



## The physicochemical properties of partially digested starch from sprouted wheat grain

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### Abstract

Partially digested starch from grain heavily damaged by sprout-induced  $\alpha$ -amylase was obtained by significantly delaying the harvest date using three wheat cultivars, Harunoakebono (HA), Haruyutaka (HY), and Hokushin (HS). In this investigation, the damaged starch was tested for physicochemical properties. The swelling power and peak viscosity determined by using a Rapid Visco-Analyzer decreased, and the digestibility of raw starch by glucoamylase increased as a result of an extremely late harvest except for HA, a sprouting-tolerant cultivar, in which visible sprouting was not seen even in the case of an extremely late harvest. However, an extremely late harvest did not significantly affect the amylose content, mean granule size, thermal properties determined by differential scanning calorimetry, and distributions of amylopectin chain length in HS and HY. Furthermore, observations by scanning electron microscopy indicated that HS and HY at an extremely late harvest contained a small amount of porous starch granules.

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### 1. Introduction

As starch accounts for over 70% of the total dry weight of wheat grain, starch properties are important for determining the eating quality of various products from wheat flour, such as bread and noodles. The physiological conditions of plant tissue from which starch is isolated affect starch properties. For example, germination has a profound effect on starch properties in cereals and legumes because  $\alpha$ -amylase activity, which is capable of degrading starch granules, increases due to germination. Several researchers have reported that germination definitely improved the digestibility of starch granules by amylase (Frias, Fornal, Ring, & Vidal-Valverde, 1998; Lorenz, Collins, & Kulp, 1981). The swelling power and amylograph viscosity decreased during

germination (Lorenz et al., 1981). In contrast, no changes or only minor ones in amylose content were observed during germination (Frias et al., 1998; Morad, Leung, Hsu, & Finney, 1980). In wheat, pre-harvest sprouting due to unfavorable weather conditions is a serious problem because it can severely reduce the quality of the grain for food products. However, very little information is available on the characteristics of partially digested starch by sprout-induced  $\alpha$ -amylase in wheat grain except for the early report of Dronzek, Hwang, and Bushuk (1972).

In this investigation, to shed more light on the characteristics of starches from wheat grain heavily damaged by sprout-induced  $\alpha$ -amylase, we examined the amylose content, mean granule size, swelling power, viscosity analyzed by rapid visco-analyzer, thermal properties by differential scanning calorimetry, enzymatic digestibility, and chain length distribution of amylopectin. Scanning electron microscopy (SEM) was used to visualize the surface structure of the starch granules. We used wheat starch samples at a significantly delayed harvest, which is

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associated with high activity of sprout-induced  $\alpha$ -amylase (Ichinose et al., 2001).

## 2. Materials and methods

### 2.1. Wheat samples at an extremely late harvest

Two spring cultivars, Harunoakebono (HA) and Haruyutaka (HY), and one winter cultivar, Hokushin (HS), were cultivated at an experimental field at the National Agricultural Center for the Hokkaido Region at Memuro, Hokkaido, Japan. For HA and HY, the harvest dates were 3rd August 2001 (early harvest) and 3rd September 2001 (extremely late harvest). For HS, the harvest dates were 22nd July 2002 (early harvest), 2nd August 2002 (late harvest), and 15th August 2002 (extremely late harvest). The appearance of sprouting grain was found in HY and HS from an extremely late harvest. In contrast, as HA is a spouting-tolerant cultivar, no visible sprouting was seen in HA even at an extremely late harvest.

### 2.2. Preparation of flour and starch

Each grain sample was milled on a Bühler experimental mill (Bühler, Inc., Uzwil, Switzerland) to produce 60% extraction flour. Starch from each flour sample was isolated by the method reported previously (Noda, Tohnooka, Taya, & Suda, 2001).

### 2.3. Starch properties

The amylose content was determined by the concanavalin A (Con A) method (Gibson, Solah, & McCleary, 1997) using an amylopectin/amylose assay kit (Megazyme International, Ireland). The reported values are means of triplicate measurements.

The granule size distribution of starch was measured using Sympatec HELOS Particle Size Analysis. The mean diameter based on volume distribution was measured. The reported values are means of duplicate measurements.

Swelling power at 60 °C was measured according to the method of Yasui, Sasaki, and Matsuki (1999). Starch (200 mg in dry weight basis) was directly weighed into a screw-cap test tube, and 5 ml of distilled water was added. The capped tubes were then placed on a vortex mixer for 10 s and incubated at 60 °C for 20 min with frequent mixing by inverting at 2 min intervals. The tubes were then cooled at 20 °C for 5 min and centrifuged at 1700g for 4 min, and the supernatant was removed with suction. The swelling power was calculated as the weight of swelled starch residue per 1 g of dry starch. The reported values are means of triplicate measurements.

Pasting properties were determined using the RVA 4D (Newport Scientific Pty, Ltd, Australia) with a starch concentration of 10% (w/w). The starch suspension was

kept at 50 °C for 1 min, heated to 95 °C at 12.2 °C/min, and kept at 95 °C for 2.5 min; it was then cooled to 50 °C at 11.8 °C/min and kept at 50 °C for 2 min. The peak viscosity, breakdown, and setback were recorded. The reported values are the means of duplicate measurements.

Gelatinization properties were determined using a DSC6100 (Seiko Instruments, Japan). Approximately 10 mg of starch sample (dry weight basis) was weighted directly into a silver pan. Distilled water was then added to give a starch concentration of 30% (dry weight basis, w/w). A cell with distilled water was used as the reference. Scans were run at a heating rate of 2 °C/min from 25 to 130 °C. The onset temperature ( $T_o$ ), peak temperature ( $T_p$ ), enthalpy ( $\Delta H$ ) of the first peak and peak temperature ( $T_p$ ), and enthalpy ( $\Delta H$ ) of the second peak were recorded. The first and second peaks are caused by the starch gelatinization and the dissociation of the amylose–lipid complex, respectively. The reported values are the means of triplicate measurements.

The enzymatic digestibility of raw starch was performed according to the modified method reported previously (Noda, Takahata, & Nagata, 1992). A commercial crystalline glucoamylase enzyme of *Rhizopus niveus* (Seikagaku Kogyo Co., Japan), which has high affinity to raw starch, was utilized for the experiments on digestion. A reaction mixture consisting of 0.5 ml of 4% (w/v) raw starch suspension, 0.25 ml of 100 mM acetate buffer (pH 5.0), and 0.25 ml of glucoamylase solution (5 unit) was incubated at 40 °C for 2 or 4 h with stirring. One unit of glucoamylase corresponds to the amount that liberates 1.0  $\mu$ mol of glucose per minute from soluble sugar at 40 °C and pH 5.0. After digestion, excess raw starch was then removed by filtration, and the glucose content of the filtrate was analyzed by the phenol sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The reported values are the means of triplicate measurements.

For HY and HS at early and extremely late harvests, the distribution of the amylopectin chain length was determined by high-performance chromatography (HPAEC) using the DX-300 system (Dionex Co., CA, USA) with a pulsed amperometric detector (PAD). The column used was a CarboPac PA-1 (4  $\times$  250 mm) with a CarboPac PA-1 guard column. Each starch was debranched with isoamylase from *Pseudomonas amyloclavata* (Seikagaku Kogyo Co., Japan) at 40 °C for 24 h as described previously (Noda, Takahata, & Sato, 1995). The debranched products were injected and eluted with a gradient of sodium acetate (0–2.5 min, increased from 150 to 200 mM; 2.5–10 min, increased from 200 to 250 mM; 10–25 min, increased from 250 to 275 mM; 25–45 min, increased from 275 to 325 mM; 45–65 min, increased from 325 to 375 mM in 150 mM NaOH with a flow rate of 1 ml/min). The area of each peak of linear chains up to DP 35 was determined using a Hitachi D-2500 Chromato integrator. Based on the area percent of each peak, the distributions (DP 6–35) of

the amylopectin chain length were calculated. The reported values are the means of triplicate measurements.

The dried starch granules from HY and HS at early and extremely late harvests were sprinkled onto double-faced tape attached to specimen stubs and coated with platinum. The mounted samples were viewed with JSM 6301F scanning electron microscope (JEOL, Japan) at an accelerating voltage of 1 kV.

### 3. Results

The effects of an extremely late harvest on the amylose content, mean granule size, swelling power, and pasting properties, as determined by RVA in three wheat cultivars, are presented in Table 1. The amylose content ranged from 17.0 to 25.5%. The amylose content in HS was lower (17.0–17.9%) than that in HA and HY (23.8–25.5%). The effect of an extremely late harvest on the amylose content was unimportant. The mean granule size ranged from 16.5 to 18.1  $\mu\text{m}$ . An extremely late harvest resulted in less change in the mean granule size. The range of the swelling power at 60 °C was 5.5–7.2 g/g starch. A low swelling power was found in HY and HS at an extremely late harvest. In contrast, an extremely late harvest did not decrease the swelling power in HA, where visible sprouting was not seen even in the case of an extremely late harvest.

From the data of pasting properties by RVA, wider ranges were observed in peak viscosity (114–358 RVU), breakdown (61–103 RVU), and setback (25–126 RVU). Similar to the data of swelling power, the peak viscosity was low in HY and HS at an extremely late harvest. Furthermore, high values of breakdown and low values of setback were found in HY and HS at an extremely late harvest. However, similarly to the results of swelling power, an extremely late harvest did not significantly affect all RVA parameters in HA.

The DSC thermal properties of starches from grain in three wheat cultivars at different stages are listed in Table 2. Starch gelatinization properties indicated that  $T_0$ ,  $T_p$ , and  $\Delta H$  were 50.2–55.5, 58.9–61.1 °C, and 10.3–11.4 J/g, respectively. The effects of an extremely late harvest on

starch gelatinization properties by DSC were not significant. Results obtained from the second peak caused by the dissociation of the amylose–lipid complex showed that  $T_p$  and  $\Delta H$  were 101.1–104.1 °C and 2.1–2.5 J/g, respectively. An extremely late harvest did not significantly affect the thermal properties in the amylose–lipid complex.

Digestibility of raw starch by crystalline glucoamylase of *R. niveus* was evaluated to understand the nature of granule surface. Glucose is the only product released from the digestion of raw starch with crystalline glucoamylase. The hydrolysis rate, which refers to the ratio of glucose in the supernatant to the total amount of sugar content in the starch on a weight basis, was estimated as described previously, and the results are presented in Fig. 1. The hydrolysis rates after 2 and 4 h of reactions were in the range of 8.9–21.3 and of 11.6–26.0%, respectively. The results of digestibility by glucoamylase indicated that starch granules extracted from grain at an extremely late harvest were digested faster in HY and HS. However, no significant difference in digestibility by glucoamylase was observed between early and extremely late harvests in HA.

As to the partially digested starches, the chain length distribution of amylopectin was investigated for a better understanding of the crystallinity and fine structure of the starch granules. The solutions of isoamylase-treated starches at early and extremely late harvests in HY and HS were analyzed by HPAEC-PAD. The results of the effects of an extremely late harvest on the distributions (DP 6–35) of amylopectin chain length calculated by this method are shown in Fig. 2. As reported earlier (Noda et al., 2001), all amylopectins showed a peak at DP 11 and had a shoulder at about DP 19. There was little difference in amylopectin chain length between early and extremely late harvests in HY and HS.

Fig. 3 shows the scanning electron micrographs of starch granules extracted from HY and HS at early and extremely late harvests. In each sample, starch granules were generally round and oval-shaped, and large (15–25  $\mu\text{m}$  in diameter) and small (<10  $\mu\text{m}$  in diameter) granules, which correspond to A- and B-type granules, respectively, were clearly observed. An extremely late harvest had a slight effect on surface structure of starch granules. Namely, at an

Table 1  
Amylose content, mean granule size and swelling power pasting properties of starches from wheat grain at different stages

	Harvest date	Amylose content (%)	Mean granule size ( $\mu\text{m}$ )	Swelling power (60 °C; g/g)	Peak viscosity (RVU)	Breakdown (RVU)	Setback (RVU)
HA	8/3	24.7 $\pm$ 2.0	17.7	7.0 $\pm$ 0.1	290	65	102
HA	9/3	23.8 $\pm$ 0.8	18.1	7.2 $\pm$ 0.1	279	64	110
HY	8/3	23.7 $\pm$ 1.5	17.1	7.1 $\pm$ 0.1	263	61	101
HY	9/3	25.5 $\pm$ 1.0	17.7	5.5 $\pm$ 0.4	114	90	25
HS	7/22	17.0 $\pm$ 0.7	17.2	7.0 $\pm$ 0.1	358	83	126
HS	8/2	16.6 $\pm$ 0.4	17.0	7.1 $\pm$ 0.1	358	72	119
HS	8/15	17.9 $\pm$ 0.9	16.5	5.8 $\pm$ 0.2	131	103	35

Table 2  
Gelatinization properties of starches wheat grain at different stages

	Harvest date	Starch gelatinization			Amylose–lipid complex	
		$T_o$ (°C)	$T_p$ (°C)	$\Delta H$ (J/g)	$T_p$ (°C)	$\Delta H$ (J/g)
HA	8/3	53.4 ± 0.1	60.4 ± 0.1	10.7 ± 0.5	103.1 ± 0.3	2.1 ± 0.1
HA	9/3	54.4 ± 0.4	61.0 ± 0.4	10.3 ± 0.1	103.3 ± 0.1	2.2 ± 0.1
HY	8/3	55.0 ± 0.6	60.9 ± 0.1	10.7 ± 0.6	103.3 ± 0.2	2.2 ± 0.2
HY	9/3	55.5 ± 0.3	61.1 ± 0.3	11.3 ± 0.8	103.6 ± 0.3	2.5 ± 0.2
HS	7/22	51.0 ± 0.2	58.9 ± 0.5	11.0 ± 0.7	101.1 ± 0.9	2.3 ± 0.3
HS	8/2	50.2 ± 0.3	58.9 ± 0.6	11.4 ± 0.4	102.5 ± 0.3	2.1 ± 0.3
HS	8/15	52.1 ± 0.2	59.7 ± 0.3	11.4 ± 0.1	104.1 ± 0.3	2.3 ± 0.1

extremely late harvest, partial starch granules were heavily damaged due to  $\alpha$ -amylase activity, whereas more than 95% of granules had no holes.

#### 4. Discussion

Many investigators have analyzed the characteristics of partially digested starch (Dronzek et al., 1972; Fernandez & Berry, 1989; Frias et al., 1998; Jiang & Liu, 2002; Kitahara, Saganuma, & Nagahama, 1994; Kuracina, Lorenz, & Kulp, 1987; Leach & Schoch, 1961; Lorenz et al., 1981; Morad et al., 1980). Some investigators have used sprouted grain, in which  $\alpha$ -amylase activity was high and partially digested starch was present (Dronzek et al., 1972; Fernandez & Berry, 1989; Frias et al., 1998; Lorenz et al., 1981; Morad et al., 1980), and some have adopted a method in which starch granules were hydrolyzed by amylase in vitro (Jiang & Liu, 2002; Kitahara et al., 1994; Kuracina et al., 1987; Leach & Schoch, 1961). We have investigated the physicochemical properties of starches from wheat grain damaged by  $\alpha$ -amylase by using samples obtained from a significantly delayed harvest date. Partial degradation of starch by endogenous  $\alpha$ -amylase appeared to have effects on the swelling power, pasting properties analyzed by RVA, and digestibility by glucoamylase.

The swelling power and amylograph viscosity of starch have been reported to decrease during sprouting in corn, barley, and triticale grain (Lorenz et al., 1981). Jiang and Liu (2002) reported that the peak viscosity and swelling power of the residues from potato starch partially hydrolyzed by pancreatic  $\alpha$ -amylase in vitro had definitely decreased. In addition, wheat starch granules modified by  $\alpha$ -amylase from bacterial, fungal, and cereal sources in vitro had lower swelling power at 90 °C and lower amylograph viscosity than the control starch (Kuracina et al., 1987). In general, a lower swelling power is associated with a lower viscosity. According to an early report by Leach and Schoch (1961), the intrinsic viscosities of the residues obtained after action of several amylases on various starches in vitro were only slightly lower than the untreated starches. Morad et al. (1980) reported that the decrease in starch viscosity during

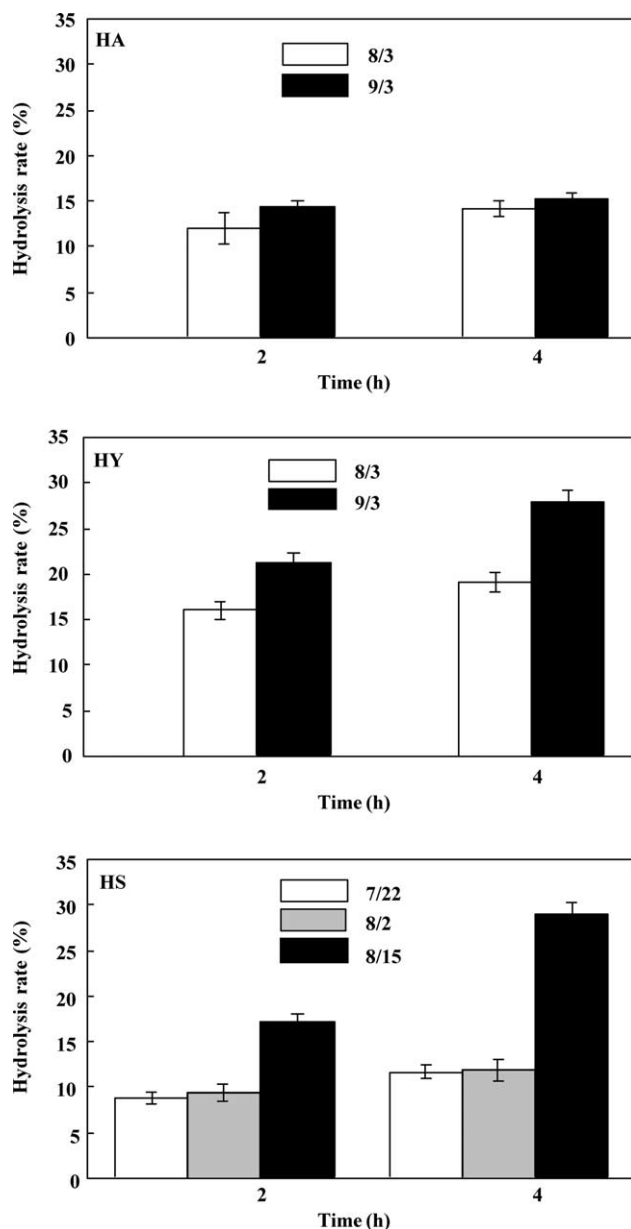


Fig. 1. Enzymatic digestibility of starch granules from wheat grain at different stages.



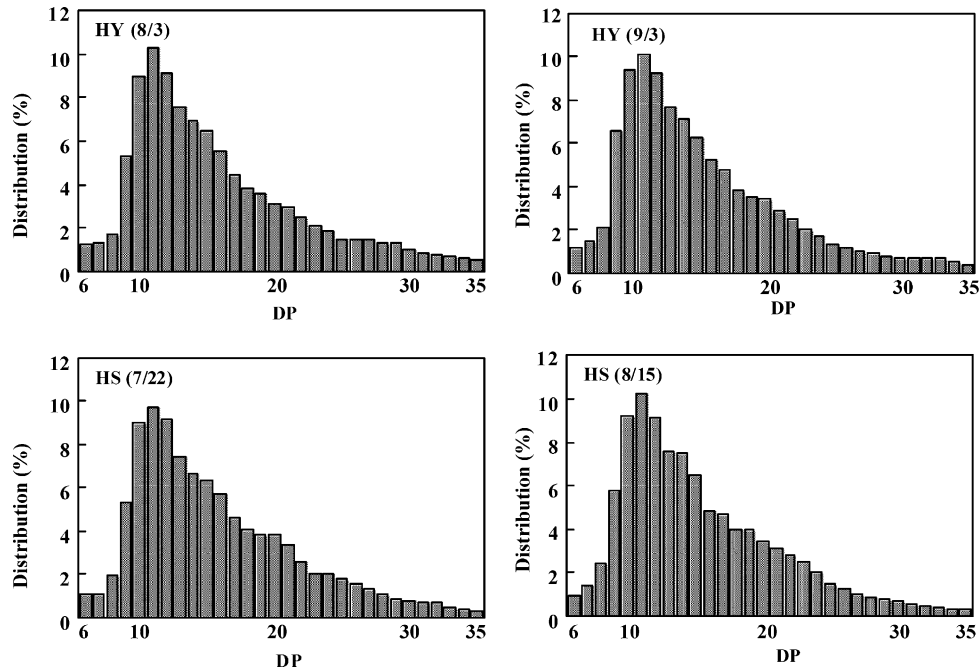


Fig. 2. Chain-length distributions (DP 6–35) of amylopectins from wheat grain at different stages.

germination was large for yellow pea but small for fava bean and lentil. This study confirmed that starches from wheat grain damaged by sprout-induced  $\alpha$ -amylase exhibited lower swelling power and peak viscosity. These results imply that degradation of starch by amylase decreases the swelling power and viscosity of starch.

It has been shown that germination sharply increases the enzymatic digestibility of starch granules from the chickpea (Fernandez & Berry, 1989) and lentil (Frias et al., 1998). In this study, starch from wheat grain heavily damaged

by  $\alpha$ -amylase was also much more susceptible than non-damaged starch to digestion by the crystalline glucoamylase of *R. niveus*. We assume that the partially digested starch has a less-ordered surface structure of granules, indicating higher enzymatic susceptibility. It was previously reported that the enzymatic susceptibility of starch granules was affected by the size of the granules (Noda, Takahata, & Nagata, 1993) and the amylose content (Juliano & Perez, 1990; Noda et al., 2002; Noda, Nishiba, Sato, & Suda, 2003). However, results from the present study demonstrate

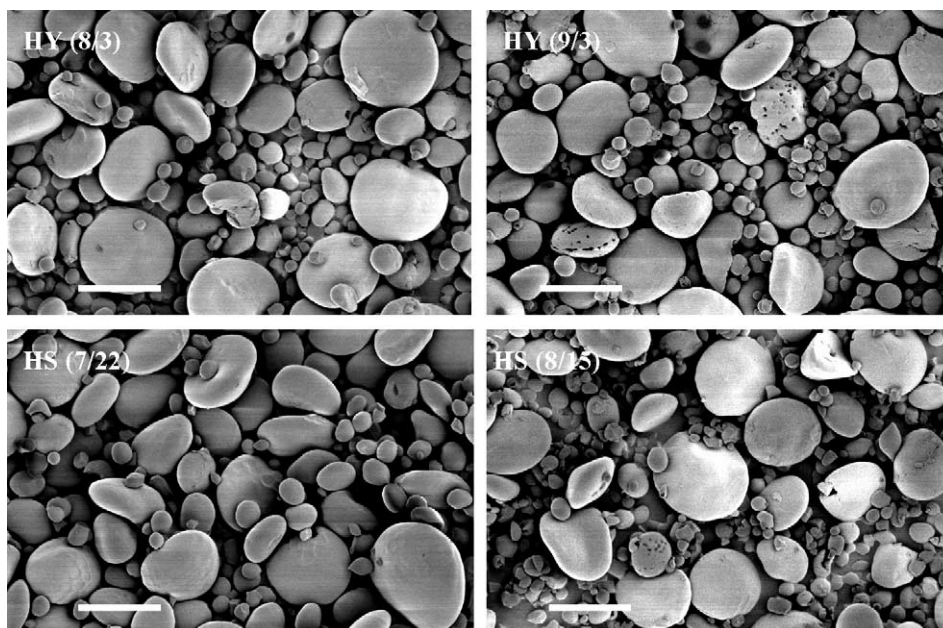


Fig. 3. Scanning electron micrographs of starch granules from wheat grain at different stages. Bar measures 20  $\mu\text{m}$ .

that the degree of starch degradation by amylase is an important factor for determining the enzymatic susceptibility of starch granules.

Commonly, the hydrolysis of starch by amylase has not resulted in a significant change in amylose content. For example, the germination process in the yellow pea (Morad et al., 1980), lentil (Frias et al., 1998; Morad et al., 1980), fava bean (Morad et al., 1980), and chickpea (Fernandez & Berry, 1989) had none or minor effects on the amylose content. According to Kitahara et al. (1994), Kuracina et al. (1987), and Leach and Schoch (1961), the amylose contents of residues obtained after amylase hydrolysis of starch in vitro were similar to those of the untreated starches. The present results also indicate little change in amylose content during starch degradation by sprout-induced  $\alpha$ -amylase in wheat grain. However, contradictory results, which show that the residues from partially hydrolyzed potato and high amylose corn starches showed lower amylose content than their control starches, were reported by Jiang and Liu (2002).

Some information on thermal properties determined by DSC of the partially digested starch has been obtained. In lentil starch, the gelatinization temperature determined by DSC did not change during germination (Frias et al., 1998). Similarly, Jiang and Liu (2002) showed that, using DSC, the gelatinization temperatures of residues from partially digested potato and high amylose corn starches in vitro were the same as those of their untreated starches. Our present data on DSC are in agreement with these previous findings. In contrast, Seneviratne and Biliaderis (1991) found manifest increases in melting temperatures determined by DSC during the digestion of wheat starch granules with *Bacillus subtilis* and hog pancreas  $\alpha$ -amylases in vitro. They also reported that hog pancreas  $\alpha$ -amylase treated starch granules exhibited distinctly lower values of gelatinization enthalpy, while in the case of *B. subtilis*  $\alpha$ -amylase such pronounced reduction was not observed. The thermal properties in an amylose–lipid complex of partially digested starch have been elucidated to date. Presumably, the amylose–lipid complexes are resistant to enzyme hydrolysis (Seneviratne & Biliaderis, 1991).

A relationship exists between the distribution of amylopectin chain length and the crystalline structure (Hizukuri, 1986). This study strongly indicates that the branch chains localized within one cluster in crystalline structure of the starch granules did not change during the germination of wheat grain. As data for longer amylopectin unit-chains with DP > 35 was not obtainable, we could not reach the conclusion that long B chains that extend through multiple clusters were attacked by sprout-induced  $\alpha$ -amylase. Frias et al. (1998) observed no effect of germination on the amylopectin structure of lentil starch, using the HPLC gel-permeation method. In contrast to our and their results, Jiang and Lui (2002) reported that residues from partially hydrolyzed potato and high-amylose corn starches had lower contents of amylopectin short chain

(DP < 16) than their untreated starches. The reasons for those differences are presumably related to the variation in the degree of hydrolysis among the starch samples used for the experiments.

There are several reports on the effect of germination on scanning electron micrographs of starch granules. Lorenz et al. (1981) observed that most of starch granules were porous in the sprouted triticale and barley. Similar finding was obtained in the sprouted wheat by Dronzek et al. (1972). According to the report of Morad et al. (1980), SEM observation indicated that the surfaces of starch granules were rough in the sprouted yellow pea, lentil and fava bean. In contrast, no alterations in the appearance of scanning electron micrographs were found due to germination in chickpea (Fernandez & Berry, 1989) and lentil (Frias et al., 1998). In this study, relatively small changes in surface structure of starch granules caused by sprout-induced  $\alpha$ -amylase in wheat grain were visualized by SEM.

## 5. Conclusions

The present work demonstrates the physicochemical properties of the partially digested starch from sprouted grain using three wheat cultivars at an extremely late harvest. The partially digested starch shows low swelling power, peak viscosity, and high digestibility of raw starch by glucoamylase. However, the germination process in wheat grain leads to no or minor change in the amylose content, mean size of granules, thermal properties by DSC, distribution of amylopectin chain length, and the appearance of scanning electron micrographs.

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