observed under a scanning electron microscope. Due to general similarities, micrographs of only the H fraction are shown in Fig. 5. The large, medium, and small granule starches were oval to round in shape with diameter ranges of 1–5, 5–15, and 15–30 μm, respectively. The surface of these granules appeared to be smooth and showed no evidence of cracks or abrasion.

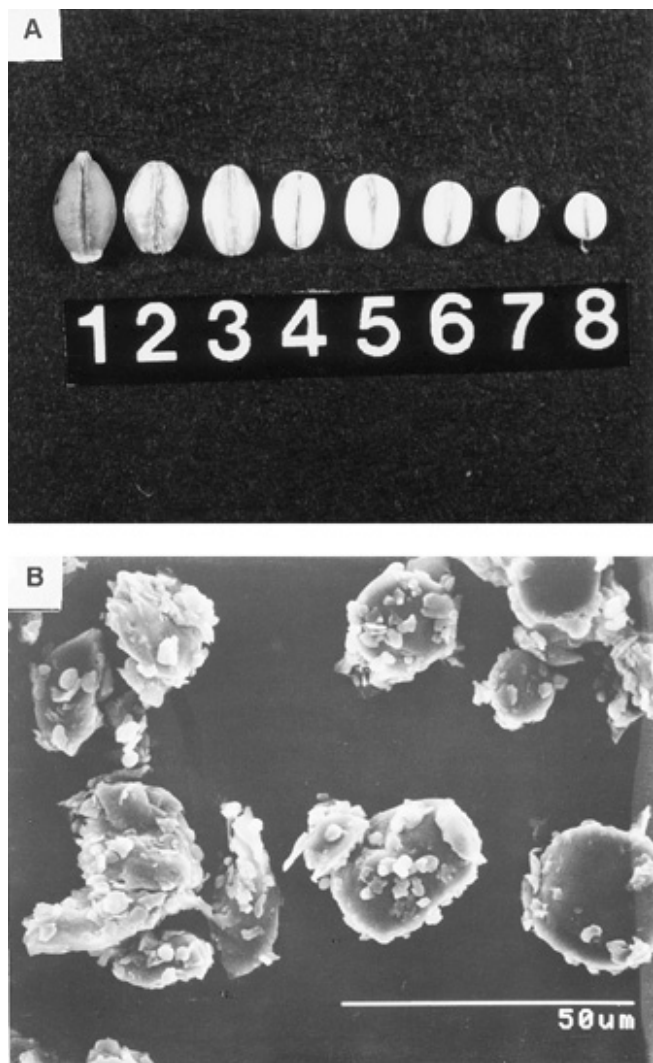


Fig. 1. Photographs of whole barley and polished grains (A) and a scanning electron micrograph of classified barley flour (B). Polished grains grew smaller uniformly, preserving their original shape. 1 = whole grain; 8 = 30% size class.

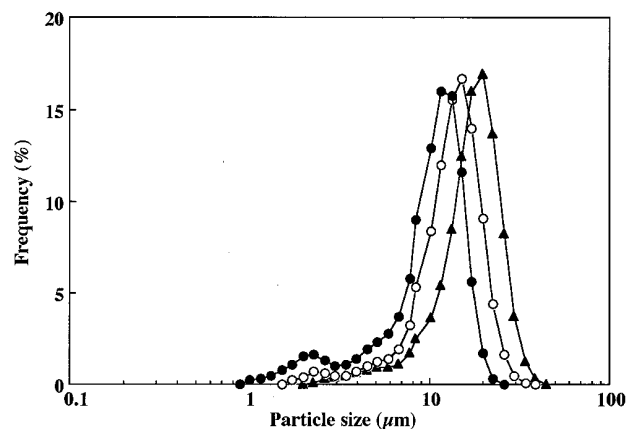


Fig. 2. Particle-size distribution of starch granules. ▲, ○, and ● = B, D, and H fractions, respectively.

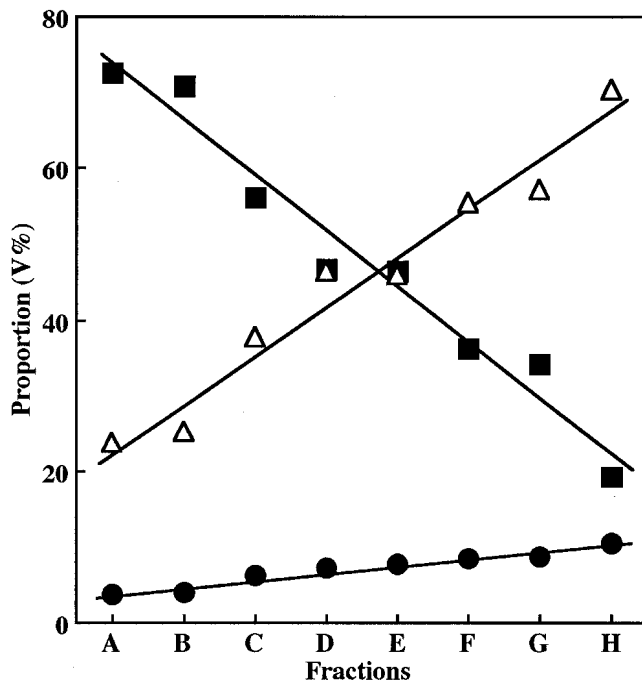


Fig. 3. Proportion of starch granules of each fraction calculated from particle-distribution data: ■ = ≥15 μm; Δ = 5–15 μm; ● = ≤5 μm.

### X-ray Diffractometry

The X-ray diffraction patterns of large, medium, and small starch granules in B, F, and H fractions are shown in Fig. 6. In all of the starches, major peaks were observed at *d*-spacings of 5.8, 5.2, 4.8, 4.4, and 3.8 Å. Zobel (1988) reported that X-ray *d*-spacings of 5.8, 5.2, and 3.8 Å are characteristic of an A-type starch crystal that is common to most cereal starches. The *d*-spacing of 4.4 Å is characteristic of amylose-lipid complex. Large granules in each fraction had a peak at 4.4 Å. The peak intensity tended to decrease with granule size. No peak was observed in small granules in each fraction. The starches showed 23.7% to 34.7% relative crystallinity. The value tended to increase from the surface to middle layer and decrease toward the center. The value also decreased with granule size. Starch granules are primarily composed of two glucose polymers, essentially linear amylose and highly branched amylopectin. The granule crystallinity is associated with the amylopectin component. Jenkins and Donald (1995) recently investigated the effect of varying amylose content on the internal structure of maize, barley, and pea starches. They indicated that amylose disrupts the structure order within the amylopectin crystallites. Klucinec and Thompson (1999) reported that each component interacts in retrogradation of dispersed starches. The difference of the crystallinity structure may be attributed to the fine structures of each starch fraction of barley grain.

### Moisture Sorption Isotherm

Figure 7 shows experimental moisture sorption data for starches of B, F, and H fractions at 20°C. The moisture contents of large and medium granules at each relative humidity tended to decrease from the surface layer to the center, but moisture contents of small granules in each fraction did not change except at 98% relative humidity. The maximum moisture contents tended to increase in the order of large, medium, and small granules in same fractions, and showed a negative correlation ( $\gamma = -0.81, n = 9$ ) with the median size of starch granules (Fig. 8). Boki et al (1990) indicated that differences of water activity for starches may be ascribed to the difference in strength of moisture-starch interaction and the submicroscopic structure of the starch granule. Hence, it may be thought that the moisture sorption properties at saturated relative

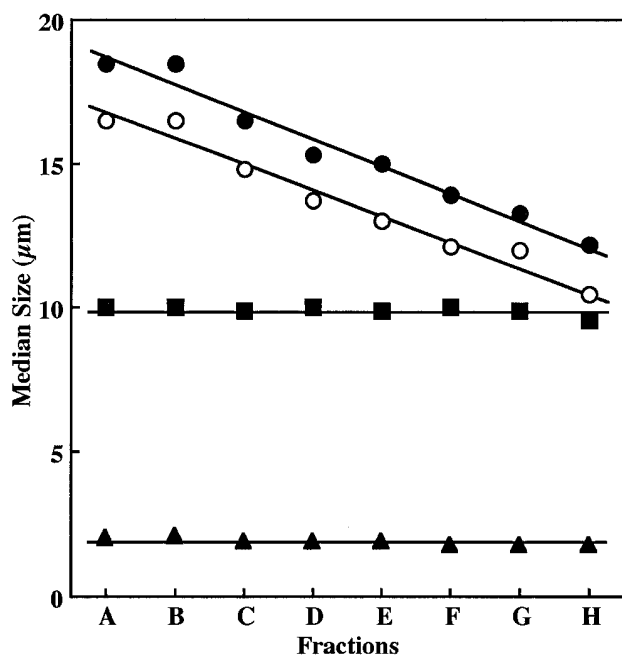


Fig. 4. Median sizes of total (○), large (●), medium (■), and small (▲) starch granules of each fraction.

humidity are primarily dependent on the surface area of starch granules but negligible for the internal structure of the granules.

### Differential Scanning Calorimetry

The transition temperatures and enthalpies of gelatinization are given in Table I. The onset, peak, and final temperatures in all starches were generally similar among the starches. The enthalpies tended to decrease in the order of large, medium, and small granules in the same fractions. Kang et al (1985) and Naka et al (1985) indicated that large granules of barley starch had a higher enthalpy of gelatinization than small granules of the same cultivar. Vasanthan and Bhatti (1996) also reported that barley small-granule starch had lower enthalpy than large-granule starch, which supports our results. There was a negative correlation between the median size of starch granule and the enthalpy, but the correlation coefficient ( $\gamma = +0.64, n = 15$ ) was relatively low (Fig. 8). How-

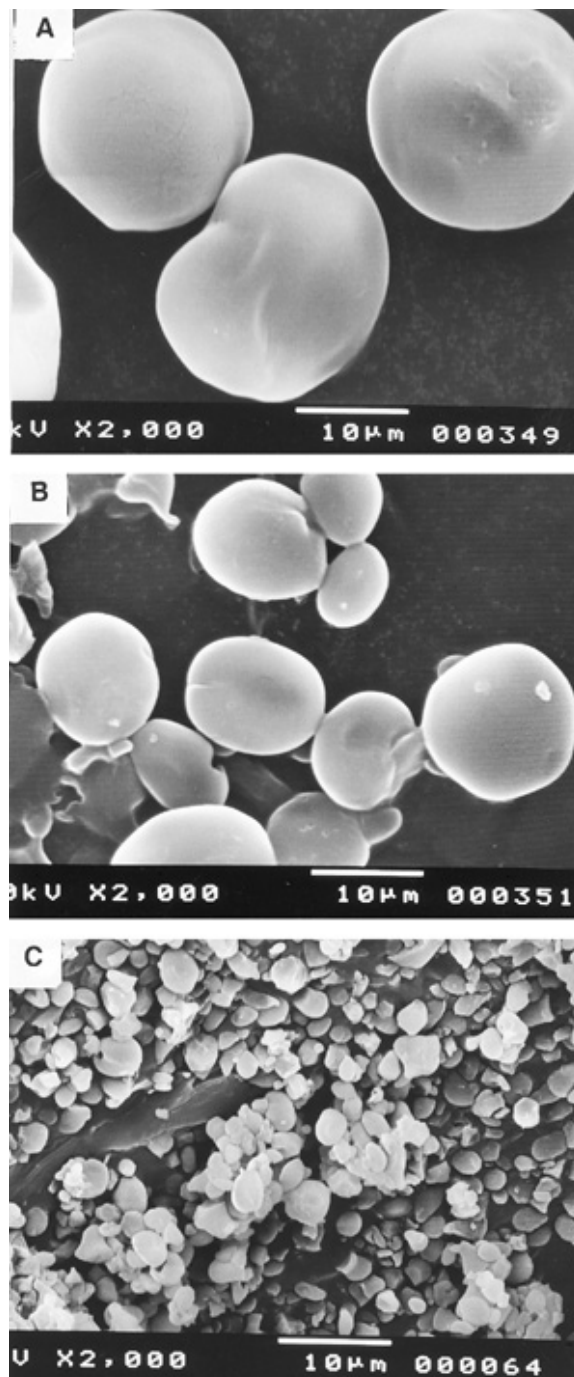


Fig. 5. Scanning electron micrographs of large (A), medium (B), and small (C) starch granules.

ever, the enthalpy showed higher correlation with the maximum moisture content ( $\gamma = -0.80$ ,  $n = 9$ ) and relative crystallinity ( $\gamma = +0.89$ ,  $n = 9$ ) of starch granules, respectively (Fig. 9). Accordingly, it may be that the gelatinization of waxy barley starch was influenced to some degree by granule size but was mainly controlled by the structure of starch granule. These findings suggested that the starch granules have differences of structure in both size and grain layer.

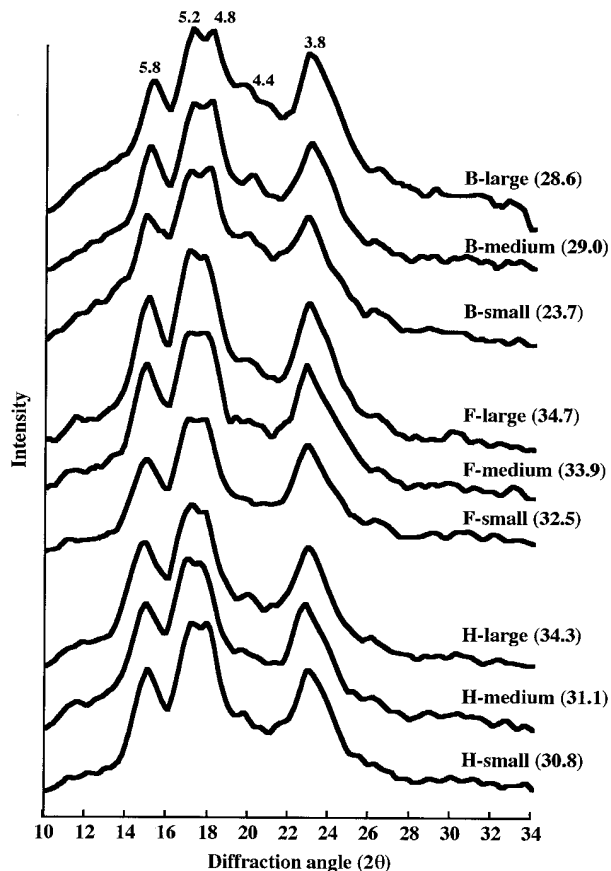


Fig. 6. X-ray diffraction patterns of large, medium and small starch granules. Particle sizes given in parentheses. Relative crystallinity (%) determined using Hermans' method (Nara et al 1978)

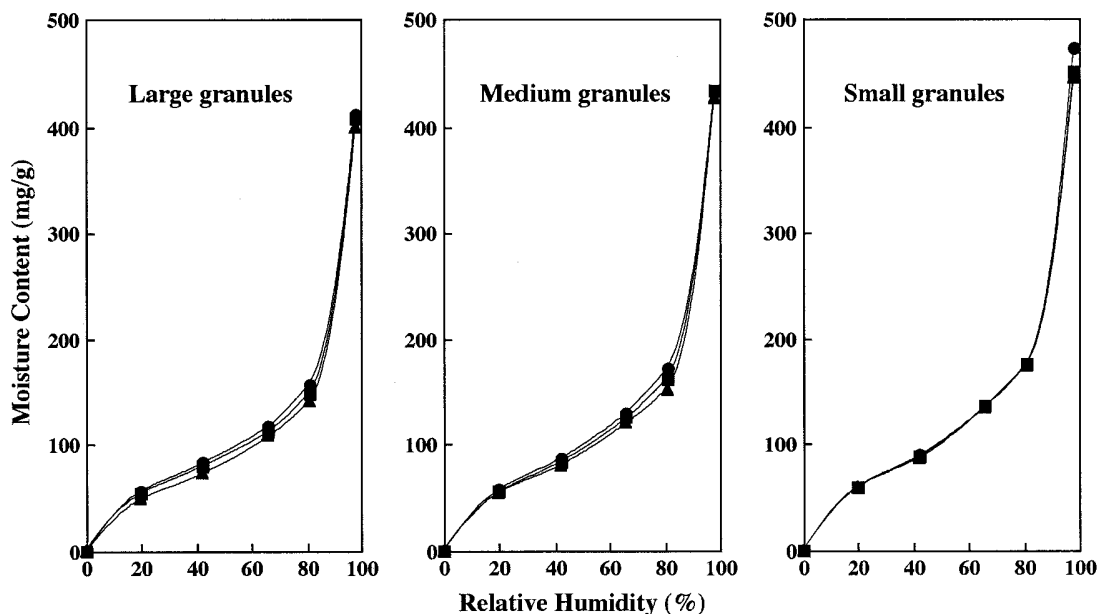


Fig. 7. Moisture sorption isotherms of large, medium, and small starch granules of B (●), F (■), and H (▲) fractions at 20°C.

## CONCLUSIONS

Waxy barley grain was divided into eight fractions from the surface to the center, and small (median size 2  $\mu\text{m}$ ), medium (10  $\mu\text{m}$ ), and large (12–19  $\mu\text{m}$ ) granule starches were isolated from each fraction. The starches had A type X-ray diffraction patterns, and the ratio of large granules decreased from the surface to the center. The uneven distribution of the granule sizes in barley grain is perhaps a hereditary characteristic. This finding may elucidate the mechanism of accumulation of barley endosperm starch.

The water activity of starch granules decreased from the surface layer to the center of the grain. The gelatinization temperatures of starch granules in each layer were approximately the same, but the enthalpies decreased in the order of large, medium, and small granules and increased from the surface layer to the center of the grain, respectively. Gelatinization property showed higher correlation with

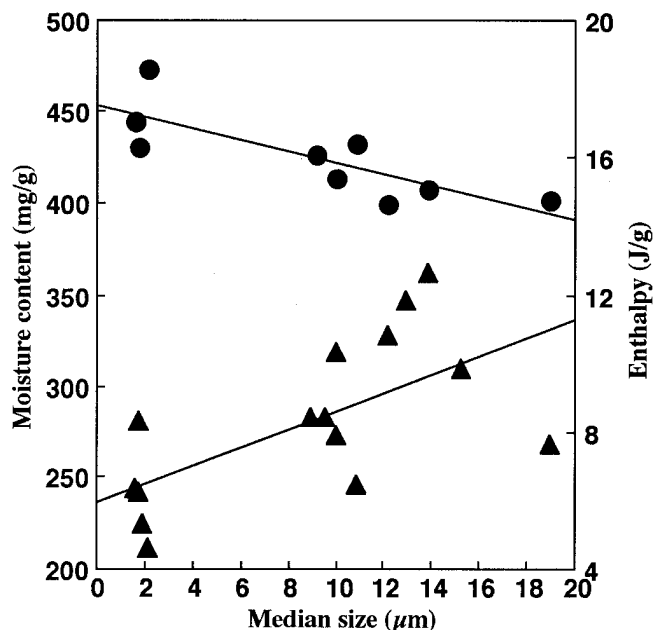


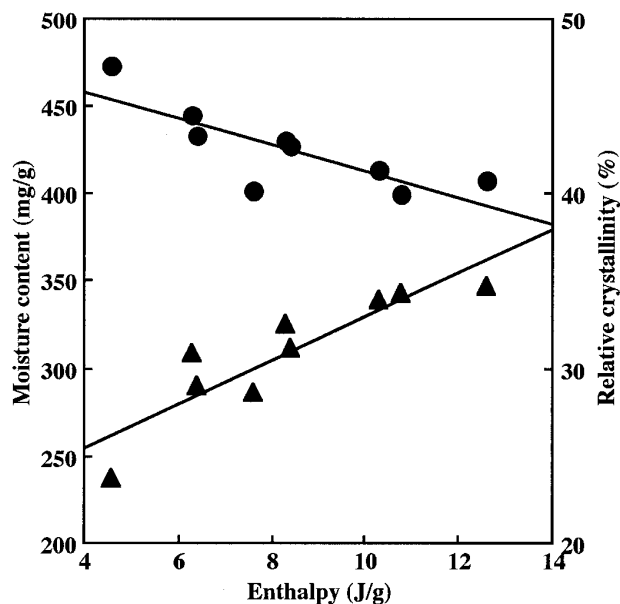
Fig. 8. Relation between median size, maximum moisture content, and enthalpy of gelatinization of starch granules. ● = Moisture content ( $y = 453.17 - 3.11x$ ,  $\gamma = -0.81$ ,  $n = 9$ ); ▲ = enthalpy ( $y = 5.96 + 0.27x$ ,  $\gamma = +0.64$ ,  $n = 15$ ).

**TABLE I**  
Gelatinization Temperatures and Enthalpy of Large, Medium, and Small Starch Granules of Each Fraction

Materials	Gelatinization Temperature <sup>a</sup> (°C)			Enthalpy $\Delta H$ (J/g)
	$T_o$	$T_p$	$T_c$	
B				
Large	57.3	63.3	70.7	7.6 ± 0.89 <sup>b</sup>
Medium	55.3	65.6	71.4	6.4 ± 0.24
Small	54.1	65.5	71.8	4.6 ± 0.40
D				
Large	60.2	65.6	71.2	9.8 ± 0.75
Medium	60.9	66.0	71.2	7.7 ± 0.14
Small	59.8	66.0	71.0	5.3 ± 0.33
F				
Large	60.5	65.2	70.4	12.6 ± 0.06
Medium	60.5	65.6	70.7	10.3 ± 0.63
Small	59.9	65.2	71.5	8.3 ± 0.84
G				
Large	60.7	65.2	70.7	11.8 ± 0.99
Medium	61.5	65.2	70.3	8.4 ± 0.05
Small	60.4	65.1	70.9	6.2 ± 0.51
H				
Large	60.4	64.8	70.0	10.8 ± 0.71
Medium	61.4	65.2	80.3	8.4 ± 0.35
Small	60.0	65.8	70.1	6.3 ± 0.05

<sup>a</sup> Onset, peak, and conclusion temperatures, respectively.

<sup>b</sup> Mean values ± standard deviation of three separate measurements.



**Fig. 9.** Relation between gelatinization enthalpy, maximum moisture content, and relative crystallinity of starch granules. ● = Moisture content ( $y = 487.67 - 7.46x$ ,  $\gamma = -0.80$ ,  $n = 9$ ); ▲, relative crystallinity ( $y = 20.54 + 1.25x$ ,  $\gamma = +0.89$ ,  $n = 9$ ).

water activity and relative crystallinity of starch granules. Accordingly, it seems that the gelatinization property was mainly controlled by structure of starch granules rather than their size. These results suggested that the starch granules have different characteristics of the structure and components in the different fractions.

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