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Characterization of storage starches from quinoa, barley and adzuki seeds

Hanjun Tang, Katsumi Watanabe, Toshio Mitsunaga*

Department of Food and Nutrition, Faculty of Agriculture, Kinki University, Nara 631-0052, Japan

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Abstract

The structures and physicochemical properties of storage starches from quinoa, barley and adzuki seeds were investigated and compared in this study. The median size of the starches were 1.0 μm for quinoa, 17.5 μm for barley and 33.0 μm for adzuki, respectively. The amylose content was 7.1% in quinoa starch, 29.2% in barley starch, 21.2% in adzuki starch. The number-average degrees of polymerization (DPn) of amylose was 900 for quinoa, 1700 for barley and 1800 for adzuki. Quinoa amylose had maximum number of branches (average number of chains per molecule (NC) 11.6) among the three starches, while adzuki amylose had almost no branches (NC 0.4). The DPn of amylopectin was 6700 for quinoa, 8000 for barley and 4500 for adzuki. Quinoa amylopectin contained extremely numerous LC (Longest Chain) (13.0%) and short chains, a few long chains, and had a unique chain length distribution as a waxy amylopectin. Adzuki amylopectin contained the fewest and the longest short chains but the highest number of long chains among the three starches. The relative crystallinity of the starch granules was 35.0% for quinoa, 19.7% for barley and 25.5% for adzuki. But, adzuki starch granules had the highest $\Delta H1$ and transition temperature, the lowest susceptibility to enzyme, and water absorption power. Starch granules were present in three complete different ultrastructures. © 2002 Published by Elsevier Science Ltd.

Keywords: Starch; Structure; Property

1. Introduction

Starch is laid down in all higher plants in the form of birefringent, semi-crystalline granules. The granules vary in crystallinity from 15 to 45% (Zobel, 1988). The granules may vary in size from <1 to >100 μm, and are primarily composed of two glucose polymers, amylose and amylopectin (Martin & Smith, 1995). Native starch granules typically contain around 20% amylose and 80% amylopectin. However, it is possible to breed plants that produce starch with different amylose and amylopectin contents. Native starch granules present three levels of organization, macromolecular structure, crystal structure and ultrastructure. The macromolecular structure of many plant starches, rice (Hizukuri, Takeda, & Maruta, 1989; Takeda, Hizukuri, & Juliano, 1987), wheat (Takeda, Suirasaka, & Hizukuri, 1984), maize (Takeda & Preiss, 1993), and barley starches (Song & Jane, 2000; Takeda, Takeda, Mizukami, & Hanasuiro, 1999; Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2001a,b; Yoshimoto, Tashiro, Takenouchi, & Takeda, 2000), have been elucidated. The starch granule crystallinity is associated with the amylopectin component. The currently accepted amylopectin structure involves short amylopectin chains forming double helices and associating into clusters (French, 1984; Hizukuri, 1986; Jenkins, Cameron, Donald, Bars, Derbyshire, & Mant et al., 1994). Three forms of crystalline structures, referred to as A, B and C type, are identified by wide angle X-ray scattering (WAXS). Shorter A chains are associated with A type crystallinity, longer A chains display B type crystallinity, while intermediate length A chains show C type crystallinity (Hizukuri, 1985). However, how amylose and amylopectin are organized within starch granule is not known clearly. Jenkins and Donald (1995) obtained the information from maize, barley and pea starches using small-angle X-ray scattering (SAXS), that, reducing the amylopectin content (increasing the amylose content) had the effect of increasing the crystalline region size. Other workers have also obtained important information from the starch granules hydrolysed by acid or enzyme, using scanning (SEM) or transmission (TEM) electron microscopy (Gallant, Bouchet, Buléon, & Pérez, 1992; Kimura & Robyt, 1995; Lauro, Forssell, Suortti, Hullenman, & Poutanen, 1999; Tang, Yoshida, Watanabe, & Mitsunaga, 1998; Vasanthan & Bhatty, 1996).

Quinoa, a cereal crop native to South America, has been cultivated for centuries. Recently, interest in quinoa was renewed as a valuable food source due to its versatility,

* Corresponding author. Fax: +81-742-43-2252.
E-mail address: mitunaga@nara.kindai.ac.jp (T. Mitsunaga).

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growth requirements and nutritive value of its protein (Mahoney, Lopez, & Hendricks, 1975). But starch is the major component of quinoa seed, and can be up to 55% (Chauhan, Eskin, & Tkachuk, 1992). Atwell, Patrick, Johnson, and Glass (1983) have reported the characterization of quinoa starch, but not in detail, especially about the fractionated amylose and amylopectin. Adzuki is the traditional food material in China and Japan, and, as the bean jam of the processing food, its popularity is high now. It is known that adzuki seeds consist mainly of starch. But there is almost no study on adzuki starch. In order to utilize these food materials effectively, it is necessary to elucidate the characteristics of the starches. In this study, we investigated structures and physicochemical properties of starches from quinoa and adzuki seeds. The properties of these starches were compared with those of normal barley starch.

2. Experimental

2.1. Materials

Quinoa seeds (Real, *Chenopodiaceae*, a product of Peru) were provided by Asai Co. Ltd. Mature barley seeds (Amaki, *Hordeum vulgare*, a six-rowed variety, a product of Okayama) were provided by Itomen Co. Ltd. Mature adzuki seeds (*Vigna angularis*, a products of Hokkaido) were obtained from Hashimoto Food Industry Co. Ltd. Beta-amylase from barley was purchased from Sigma Chemical Co (St. Louis, MO). Isoamylase from *Pseudomonas amyloderamosa* was purchased from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). Other chemicals, all reagent grade, were used without further purification.

2.2. Preparation of starches

The starch granules were isolated by the alkali method from quinoa, barley and adzuki seeds as described previously (Takeda et al., 1999; Tang et al., 1998; Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2000). Fractionation of amylose and amylopectin was carried out by following the procedure of Takeda, Hizukuri, and Juliano (1986).

2.3. Particle size distribution of starch granules

The prepared starch granules were fully suspended in water, and then the particle size distribution was examined by a particle size analyzer (Horiba, Ltd. LA-700, Japan) (Tang et al., 1998).

2.4. Analysis of fine structures of starches

Iodine absorption spectra were measured by the method reported previously (Takeda, Takeda, & Hizukuri, 1983). The amylose content and apparent content in starch were calculated from blue value (BV) with the following equations, in which amylose content was equal to: [BV(starch − amylopectin)/BV(amylose − amylopectin)] × 100, and apparent content was equal to: [BV(starch)/BV(amylose)] × 100, assuming the amylose BV to be 1.2 (Takeda et al., 1983; Tang et al., 2001a,b). The number-average degrees of polymerization (DPn) by the modified Park–Johnson method (Hizukuri, Takeda, Yasuda, & Suzuki, 1981; Suzuki, Hizukari, & Takeda, 1981), and the average chain length (CL) were also determined by the same method after isoamylolysis. The average number of chains per molecule (NC) was the value of ((DPn/CL) − 1). The isoamylolysis of starch was performed by the method reported previously (Tang et al., 2001a,b). The chain-length distribution of amylopectin was done by HPLC on two sequentially linked columns (TSK gel G3000SW + G2000SW, 7.5 *mm* × 60 *cm*) at 35°C by the method reported previously (Tang et al., 2001a,b). Total carbohydrate was measured by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.5. Wide-angle X-ray diffraction of starch granules

X-ray diffraction was performed by an X-ray diffractometer of Rint-2000 type (Rigaku Denki, Co. Ltd., Tokyo) operating at 40 kV and 80 mA. Diffractograms were obtained from 4° 2θ to 40° 2θ with a scanning speed of 8°/min and scanning step of 0.02° 2θ. The degree of crystallinity for the starch granules was evaluated as the ratio of the areas of crystalline and amorphous regions of the X-ray diffractograms with Hermans' method (Nara, Mori, & Komiya, 1978).

2.6. Gelatinization properties of starch granules

Gelatinization properties of the prepared starch granules were measured by sensitive DSC-8240D (Rigaku Denki, Co. Ltd., Tokyo). The samples with a starch-to-water ratio of 7–10 mg to 15 μl were sealed hermetically into an aluminum pan of 30 μl. Distilled water was used as the reference material. The temperature was raised from room temperature (about 25°C) to 140°C at a heating rate of 5°C/min (Takaya, Sano, & Nishinari, 2000).

2.7. Starch granule susceptibilities to enzymes

To sample (25 mg), was added, successively, 1 ml of 0.1 M acetate buffer (pH 4.8), 100 units of beta-amylase and 700 units of isoamylase. The reaction was initiated at 37°C with shaking for 0–30 h. It was stopped by the addition of 50 μl of 1 M HCl and then the pH was retured to 7.0 with 1 M NaOH solution. The reaction mixture was pipetted into 0.5 ml of 95% ethanol, and then centrifuged at 1500 g for 10 min. The supernatant was analyzed for soluble carbohydrates by the phenol–sulfuric acid method (Dubois et al., 1956). Percent hydrolysis was expressed as milligrams of maltose released per 100 mg of dry starch. Appropriate controls without the enzymes were prepared. The precipitate was washed with ethanol and diethyl ether, and dried in a

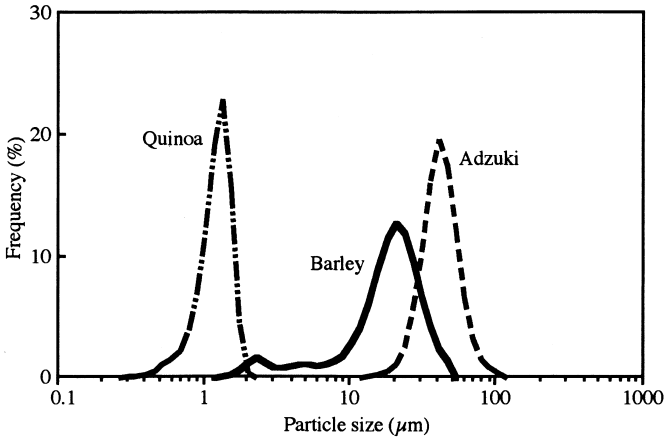

Fig. 1. Distribution of particle size of starch granules.

vacuum desiccator. It was observed by SEM (Datam JSM-5400 LV, Japan) at an accelerating voltage of 15–30 kV (Tang et al., 1998).

2.8. Transmission electron microscopy of starch granules

The structures of starch granules were investigated by a transmission electron microscope (JEOL DOEL, JEM-3010) at accelerating voltage 300 kV, current 14.8 pA and electrical transcription 1.4 s.

2.9. Water absorption power of starch granules

Starch granules were dried in a vacuum desiccator at 10^{-3} mmHg absolute pressure for one week, at room temperature (Boki, Ohno, & Shinoda, 1989 and Boki, Ohno, & Shinoda 1990). The dried granules were analyzed for the amount of equilibrium moisture sorption at 20°C for one week under 20, 42, 66, 81 and 98% relative humidity.

2.10. Statistical analysis

Statistical analysis of all the data was performed using Microsoft Excel.

Table 1
Absorbance of the starch–iodine complex and amylose contents

Materials	λ_{max} (nm)[a]	BV[b]	Amylose content (%)[c]
Quinoa	609 ± 4.7	0.305 ± 0.006	7.1 (25.4)
Barley	622 ± 0.3	0.456 ± 0.008	29.2 (38.0)
Adzuki	606 ± 0.9	0.498 ± 0.006	21.2 (41.5)

[a] Maximum absorption wavelength; values are the mean ± SD of three separate measurements.

[b] Blue value at 680 nm; values are the mean ± SD of three separate measurements.

[c] Amylose content (%) = [BV(starch-amylopectin)/BV(amylose-amylopectin)] × 100; (): apparent content (%) = [BV(starch) / BV(amylose)] × 100, assuming the amylose BV to be 1.2.

3. Results

3.1. Particle size distribution

The distribution patterns of quinoa, barley and adzuki starch granules are shown in Fig. 1. The sizes of the starch granules ranged from 1 to 100 μm as measured by a particle size analyzer. Quinoa and adzuki starches showed a single peak, while barley starch showed three peaks and the broadest distribution. Among the starch granules, adzuki was around 33 μm in median size and the largest, quinoa was around 1 μm and the smallest. Barley was around 17.5 μm and was intermediate between them.

3.2. Iodine absorption spectra of starch, and amylose content

Measurements of iodine absorption spectra of the defatted starches are given in Table 1. The λ_{max} (maximum absorption wavelength) and BV of the starches were 609 nm and 0.305 for quinoa, 622 nm and 0.456 for barley, and 606 nm and 0.498 for adzuki. The true and apparent amylose contents of the starches were 7.1 and 25.4% for quinoa, 29.2 and 38.0% for barley, and 21.2 and 41.5% for adzuki. The apparent amylose content for quinoa was around 3 times higher than the true content, and around 2 times higher for adzuki. Quinoa starch with a low true amylose content showed higher BV. But barley starch with high ($P < 0.005$) true amylose content had low ($p < 0.005$) BV, compared to adzuki starch.

3.3. Properties of amylose and amylopectin molecules

The properties of the amyloses and amylopectins isolated from quinoa, barley and adzuki starches are listed in Table 2. The λ_{max} for the amyloses was between 655 and 663 nm. The BV and DPn of the amyloses were 1.014 and 921 for quinoa, 1.240 and 1695 for barley, and 1.428 and 1788 for adzuki. The DPn for quinoa was the smallest ($P < 0.001$) among the three amyloses. The DPn of adzuki was similar to

Table 2
Properties of amylose and amylopectin molecules (values are the mean ± SD of three separate measurements)

Materials	λ_{max} (nm)[a]	BV[b]	DPn[c]	CL[d]	NC[e]
Amylose					
Quinoa	663 ± 2.9	1.014 ± 0.029	921 ± 58	73 ± 6.0	11.6
Barley	659 ± 2.4	1.240 ± 0.011	1659 ± 118	167 ± 14.3	9.0
Adzuki	655 ± 2.9	1.428 ± 0.024	1788 ± 78	1267 ± 70.1	0.4
Amylopectin					
Quinoa	595 ± 2.9	0.251 ± 0.029	6675 ± 376	21 ± 1.8	317
Barley	542 ± 2.4	0.132 ± 0.011	7963 ± 503	20 ± 1.2	397
Adzuki	569 ± 2.9	0.248 ± 0.004	4465 ± 362	25 ± 2.0	178

[a] Maximum absorption wavelength.
[b] Blue value at 680 nm.
[c] Number-average degrees of polymerization.
[d] Average chain-length.
[e] Average number of chains per molecule = (DPn/CL) − 1.

that of barley. Quinoa amylose also had the most number of branches (average 11.6 chains), while adzuki amylose had almost no branches (average 0.4 chains). Barley amylose averaged 9.0 chains. Quinoa amylose had the lowest BV ($P < 0.001$), probably due to its DPn and branching. The λ_{max} and BV of the amylopectins were 595 nm and 0.251 for quinoa, 542 nm and 0.132 for barley, and 569 nm and 0.248 for adzuki. The DPn was around 6700 for quinoa, 8000 for barley, and 4500 for adzuki. The CL and NC were 21 residues and 317 chains for quinoa, 20 residues and 397 chains for barley, and 25 residues and 178 chains for adzuki.

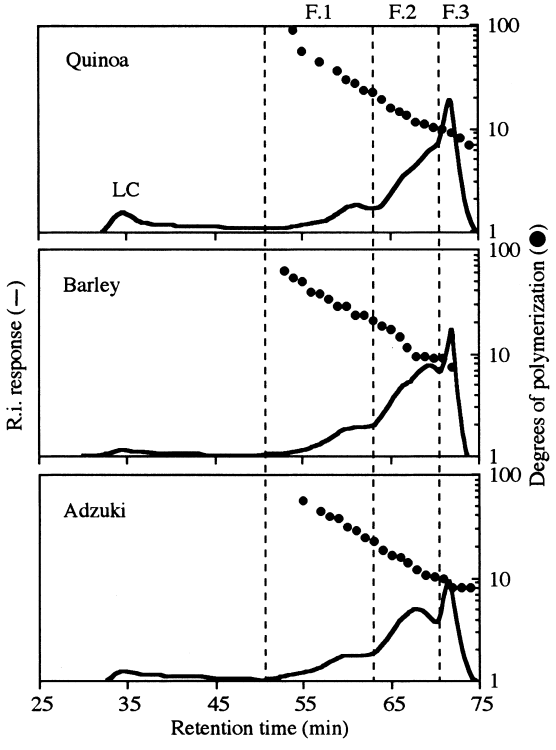

Fig. 2. Gel-permeation HPLC of isoamylase-debranched amylopectins. LC, longest chain. Columns, TSK gel G3000SW and G2000SW, 7.5 × 600 mm at 35°C; buffer, 0.1 M NaOAc containing 0.02% NaN3 (pH 6.2); flow rate, 0.6 ml/min.

Among the three samples, quinoa amylopectin had the highest λ_{max} ($P < 0.001$), but the BV was similar to that of adzuki. Adzuki amylopectin with the smallest DPn ($P < 0.01$) displayed the longest CL ($P < 0.01$) and the smallest NC ($P < 0.005$).

3.4. Chain-length distribution of amylopectin

The chain-length distribution profiles of the amylopectins determined by gel-permeation HPLC are shown in Fig. 2. The LC fraction was present with the three amylopectins and had a trimodal distribution curve (F.1–F.3). The distribution profiles had clearly different characteristics

Table 3
Properties of chain length distribution of amylopectin molecules (values are the mean of two separate measurements)

	Quinoa	Barley	Adzuki
LC (W%)[a]	13.0	5.4	9.7
F. 1			
Weight%	19.5	25.9	25.3
Molar%	7.8	10.1	12.9
DP[b]	40.5	42.2	40.5
F. 2			
Weight%	41.8	51.4	47.5
Molar%	51.5	55.5	53.8
DP	16.5	17.1	18.5
F. 3			
Weight%	25.7	17.3	17.5
Molar%	40.7	34.4	33.3
DP	11.1	10.0	11.3
W% of short chains[c]	67.5	68.7	65.0
Average short chain length[d]	13.6	14.2	15.7
Ratio of short chains per cluster[e]	12	9	7
Number of clusters per molecule[f]	28	42	25

[a] Longest chain.
[b] Average degrees of polymerization.
[c] W% of F.2 and F.3.
[d] DP of short chains.
[e] M% of F.2 and F.3/M% of F.1.
[f] (W% of short chains × DPn)/(ratio of short chains per cluster × average short chain length).

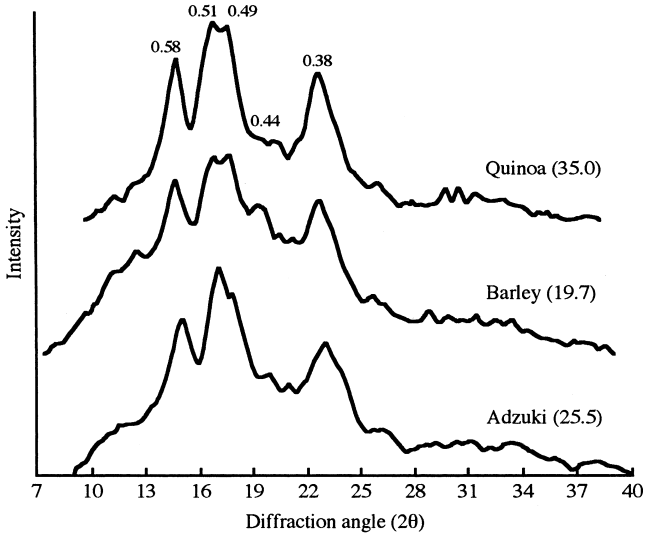

Fig. 3. Wide angle X-ray diffraction patterns of starch granules. Relative crystallinity (%); mean values of three separate measurements.

depending on the plant. The characteristics are given in Table 3. The carbohydrate proportion (W%) of LC was 13.0% for quinoa, 5.4% for barley, and 9.7% for adzuki. For F.1, quinoa had the smallest W% and molar ratio of the chains (M%) among the three amylopectins, while adzuki had the largest W%, M%. For F.2, quinoa amylopectin had the smallest W%, M% and DP among the three amylopectins, while barley had the largest W% and M%, and adzuki amylopectin had the largest DP. For F.3, the W% and M% values of quinoa were high, compared to those of barley and adzuki. The cluster length was 13.6 glucose residues for quinoa, 14.2 for barley and 15.7 for adzuki. Quinoa had the highest number of chains in a cluster ($p < 0.05$), and adzuki the fewest ($p < 0.05$), among the three amylopectins.

3.5. Wide-angle X-ray diffraction of starch granules

The X-ray diffraction patterns of the starch granules are given in Fig. 3. Quinoa and barley had characteristics of an A type crystal (Atwell et al., 1983; Tang et al., 2000; Zheng, Han, & Bhatty, 1998). Adzuki starch showed a C type crystal that is common to most legume starches (Gallant et al., 1992). The *d*-spacing of 0.44 nm is characteristic of amylose–lipid complex (Zobel, 1988). Barley displayed the largest intensity at *d*-spacing of 0.44 nm. The relative crystallinity for quinoa starch granules was the highest ($35.0 \pm 2.5\%$, $P < 0.005$), that for barley starch granules was the lowest ($19.7 \pm 1.6\%$, $P < 0.05$), and that for adzuki starch granules was a value in-between ($25.5 \pm 2.0\%$). These results corresponded with their amylose content (Table 1).

3.6. Gelatinization properties of starch granules

Differential scanning calorimetry (DSC) thermal curves

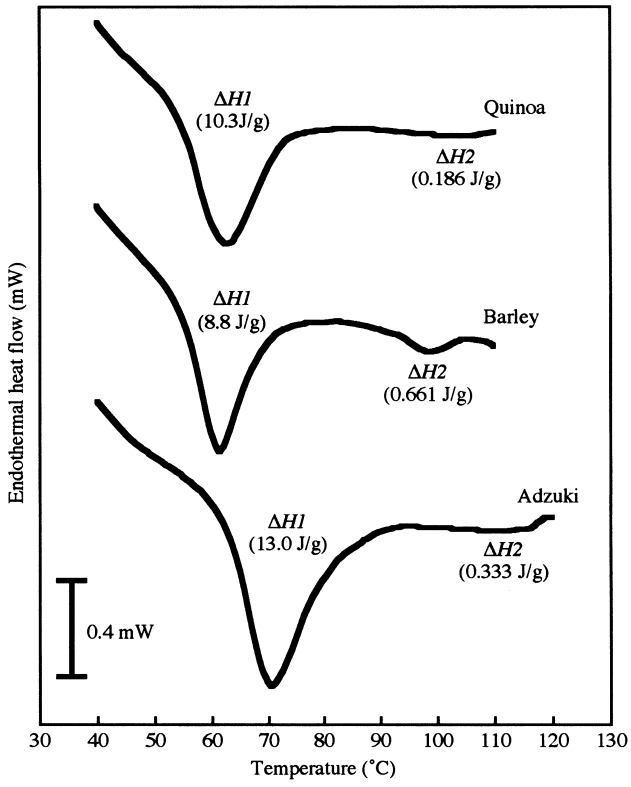

Fig. 4. Differential scanning calorimetry thermal curves of starch granules. Values of ΔH were the mean ± SD of three separate measurements.

of the starch granules are given in Fig. 4. Each starch granule displayed a characteristic DSC thermogram. Barley showed clearly two narrow endotherms. The endotherms of quinoa and adzuki were broader, particularly the second endotherm. The first endotherm depends on crystallinity of starch granule, and the second endotherm relates to amylose–lipid complex (Czuchajowska, Klamczynski, Paszczynska, & Baik, 1998; Atwell et al., 1983. In first endotherm, onset, peak and final temperatures were 54.5, 62.6 and 71.3°C for quinoa, 55.9, 61.5 and 67.6°C for barley, 63.3, 70.6 and 82.2°C for adzuki. The first enthalpy changes ($\Delta H1$) were 10.3(±0.2) J/g for quinoa, 8.8(±0.1) J/g for barley and 13.0(±0.3) J/g for adzuki. The second enthalpy changes ($\Delta H2$) were 0.186(±0.015) J/g for quinoa, 0.661(±0.034) J/g for barley and 0.333(±0.039) J/g for adzuki. Adzuki starch displayed the largest $\Delta H1$ ($P < 0.0001$) and the highest transition temperature among the three starches, although its relative crystallinity was lower than that of quinoa (Fig. 3). Barley starch had the smallest $\Delta H1$ ($P < 0.001$), and the largest $\Delta H2$ ($P < 0.001$) among the three starches, which corresponded with its amylose content (Table 1) and diffraction intensity at a *d*-spacing of 0.44 nm (Fig. 3).

3.7. Starch granule susceptibilities to enzymes

The hydrolysis rates of the starch granules as measured from their conjugation with beta-amylase and isoamylase

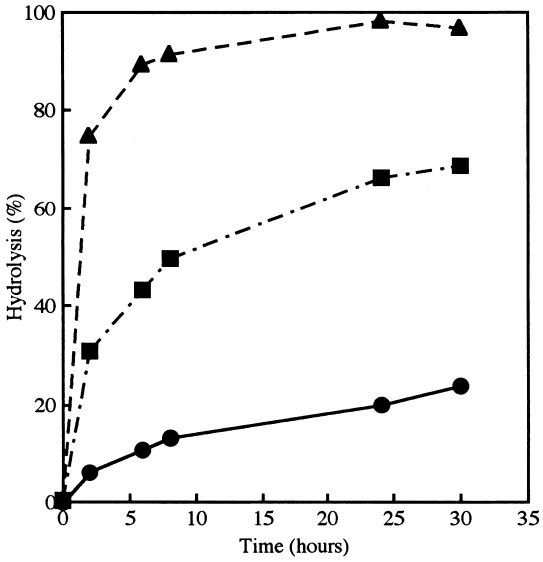

Fig. 5. Enzyme hydrolysis of starch granules. Values were the mean of three separate measurements. SD at 2, 6, 8, 24 and 30 h for quinoa (▲) is 1.4, 0.6, 2.7, 1.4 and 1.4%; for barley (■) is 0.4, 3.0, 0.5, 2.1 and 0.4%; and for adzuki (●) is 0.3, 0.3, 0.5, 0.6 and 0.6%.

are given in Fig. 5. A relatively fast rate of hydrolysis initially (from 0 to 2 h), followed by a slower rate up until 30 h, was observed with the three starch granules. But the degree of hydrolysis differed completely among them. At 30 h, quinoa starch was almost hydrolyzed, whereas the degree of hydrolysis was 68% for barley starch and only 23% for adzuki starch.

The appearance of the hydrolyzed granules was also studied with SEM (Fig. 6). For quinoa starch granules, only exocorrosion was observed, suggesting a homogeneous structure in the outer layer. Barley starch granules showed numerous deep holes into the inside of the granule, and the cores of the granules were hydrolyzed by the enzymes. Adzuki starch granules were first disrupted by the enzymes at the point of fusion forming a compound granule; they were also slightly eroded by exocorrosion. The soft part of the disrupted starch granules was then cut out. The granules in both barley and adzuki appeared to be composed of small, more or less spherical blocklets coming together tangentially. But the blocklets in adzuki were larger than those of barley. These results corresponded with those reported previously (Gallant et al., 1992; Kimura & Robyt 1995; Lauro et al., 1999; Tang et al., 1998; Vasanthan and Bhatty 1996). However, in the disrupted adzuki starch granules, numerous extremely small pits were also observed even in the blocklets.

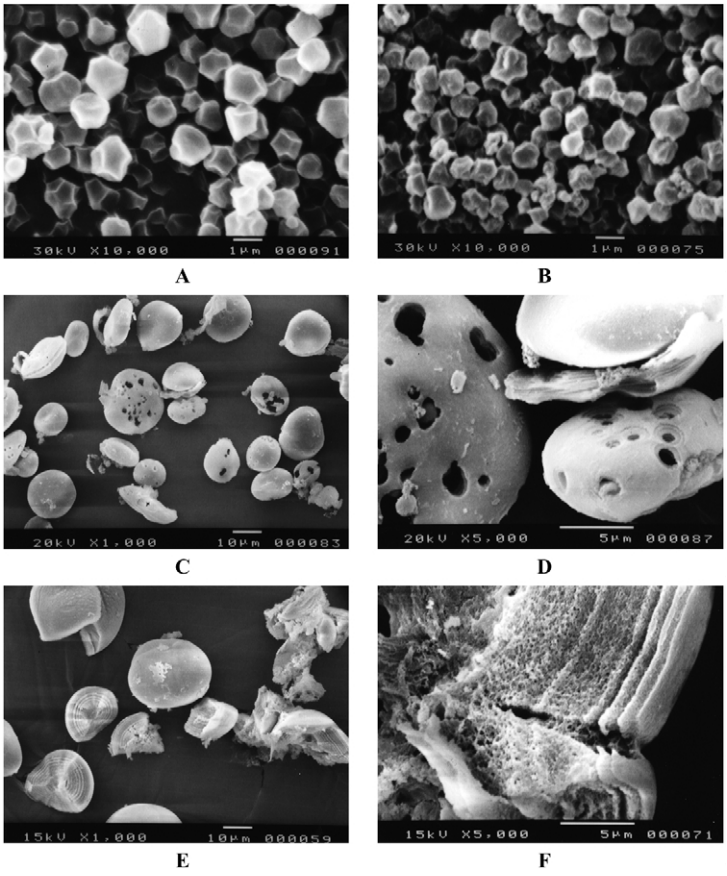

Fig. 6. Scanning electron microscopy of starch granules. A, quinoa starch granules; B, quinoa starch hydrolyzed for 2 h; C and D, barley starch hydrolyzed for 8 h; E and F, adzuki starch hydrolyzed for 30 h.

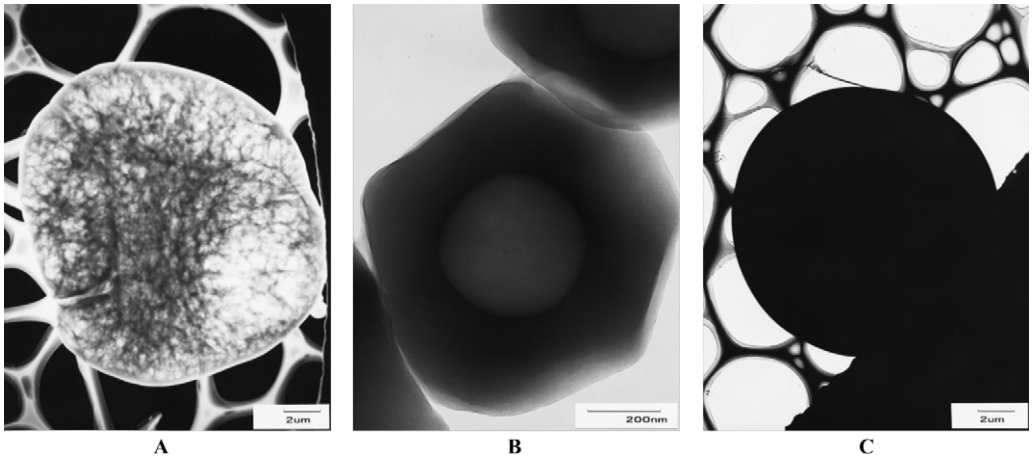

Fig. 7. Transmission electron microscopy of starch granules. Shown is a photograph of a dark field of vision for barley starch (A), and a bright field of vision for quinoa (B) and adzuki (C) starches.

3.8. Transmission electron microscopy of starch granules

We observed quinoa, barley and adzuki starch granules using TEM directly. The photographs of a dark field of vision in barley starch and a bright field of vision in quinoa and adzuki starches were obtained (Fig. 7). For quinoa, the organic density in the outer layer of the granule was homogeneous but greater than that of the inner layer. Adzuki starch granules had a homogeneous and highly dense tissue. However, barley starch granules had a heterogeneous tissue, and a clear network structure.

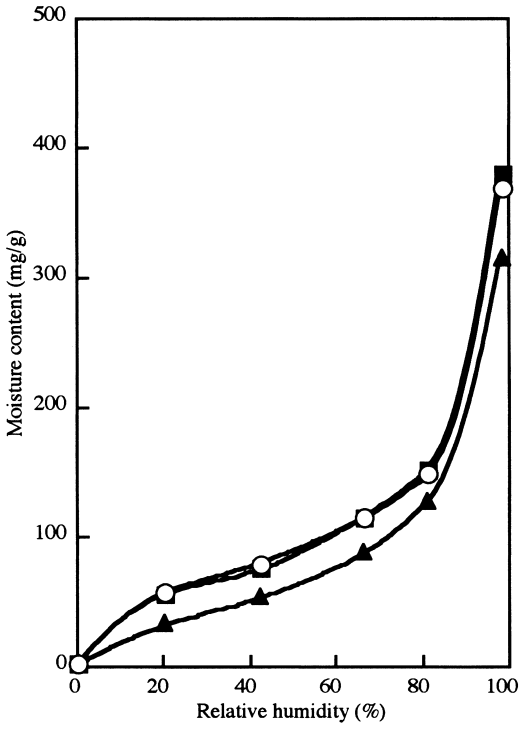

Fig. 8. Moisture sorption isotherms of starch granules at 20°C. ■ quinoa; ○ barley; ▲ adzuki.

3.9. Water absorption power of starch granules

The water absorption power of the starch granules in quinoa, barley and adzuki seeds was measured by a gravimetric method, and their moisture sorption isotherms are shown in Fig. 8. According to Van den Berg and Bruin (1981), a general sigmoid sorption isotherm can be divided into three parts, range I (relative humidity, 0–22%), II (22–73%) and III (73–100%). In ranges II and III, water molecules sorb or penetrate into newly created pores of the already swollen structure and are mechanically entrapped in the void spaces of starch. Adzuki starch granules showed the lowest moisture content over the three ranges among the three starches. Barley starch granules showed a similar water absorption power to quinoa starch granules, although their particle sizes (Fig. 1) were quite different.

4. Discussion

Quinoa, barley and adzuki seed starches studied here differed greatly in structures and properties. Quinoa starch was very small in granule size (Atwell et al., 1983), and barley starch had a characteristic distribution of three peaks (Tang et al., 1998 and Tang et al., 2000), which corresponded with earlier reports. Adzuki starch was very large in granule size and similar to potato (Kimura & Robyt, 1995). Quinoa starch had lower amylose content, and was waxy. Adzuki starch was lower in amylose content than barley starch but similar to that of normal starches such as rice (Hizukuri et al., 1989), wheat (Takeda et al., 1984) and corn (Takeda, Suzuki, & Hizukuri, 1988). Quinoa amylose had a small DPn but many branches, compared to barley and adzuki. Adzuki amylose was similar to barley amylose in DPn, but had almost no branches. Quinoa amylose had the lowest BV among the three amyloses, and adzuki amylose the highest, probably due to their DPn and branching chains. Barley amylopectin had the largest DPn and NC, but was

similar to quinoa amylopectin in the CL. Adzuki amylopectin had the longest CL, and the smallest DPn and NC. The amylopectin with a smaller DPn had a longer CL and larger BV, which corresponded with the earlier reports (Schulman, Tomooka, Suzuki, Myllarinen, & Hizukuri, 1995; Takeda et al., 1987, 1988, and 1999). The chain length distribution ranges in the short chain fractions of the amylopectins were narrow as measured by HPLC, compared to results of other studies (Hizukuri & Maehara, 1990; Schulman et al., 1995; Takeda et al., 1987, 1988 and 1999), probably due to differences in the column and flow rate used (Tang et al., 2001a,b). LC was present with the three amylopectins, but the amount of LC differed with the plant. The amount of LC in quinoa was similar to that in corn (Takeda et al., 1988). This is the first time LC has been clearly identified in waxy amylopectin. However, barley and adzuki amylopectin are consistent with the general characteristics of amylopectins reported previously (Hizukuri & Maehara, 1990; Schulman et al., 1995; Takeda et al., 1987, 1988 and 1999; Tang et al., 2001a,b). These structural characteristics corresponded with the λ_{max} and BV of the amylopectins, and were reflected clearly in the properties (Table 1) of the starches.

Quinoa and barley starches exhibited the A X-ray diffraction pattern common to native cereal starches (Atwell et al., 1983; Tang et al., 2000; Zheng et al., 1998). Adzuki was a C-type crystal that is common to most legume starches (Gallant et al., 1992). The *d*-spacing of 0.44 nm is characteristic of amylose–lipid complex (Zobel, 1988). Among the three starches, barley had the largest intensity at a *d*-spacing of 0.44 nm and the lowest relative crystallinity, while quinoa displayed the lowest intensity at a *d*-spacing of 0.44 nm and the highest relative crystallinity. These results corresponded with the amylose contents of the starches, because the crystallinity of the starch granule mainly depends on the amylopectin component.

Gelatinization of starch granule was a process of melting of double helices and loss of crystallinity (Jenkins et al., 1994), and represents the sum of individual crystal meltings. The first endotherm depends on the crystallinity of the granule (Czuchajowska et al., 1998). Waxy starches display larger gelatinization enthalpy, reflecting a higher percentage crystallinity of amylopectin; starches with longer amylopectin chain lengths also display larger enthalpy changes (Jane, Chen, Lee, McPherson, Wong, & Radosaljevic et al., 1999). Adzuki had the largest $\Delta H1$ among the three starches, probably due to relatively high crystallinity and long amylopectin chain length. However, quinoa starch with the highest relative crystallinity had relatively small $\Delta H1$ and the highest susceptibility to enzymes among the three starches. Within an amylopectin molecule, at least two residues adjacent to a branch point can not participate in double helix formation due to steric constrains (Umeki & Kainuma, 1981). The proportion of shorter chains able to participate in double helix formation may therefore be smaller (Craig, Lloyd, Tomlinson, Barber, Edwards, & wang et al., 1998). The relatively high ratios of the intensities of peak 1 (DP 12-14) to the shoulder (DP 18-21) in the chain length distribution of amylopectins with HPAEC-ENZ-PAD suggest a defective crystalline structure (Jane et al., 1999). The long chains are believed to span two or three of the clusters formed by short chains (Hizukuri, 1986). Accordingly, it was thought that quinoa amylopectin with many short chains and few long chains may not be useful to form a stable cluster structure and crystalline network.

In the observation using TEM, quinoa starch granule showed a homogeneous organic density in the outer layer, which was greater than that in the inner layer. Adzuki starch granules showed a homogeneous and highly dense organization overall. However, barley starch granules displayed a heterogeneous organic density, and a clear network structure. These may explain why the hydrolysis form with the enzymes differs among the starch granules. The hydrolysis forms were similar to those obtained with glucoamylase, alpha-amylase or beta-amylase (Gallant et al., 1992; Kimura & Robyt, 1995; Lauro et al., 1999; Tang et al., 1998; Vasanthan & Bhatty, 1996). These suggested that the hydrolysis of starch granules does not relate to the characteristics of the enzyme, but rather, to the structural characteristics of the granule. Also, the blocklets in adzuki starch granules were larger than those in barley starch granules. An average amylopectin molecule is 200–400 nm long (20–40 clusters) and 10–15 nm wide (Kainuma, 1988; Smith & Martin, 1993). The length of the amylopectin molecules is believed to correspond to the thickness of the growth rings of the starch granule (Jenkins et al., 1994; Jenkins & Donald, 1995; Martin & Smith, 1995). Following these reports and the model of Hizukuri (1986), the number of clusters per amylopectin molecule was calculated from the W% of short chains, DPn, ratio of short chains per cluster and the average short chain length. The amylopectin molecules had 28 clusters for quinoa, 42 clusters for barley, and 25 clusters for adzuki (Table 3). Although the relative size of crystalline and amorphous lamellae varies with variety, the combined repeat distance (9 nm) remains constant (Jenkins & Donald, 1995). Therefore, if the clusters are arranged in alignment (Kainuma, 1988; Martin & Smith 1995; Smith & Martin, 1993), barley amylopectin molecule is the longest, and adzuki amylopectin molecule the shortest among the three starches. But these lengths seemed not to correspond with the thickness of the hard parts (blocklets) observed with SEM. This suggested that the clusters in an amylopectin molecule are not always arranged in alignment, or, all the double helix axes of amylopectin are not always oriented perpendicular to the growth rings of the granule.

The water absorption power of the starch granules was measured by a gravimetric method. Van den Berg and Bruin (1981) indicated that water molecules sorb or penetrate into the newly created pores of an already swollen structure and are mechanically entrapped in the void spaces of starch in ranges II and III of the moisture sorption isotherm. In other words, the water absorption power of starch granules relates

to the stability of the granular organization. Quinoa starch had a similar water absorption power as that of barley starch, although their particle sizes were quite different. But adzuki starch had a lower water absorption power in all the three ranges. The results reflected amylose content, organic density and granular size of the starch granules.

5. Conclusion

Quinoa, barley and adzuki starches were present in completely different structures. Not only the amylose content differed within the starch granules, but also the localization of the amylose. Quinoa amylopectin had the largest ratio of short chain to long chain and the highest LC among the three starches. Quinoa starch granules may be weak in the stability of the cluster structure and crystalline network. Barley amylopectin had the most clusters among three starches. Barley starch granules may have the most unstable ultrastructure. Adzuki amylopectin had the longest short chains and the smallest ratio of short chain to long chain among three starches. Adzuki starch granules were the strongest both in crystal structure and ultrastructure. It was thought that the results might be useful in elucidating a relationship among the three structural levels of native starch granules.

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