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The Pasting Properties of Flour and Starch in Wheat Grain Damaged by α-Amylase

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We sought to determine the effects of sprout-induced α-amylase on the pasting properties of flour and starch in wheat grain. To accomplish this, we used grain from five winter and two spring wheat cultivars obtained by significantly delaying the harvest date. Furthermore, grain from one winter wheat cultivar was soaked in water for 24 h at 5°C and then stored up to 48 h at 20°C. α-Amylase activity and the pasting properties of flour and starch were examined in each wheat sample mentioned above. Extremely late harvesting was associated with higher α-amylase activity and lower peak viscosity of flour in general. However, little change in starch peak viscosity was observed during the late stage of development in most cultivars. A wetting treatment also indicated that an excess accumulation of α-amylase and a marked decrease in flour peak viscosity did not coincide with a large decline in starch peak viscosity.

Keywords: α-amylase, wheat flour, wheat starch, pasting properties

Pre-harvest sprouting is a serious problem for wheat production in Japan. In Hokkaido, the main northern island of Japan, wheat often suffers from pre-harvest sprouting when it matures under conditions that are cool and humid for ripening. Wide differences in susceptibility to pre-harvest sprouting have been observed among wheat cultivars. Extremely late harvesting is generally linked with pre-harvest sprouting. Therefore, early harvesting is thought to be important to avoid pre-harvest sprouting. α-Amylase, an enzyme that is widely distributed in plants, catalyzes the endo-hydrolysis of α-1, 4 glucan bonds and plays a major role during the degradation of native starch granules. Pre-harvest sprouting has been closely associated with excessive α-amylase activity in wheat grain. It is known that the presence of α-amylase in wheat flour seriously damages flour quality. Flour pasting properties have been reported to correlate with the quality of various products from wheat flour, such as noodles (Crosbie, 1991; Panozzo & McCormick, 1993). Viscometric methods, such as the Brabender amylograph (Hutchinson, 1966; D'Appolonia *et al*., 1982), the falling number apparatus (Hutchinson, 1966; D'Appolonia *et al*., 1982; Perten, 1964; Finney, 1985; Moot & Every, 1990), and the rapid visco-analyzer (Ross *et al*., 1987), have been widely used for determining the quality of wheat flour. While the slurry of wheat flour and water with high α-amylase activity is heated in these instruments, starch granules in wheat flour are degraded by endogenous α-amylase. Consequently, flour viscosity determined by these instruments appears to reflect the level of α-amylase present in the flour (Hutchinson, 1966; D'Appolonia *et al*., 1982; Perten, 1964; Finney, 1985; Moot & Every, 1990). On the other hand, differences in starch pasting properties are found between different wheat samples. Therefore, when α-amylase activity is extremely low, the viscosity of starch itself, not α-amylase activity, in wheat flour is an indicative factor in determining the flour viscosity. It appears that damaged starch is present in wheat grain with a high activity of sprout-induced α-amylase, which can degrade native starch granules. However, inactivation of α-amylase by several methods, such as acidification (Meredith, 1970a) and the use of salts of heavy metal (Meredith, 1970b), enhances the viscosity of wheat flour damaged by sprout-induced α-amylase, leading to the assumption that wheat flour with high activity of sprout-induced α-amylase does not contain starch with highly reduced viscosity. There has been an obvious lack of information concerning the effect of sprout-induced α-amylase on starch pasting properties using the isolated starch from wheat flour.

In this study, we attempted to estimate the pasting properties of flours and starches from wheat grain severely damaged by α-amylase caused by the stimulation of pre-harvest sprouting. To obtain wheat samples with high α-amylase activity, wheat grain exposed to pre-harvest sprouting conditions was used. First, wheat grain at extremely late harvesting was used, including sprouting susceptible cultivars; we then induced germination using a water treatment.

Materials and Methods

Wheat samples at extremely late harvest Five winter wheat cultivars, Satanta (ST), Hokushin (HS), Chihokukomugi (CK), Lancer (LC), and Clerkscream (CS), and two spring wheat cultivars, Harunoakebono (HA) and Haruyutaka (HY), were cultivated in a standard manner in 2000–2001 for winter cultivars and in 2001 for spring cultivars, respectively, at the experimental farm of the National Agricultural Research Center for the Hokkaido Region at Memuro, Hokkaido, and used for this study. Among the five winter cultivars, the earliest maturity dates were July 20 for ST and July 21 for HS. CK matured on July 25, LC on July 31, and CS on July 25. The maturity date was August 10 for the two spring cultivars. CK and HY are susceptible to pre-

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harvest sprouting, while ST and HA are sprouting-tolerant cultivars. LC is a unique cultivar in that it often exhibits α-amylase activity in spite of a high tolerance to pre-harvest sprouting (Nakatsu, 1999). These 7 cultivars were harvested at 5–10 day intervals throughout the period from pre- to extremely late maturity.

Soaking wheat samples As HS is the most typical wheat cultivar in the Hokkaido region, it was selected for this study. It was cultivated in the standard manner in 1999–2000 at the above experimental farm. HS harvested at harvest ripeness was used for this study. Seeds were stored at 4°C and used within 2 months. A wetting treatment was performed by the method of Ichinose *et al.* (2001). Distilled water was poured on the wheat seeds. After 24 h of water absorption at 5°C, the distilled water was removed from the seeds. The treated seeds were then stored at 20°C for 0, 6, 12, 24, 36, and 48 h to germinate and induce α-amylase activity.

Preparation of flour and starch Each wheat sample was washed, tempered, and milled on a Buhler test mill (Buhler, Inc., Sannhof, Switzerland) to obtain a flour of 60% extraction rate. Starch from each sample was isolated by a previously reported method (Noda *et al.*, 2001). A dough (100 parts flour, 60 parts water) was allowed to rest in cold distilled water for 1 h and kneaded under water until the washings were clear. The starch washings were combined and passed through a 45 μm sieve. The filtrate, starch suspension, was allowed to stand for 3 h. The supernatant was discarded, and the remaining starch pellet was washed successively with water twice, then with ethanol, and air-dried.

Enzyme assay For the extraction of each enzyme, 0.2 g of each flour or starch was added to 1 ml of a 100 mM HEPES (*N*-2-hydroxyethyl-piperazine-*N*′-2-ethanesulfonic acid) KOH buffer (pH 7.5) containing 1 mM EDTA, 5 mM DTT, 5 mM $MgCl_2$, and 10 mM $NaHSO_3$, and the mixture was continuously and thoroughly stirred. After centrifugation (13,000 g×15 min), the supernatant was used for different enzyme assays. The α-amylase activity of each flour was determined using blocked *p*-nitrophenyl-maltoheptaoside as a substrate (McCleary & Sheehan, 1987). The absorbance was determined at 400 nm, and a value of 18.4 was used for the extinction coefficient of a 1 mM solution of *p*-nitrophenol. One unit of activity was defined as the amount of enzyme releasing 1 μmol of *p*-nitrophenol per minute.

RVA analysis RVA paste viscosity was determined using the RVA-Super3 (Newport Scientific Pty, Ltd., Australia) as follows. Each flour or starch was added to 25 ml of distilled water to create a 10% suspension (dry matter, w/w). The 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; then, it was cooled to 50°C at 11.8°C/min and kept at 50°C for 2 min. The RVA experiments were performed in duplicate.

Results and Discussion

Developmental test Since the hydrolysis of native starch granules, which occurs in wheat grain that is sprouting pre-harvest, is believed to be mainly due to α-amylase (Sargeant & Walker, 1978; MacGregor & Matsuo, 1982), we focused on the α-amylase activity in wheat flour in this investigation. To obtain wheat grain damaged by sprout-induced α-amylase, we first used grain that had been obtained by significantly delaying the harvest. The developmental changes in the α-amylase activity of flours from 7 wheat cultivars are presented in Fig. 1. Notable increases in α-amylase activity were not found until the third harvest of the five winter cultivars and the fourth harvest of the two spring cultivars. The α-amylase activity varied among the cultivars until the third harvest of the five winter cultivars and the fourth harvest of the two spring cultivars, which were mentioned above. The highest α-amylase activity was observed in HY (672–991 mU/g) while the lowest in ST (161–177 mU/g). In samples other than HY, CK exhibited over 500 mU/g at the second harvest. Nakatsu *et al.* (1997) concluded that the ground whole wheat with α-amylase activity over 500 mU/g exhibited an unacceptable level of amylograph viscosity and was not satisfactory for making products such as white salted noodles. The last harvest was clearly associated with high α-amylase activity, especially in the sprouting of the susceptible cultivars, HY (2750 mU/g) and CK (1801 mU/g). Relatively higher α-amylase activity was detected in LC (996 mU/g), whose α-amylase is often activated in the absence of germination (Nakatsu, 1999). In contrast, only tiny increases in α-amylase activity were found during extremely late harvesting in ST and HA, which are sprouting-tolerant cultivars. Thus, there was tremendous variation in α-amylase activity (188–2750 mU/g) among the cultivars at the last

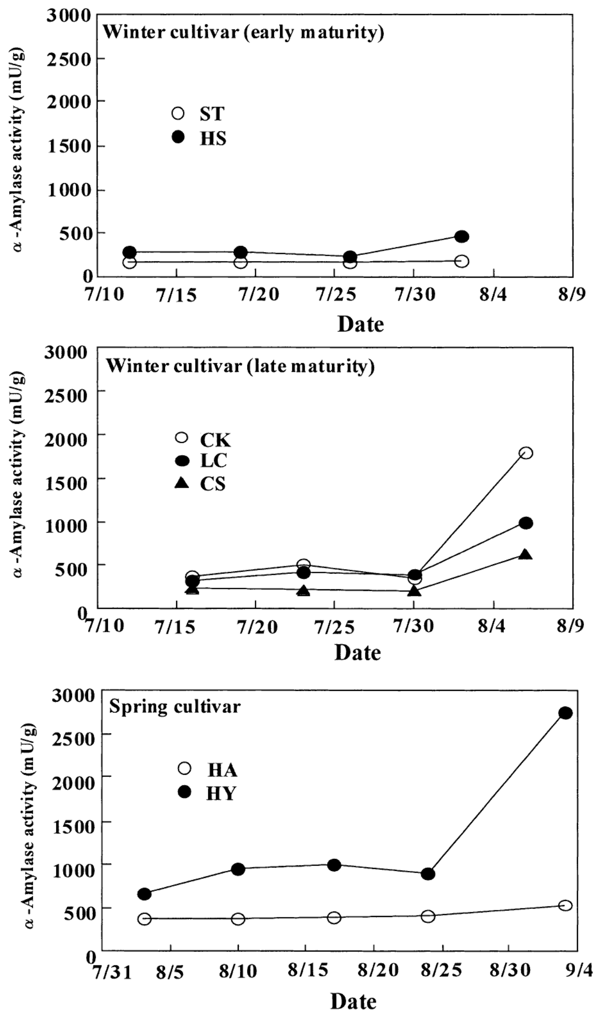

Fig. 1. Changes in α-amylase activity of flour during late development of wheat grain.

harvest.

It is widely recognized that α-amylase activity influences the flour pasting properties in wheat grain. Therefore, we determined the pasting properties of wheat flours, and the results are shown in Table 1. Although all cultivars showed significant decreases in peak viscosity and breakdown during development, the pattern of decrease differed among cultivars. Among the five winter cultivars, ST and HS ranked highest in peak viscosity (216–261 RVU) and breakdown (111–123 RVU), not showing their reduction until the third harvest. In contrast, the values of peak viscosity and breakdown tended to drop during early-middle harvest in HY and LC. CK exhibited definite reductions in peak viscosity and breakdown at the second harvest. Unexpectedly, increases in peak viscosity and breakdown were found at the third harvest in CK. The last harvest was associated with the lowest values of peak viscosity and breakdown in all winter cultivars. Concerning the two spring cultivars, higher values of peak viscosity (97–198 RVU) and breakdown (72–105 RVU) were observed in HA at all harvests than in HY (peak viscosity, 39–96 RVU; breakdown, 21–69 RVU). Peak viscosity was almost constant until the third harvest in HA, while it tended to decrease during early development in HY. At the last harvest, the orders of peak viscosity and breakdown among cultivars were ST>HA>HS>LC=CS>CK=HY, which was almost the same as the order of α-amylase activity.

As mentioned above, we assumed that the reduction in flour peak viscosity was primarily caused by the enhancement of the α-amylase activity. To investigate if a clear decline in flour viscosity is reflected in part by the reduced viscosity of starch in wheat flour, the pasting properties of the starches isolated from the flours were analyzed by delaying the harvest date. The results are presented in Table 1. A clear decrease in peak viscosity was observed throughout the development in HY. Therefore, the peak viscosity was low (116 RVU) in HY at the last harvest, which displayed high α-amylase activity (2750 mU/g). In contrast, developmental changes in peak viscosity and breakdown were not distinctly found in the cultivars, except for the data of peak viscosity in HY, which was different from the data of flour viscosity. Even CK at the last harvest, which displayed high α-amylase activity (1801 mU/g), exhibited relatively higher values of peak viscosity (278 RVU) and breakdown (106 RVU). It has been reported that α-amylase from germinated wheat adsorbs onto starch granules (Sargeant & Walker, 1978). Therefore, we examined α-amylase activity adsorbed onto these granules. No or very little α-amylase activity (less than 25 mU/g) in starch granules was found in all samples except HY at the last harvest, in which high α-amylase activity (534 mU/g) was detected in these granules. In HY at the last harvest, starch granules were presumably degraded by the adsorbed α-amylase, which led to a significant reduction in starch peak viscosity, while the slurry of starch and water was heated in RVA. Abou-Guendia and D'Appolonia (1973) reported an increase in starch peak viscosity during maturation of wheat grain. It was, however, impossible to compare their data with ours, as our research was performed using wheat grain obtained from the extremely late harvest period.

Wetting treatment Wheat seeds (HS) were soaked in water for 24 h at 5°C and dried for 48 h at 20°C to stimulate preharvest sprouting conditions. The results of α-amylase activity and the pasting properties of flour and starch are presented in Fig. 2. α-Amylase activity of flour was low (32–69 mU/g) until

Table 1. Changes in the pasting properties of flour and starch during late development of wheat grain.

Cultivar	Harvest date	Flour		Starch	
		Peak viscosity (RVU)	Breakdown (RVU)	Peak viscosity (RVU)	Breakdown (RVU)
Winter cultivar					
ST	7/12	251	123	295	79
	7/19	261	121	314	109
	7/26	261	124	316	107
	8/2	178	103	302	109
HS	7/12	235	115	294	96
	7/19	207	112	280	80
	7/26	216	111	287	72
	8/2	79	57	295	58
CK	7/16	174	84	262	98
	7/23	67	42	284	89
	7/30	169	82	285	124
	8/6	35	17	278	106
LC	7/16	129	81	249	85
	7/23	79	52	255	115
	7/30	83	54	287	88
	8/6	44	26	302	101
CS	7/16	181	79	290	98
	7/23	178	73	280	96
	7/30	135	69	268	93
	8/6	48	27	293	101
Spring cultivar					
HA	8/3	176	94	290	113
	8/10	173	97	300	119
	8/17	198	105	287	118
	8/24	156	93	296	116
	9/3	97	72	262	121
HY	8/3	96	69	247	108
	8/10	68	47	187	128
	8/17	66	45	240	106
	8/24	76	52	204	102
	9/3	39	21	116	95

12 h and began to increase sharply after that, reaching as high as 4512 mU/g after 48 h. According to the report of Ichinose *et al.* (2001), higher α-amylase activity (536–3821 mU/g) was observed in the flours from wheat grain of three cultivars preserved for 24 h at 20°C after soaking in water for 24 h at 5°C. From the results of flour pasting properties, there was little change in peak viscosity (214–231 RVU) and breakdown (102–110 RVU) up to 12 h. Distinct decreases in peak viscosity and breakdown were observed between 12 and 48 h. Thus, there was a correspondence of decreases in peak viscosity and breakdown with an increase in α-amylase activity. The results of starch pasting properties indicated that peak viscosity decreased slightly by 24 h and that breakdown increased by 6 h and then decreased gradually between 6 and 36 h. Relatively high values of peak viscosity (255 RVU) and breakdown (133 RVU) were found even after 48 h. The data that we obtained with a wetting test also revealed that excess accumulation of α-amylase did not occur with a large decline in the peak viscosity of starch. Lorenz *et al.* (1981) reported that, after 2 days of sprouting, the viscosity of starch in triticale and barley was reduced to almost zero according to the Brabender amylograph. They also reported that, in corn, no decrease in starch viscosity was observed until 2 days of sprouting, while a significant reduction in starch viscosity was detected from 4–7 days of sprouting. No α-amylase activity in starch granules was found in any of the samples (data not shown).

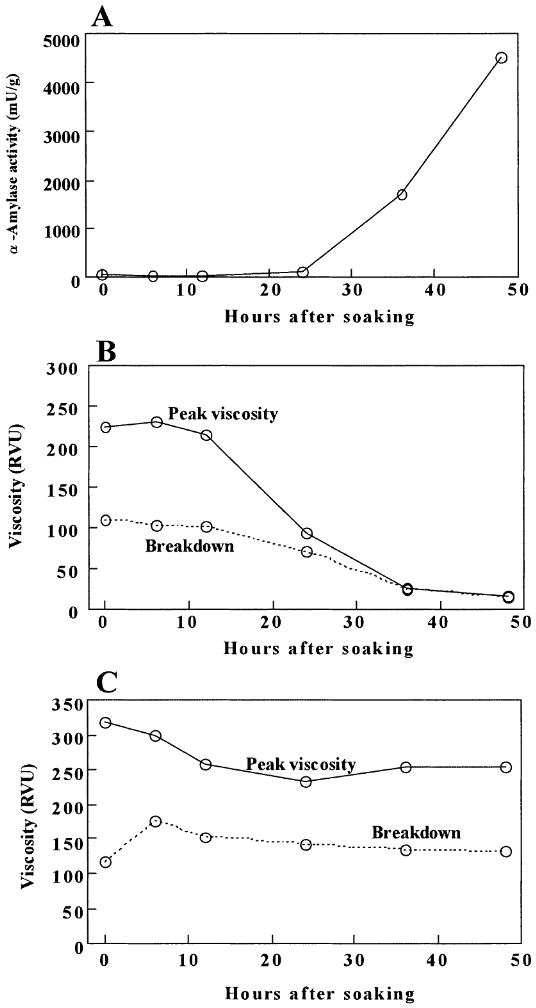

Fig. 2. Effects of period after soaking on α-amylase activity of flour (A) and the pasting properties of flour (B) and starch (C) in wheat grains.

Correlation between α-amylase activity and pasting properties The Brabender amylograph (Hutchinson, 1966; D'Appolonia *et al.*, 1982) and falling number (Hutchinson, 1966; D'Appolonia *et al.*, 1982; Perten, 1964; Finney, 1985; Moot & Every, 1990) have long been used to estimate α-amylase activity in studies on the relationship between this activity and wheat flour's viscometric properties. Additionally, RVA has been applied to screen sprout-damaged wheat (Ross *et al.*, 1987). The aim of this investigation was to determine the contribution of α-amylase activity to the RVA properties of the isolated starch as well as those of flour, using the data (n=36) obtained from developmental and wetting treatment tests. A curvilinear relationship was found between flour peak viscosity and α-amylase activity. Similarly, flour breakdown showed a curvilinear relationship with α-amylase activity. As the $\log_e$ of the results was plotted, notably higher correlation coefficients were found between flour peak viscosity and α-amylase activity ($r=-0.937$)

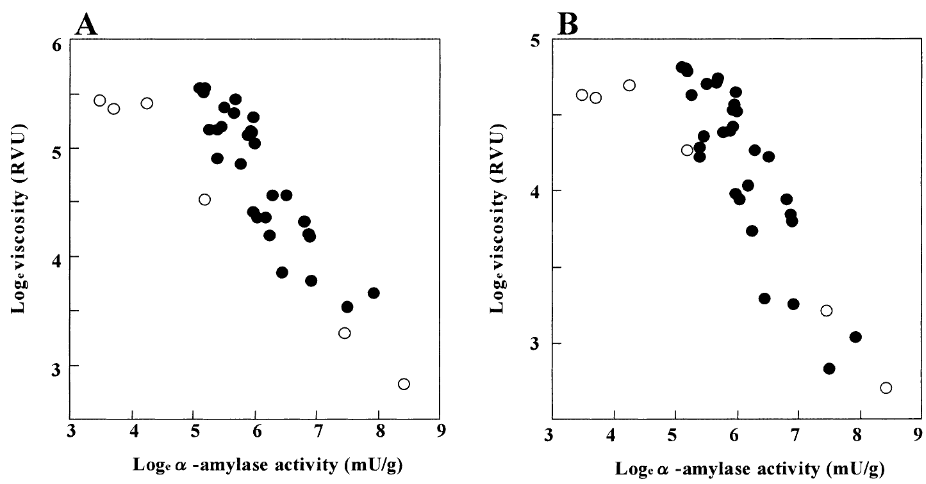

Fig. 3. Relationship between either $\log_e$ flour peak viscosity (A) or $\log_e$ flour breakdown (B) and $\log_e$ α-amylase activity of flour. ● and ○ indicate samples obtained from developmental and wetting treatment tests, respectively.

and between flour breakdown and α-amylase activity ($r=-0.908$) (Fig. 3). The trends were similar to the reported data on the relation of α-amylase activity to flour peak viscosity (D'Appolonia *et al*., 1982). Similarly, the $\log_e$ of the falling number was reported to be negatively correlated with the $\log_e$ of α-amylase activity (D'Appolonia *et al*., 1982; Moot & Every, 1990). These results mean that the starch degraded by α-amylase activity affects the flour viscosity and the falling number. The degradation of protein by endo-protease activity in wheat grain may have an effect on flour viscosity. However, Ichinose *et al*. (2002) reported that endo-protease activity was induced in the later stages of germination than α-amylase activity. They suggested that the contribution of endo-protease to flour viscosity was small. When flour peak viscosity and flour breakdown were higher than 200 RVU ($\log_e$ of viscosity, 5.30 RVU) and 100 RVU ($\log_e$ of viscosity, 4.61 RVU), respectively, predicting α-amylase activity was less satisfactory. If wheat flour has low α-amylase activity, its viscosity is assumed to be reflected by the viscosity of starch itself but not by this activity.

Compared to flour viscosity data, the relationships obtained with the isolated starch between peak viscosity and α-amylase activity and between breakdown and α-amylase activity were not as definite, although a significant negative correlation coefficient ($r=-0.484$) was found between the former two. It is reasonable that wheat flour with high α-amylase activity contains starch with low viscosity. In contrast, remarkable differences in starch peak viscosity (116–278 RVU) were observed among the flours with α-amylase activity higher than 1000 mU/g. In 1970, using sprout-damaged wheat, Meredith confirmed that the inactivation of α-amylase by acidification (Meredith, 1970a) and use of salts of heavy metal (Meredith, 1970b), such as silver nitrate and mercuric chloride, increased flour peak viscosity. These results, including ours, indicated that damaged starch was not necessarily produced in wheat grain with a high activity of sprout-induced α-amylase. Presumably, HY at the last harvest, which exhibited high α-amylase activity and low starch peak viscosity, was regarded as a rather rare case. Further investigation will be needed to elucidate several physicochemical properties of partially digested starch from HY at the last harvest.

In this study, wetting treatments and delayed harvests were used on wheat grain to examine the effects of sprout-induced α-amylase on the pasting properties, determined by RVA, of isolated starch and flour. From the results of all experiments, α-amylase activity that is present in wheat flour plays an important part in determining flour peak viscosity, while it has a lesser effect on starch peak viscosity.

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