Starch Pasting Properties and Amylose Content from 17 Waxy Barley Lines

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ABSTRACT

Starch pasting properties and amylose content from 17 waxy barley lines (*waxy* gene originating from indigenous lines and an artificial mutant) were analyzed using rapid viscosity analysis (Rapid Visco Analyser [RVA]). Amylose contents varied from 0% (Shikoku-hadaka 97) to 9.5% (Shikoku-hadaka 96) compared with 30% for normal barley. Eight parameters were obtained from RVA profiles of these lines and correlation between each of these parameters and amylose content were evaluated. These parameters include pasting temperature (PT), peak viscosity (PV), temperature at PV, minimum viscosity (MV), final viscosity (FV), breakdown (BD), setback (SB), and time maintained at >80% PV (hot paste stability [HPS]). Significant correlations (0.64 and 0.61) were found between amylose content and FV and SB, respectively. High correlation (0.72) was found between amylose content and temperature at PV. HPS calculated from RVA profiles showed the highest correlation (0.79) to amylose content. Outer part of barley grains contained higher amounts of amylose than the inner part. There was a tendency that both PT and FV positively correlated to the amylose content of these parts.

Cereal endosperm starch generally consists of amylose and amylopectin. Endosperm amylose content plays an important role in the quality of cereal products because it affects starch properties. Indigenous waxy barley lines have 2–10% amylose (Ishikawa et al 1994; Washington et al 2000), while waxy lines such as rice and corn have been reported to have no amylose. Indigenous waxy barley lines originated in East Asia and most are hull-less. Hull-less barley is used for food such as miso paste and boiled pearled rice and barley. Waxy barley is conventionally used for specialized foods in Japan and Korea. The waxy phenotype produces a texture stickiness that most Japanese prefer, so the potential uses of waxy barley is expected to expand. The 403bp deletion in the 5'-untranslated region of the *waxy* gene was found invariably in waxy barley lines (Domon et al 2002a). The agriculturally improved waxy barley 'Daishimochi' (Doi et al 1999) was developed and released by introgressing the indigenous *waxy* gene of 'Mochimugi D' into the cultivar. Daishimochi flour and wheat flour mixtures are used in bread, biscuits, cake, and noodles. In addition, amylose-free waxy mutant barley was bred by chemical mutagen (Ishikawa et al 1994) to expand the use of waxy barley in food. Patron et al (2002) and Domon et al (2002b) found that a stop codon had been introduced in the coding region of *waxy* gene, causing inactivation of the gene. The highly variable amylose content of starch granules from waxy phenotypes of barley is important in assessing the physicochemical properties of starch.

We studied starch pasting properties of 17 waxy barleys using rapid viscosity analysis (RVA) (Rapid Visco Analyser RVA-4, Newport Scientific, Warriewood, Australia). Amylose contents and RVA profiles were obtained and the relationship between amylose contents and RVA parameters was calculated and evaluated. We also studied starch properties from different pearled fractions of some waxy lines.

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MATERIALS AND METHODS

Table I lists the nine indigenous waxy lines, seven introgressed waxy lines, and a mutant waxy line together with one normal line used in our work. Waxy phenotype of introgressed waxy lines was introduced from an indigenous waxy line. Waxy origin of some introgressed lines cannot be clearly specified.

Starch Extraction

Grains were pearled to 60% weight (hull-less) or 55% (hulled) using a small pearling machine (Kett Electronic Laboratory, Tokyo, Japan), then ground using a cyclotec mill (Cyclotec Tecator 1093, Tecator AB, Höganäs, Sweden) to obtain a whole grain fraction. For fractionating grains, all grains were pearled to 40% weight. The obtained bran was used for extracting starch from the outer part (40%). The grains were pearled to 20% weight and ground to obtain starch from the inner part (0–20%). Barley flour was homogenized with 0.08% NaOH solution and centrifuged at 3,000 rpm for 10 min. Starch residue was washed three times with 0.08% NaOH, neutralized, and washed with distilled water. Starch was defatted with 80% methanol.

Apparent Amylose Content and Iodine Staining

Apparent starch amylose content was determined using a spectrophotometer based on colorimetry of the iodine-starch complex (A_{620nm}). 95% Ethanol (1 mL) was added to 100 mg of starch. After dispersion by a vortex mixer, 9 mL of 0.1*N* NaOH was added to the suspension, which was then heated in a boiling water bath for 10 min to give clear solution. This solution was quantitatively transferred to a 100-mL volumetric flask and diluted with distilled water. After transferring 1.25 mL of the solution to another tube, 0.25 mL of 1*N* acetic acid and 0.5 mL of a potassium iodide and iodine solution (0.2% KI, 0.04% I_2, w/v) were added. Finally, the solution was quantitatively transferred to a 25-mL volumetric flask. The absorbance at 620 nm was measured in 50 μg/mL of starch solution. A potato amylose (Sigma Type III) (100% amylose) and mutant waxy barley (total fraction of starch from Shikoku-hadaka 97 [0% amylose]) (Ishikawa et al 1994) starch mixture was used as the standard. Apparent indicates that the contribution of amylopectin to absorbance was not considered. Experiments were done at least five times. Endosperm starch granules were stained with a potassium iodide and iodine solution (0.2% KI, 0.04% I_2 w/v) and observed by light microscopy.

Starch Pasting Properties

Starch paste viscosity was measured using rapid viscosity analysis (RVA) (Rapid Visco Analyser RVA-4, Newport Scientific, Warriewood, Australia). Starch (2.5 g, 13.5% wb) was mixed with 25 mL of distilled water. The suspension was heated from 35 to

95°C at 3°C/min and held at 95°C for 5 min, then cooled to 35°C at 3°C/min. Peak viscosity (PV), minimum viscosity (MV), and final viscosity (FV) were recorded, and breakdown (BD: PV minus MV) and setback (SB: FV minus MV) were calculated. The temperature at PV and time maintained at >80% of PV (hot paste stability; HPS) (Kiribuchi-Otobe et al 2001) were also determined.

RESULTS

Apparent Amylose Content and Starch Pasting Properties

Amylose content of starch from 17 waxy lines and one normal line are shown in Table I. The value range was 0–9.5% for waxy lines, far lower than the value (29.0%) of normal barley.

RVA profiles of lines with highest (Shikoku-hadaka 96), medium (Daishimochi), and lowest (Shikoku-hadaka 97) amylose content are shown in Fig. 1. There are considerable differences among the three lines. RVA profiles of all other lines were also obtained and parameters of these profiles are summarized in Table I. Compared with the Shikoku-hadaka 97, values for HPS, temperature at PV, FV, and SB were higher in Shikoku-hadaka 96. The correlation coefficient between RVA parameters and amylose content was calculated. There was no correlation between amylose content and PT, PV, MV, or BD. Significant correlation was observed between SB (0.61), FV (0.64), and amylose content. High correlation (0.72) was observed between temperature at PV and amylose content. HPS value had a range of 101.7–293.7 sec, which showed the highest correlation (0.79) to amylose content.Starch Pasting Properties of Different Pearled Fractions

RVA profiles of the inner and outer parts of Shikoku-hadaka 96 are shown in Fig. 2a and b. Amylose contents and RVA parameters of starch from the outer and inner part of Daishimochi, Waxy Odorbrucker, and Shikoku-hadaka 96 are listed in Table II. Amylose content in the outer part is higher than that in the inner part. With all three lines, starches of the outer part, which have higher amylose contents than the inner part, showed higher temperature at PV in accordance with the tendency found in 17 waxy lines.

Outer parts of Shikoku-hadaka 96 and Waxy Odorbrucker showed higher FV than the inner parts. But this relationship did not hold in Daishimochi. Surprisingly, no relationship was observed between HPS and amylose content.

Under microscopic observation, all starch granules of Shikoku-hadaka 97 (Fig. 3A) stained with KI-I_2 are reddish brown, although starch granules stained with KI-I_2 are a mixture of reddish brown and blue in Shikoku-hadaka 46 (Fig. 3B) and Shikoku-hadaka 96 (Fig. 3C).

DISCUSSION

Amylose Effect on Starch Pasting Properties in Waxy Barley

RVA parameters such as FV and SB were correlated with amylose content. Zeng et al (1997) reported significant correlation between apparent amylose content and RVA parameters in wheat. The correlation coefficients between RVA parameters and apparent amylose content were reported at –0.91 (PV), 0.87 (MV), 0.97 (FV), –0.92 (BD), and 0.95 (SB). Yamamori et al (2000) also pointed out that these RVA parameters correlated with amylose content in wheat. The correlation coefficients between RVA parameters and amylose content were reported at –0.58 (PV), 0.85 (MV), 0.96 (FV), –0.78 (BD), and 0.93 (SB). Apparent amylose content of samples was 17.5–23.5% in Zeng et al (1997)

TABLE I
RVA Parameters and Amylose Content for Waxy Barley Lines and Correlation Coefficients of Each Parameter to Amylose Content[a]

Line Name	Waxy Origin	Amylose Content (%, db)	PT (°C)	Temp at PV (°C)	PV (SNU)	MV (SNU)	FV (SNU)	BD (SNU)	SB (SNU)	HPS (sec)
Shikoku-hadaka 97	Mutant	0.0	63.1	69.1	295.7	73.8	164.3	221.9	90.5	113.4
Shikoku-hadaka 46	Introgressed	1.8	63.9	69.1	318.7	61.3	153.1	257.3	91.8	101.7
Mochimugi D	Indigenous	2.3	66.8	73.9	326.3	66.6	138.4	259.7	71.8	172.1
Tokushima mochimugi	Indigenous	3.1	66.4	72.8	304.8	64.9	157.1	239.9	92.2	152.6
Dango mugi	Indigenous	3.6	65.0	71.8	360.5	79.3	169.1	281.3	89.8	144.7
Daishimochi	Introgressed	4.0	65.7	71.3	329.2	55.0	175.1	274.2	120.1	105.6
Yatomimochi	Indigenous	4.1	66.8	71.3	286.3	54.9	145.8	231.4	90.8	117.3
Himekei 605	Introgressed	6.1	66.8	73.8	325.2	64.7	145.5	260.5	80.8	136.9
Sumiremochi	Indigenous	6.2	65.6	74.4	280.3	65.6	193.7	214.7	128.1	293.7
Yonezawamochi	Introgressed	6.5	65.8	72.8	321.8	69.2	175.8	252.7	106.6	174.0
Hizen	Indigenous	7.2	65.8	74.0	354.3	60.9	151.6	293.4	90.7	197.5
Awamugi	Indigenous	7.8	67.1	75.0	334.3	79.6	189.7	254.7	110.1	287.5
Bouzu mochi	Indigenous	7.8	66.6	74.1	296.6	71.3	177.7	225.3	106.4	209.3
Hiroshima mochimugi	Indigenous	8.7	66.1	74.4	364.9	70.9	165.8	294.0	94.8	217.1
Waxy Betzes	Introgressed	8.8	65.8	86.6	298.7	76.8	198.1	221.9	121.3	269.9
Waxy Odorbrucker	Introgressed	9.0	65.1	90.1	247.8	72.3	203.5	175.4	131.2	264.0
Shikoku-hadaka 96	Introgressed	9.5	65.0	84.4	290.6	71.8	201.5	218.8	129.8	268.0
Correlation coeffcient			0.37	0.72**[b]	–0.11	0.32	0.64**	–0.18	0.61**	0.79**
Shikoku-hadaka 84	Nonwaxy	29.0	73.4	89.3	103.3	65.5	297.7	37.8	232.2	219.0

[a] PT, pasting temperature; PV, peak viscosity; MV, minimum viscosity; FV, final viscosity; BD, breakdown; SB, setback; HPS, hot paste stability (time maintained >80% of PV); SNU, stirring number unit.
[b] Significant at $P < 0.01$ (**).

TABLE II
RVA Parameters and Amylose Content of Different Pearled Parts in the Same Waxy Lines[a]

Line Name	Pearled Fraction	Amylose Content (%, db)	PT (°C)	Temp at PV (°C)	PV (SNU)	MV (SNU)	FV (SNU)	BD (SNU)	SB (SNU)	HPS (sec)
Shikoku-hadaka 96	0–20%	4.7	64.9	78.2	249.7	64.3	185.3	152.8	121.0	391.1
	40%	8.5	62.6	90.3	251.8	54.6	197.3	192.7	142.7	183.9
Waxy Odorbrucker	0–20%	6.1	64.8	86.0	245.3	75.6	175.7	169.8	100.1	412.7
	40%	9.5	64.8	93.8	266.3	82.0	263.7	184.3	181.7	121.3
Daishimochi	0–20%	3.4	63.8	70.6	297.0	50.4	137.7	246.6	87.3	115.3
	40%	5.7	64.7	72.4	274.8	60.8	131.6	214.0	70.8	217.0

[a] PT, pasting temperature; PV, peak viscosity; MV, minimum viscosity; FV, final viscosity; BD, breakdown; SB, setback; HPS, hot paste stability (time maintained >80% of PV); SNU, stirring number unit.

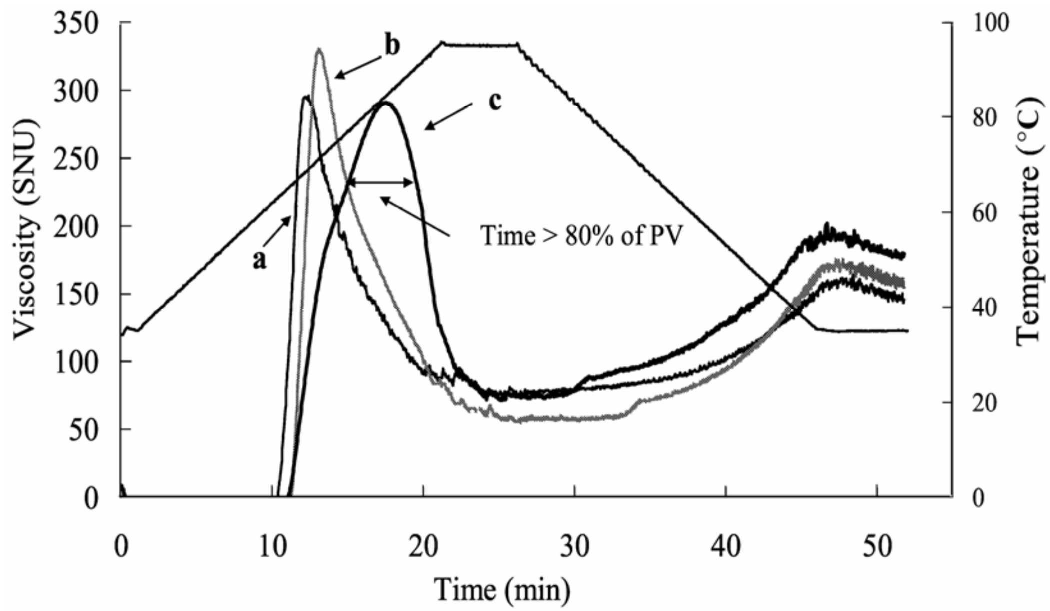

Fig. 1. Starch pasting profiles of three waxy barley lines: a, Shikoku-hadaka 97; b, Daishimochi; c, Shikoku-hadaka 96.

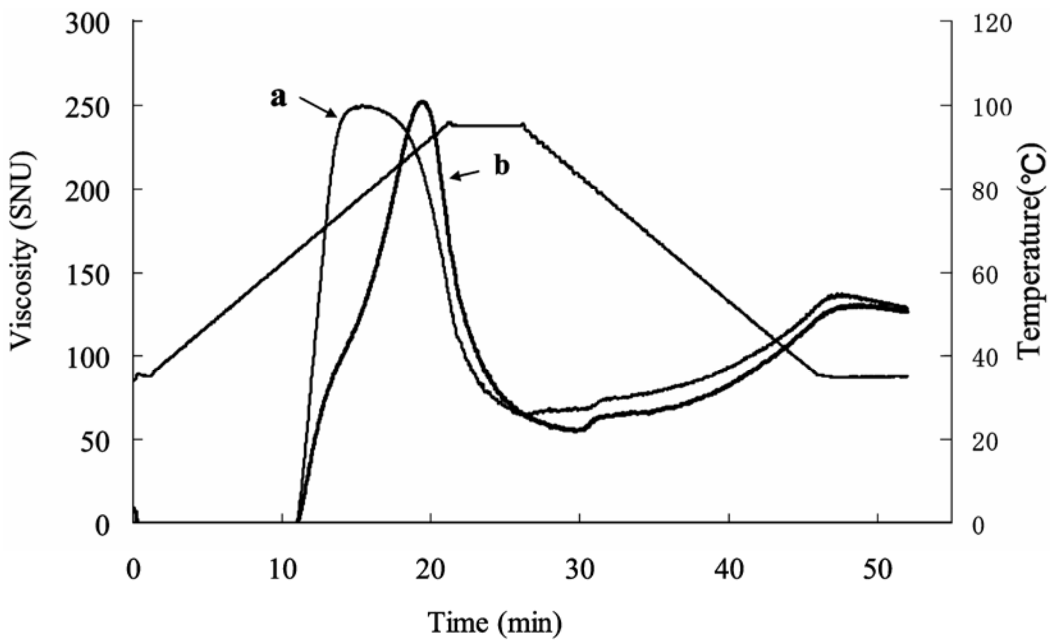

Fig. 2. Starch pasting profiles of the inner and outer parts of Shikoku-hadaka 96 grains: a, inner part (0–20%); b, outer part (40%).

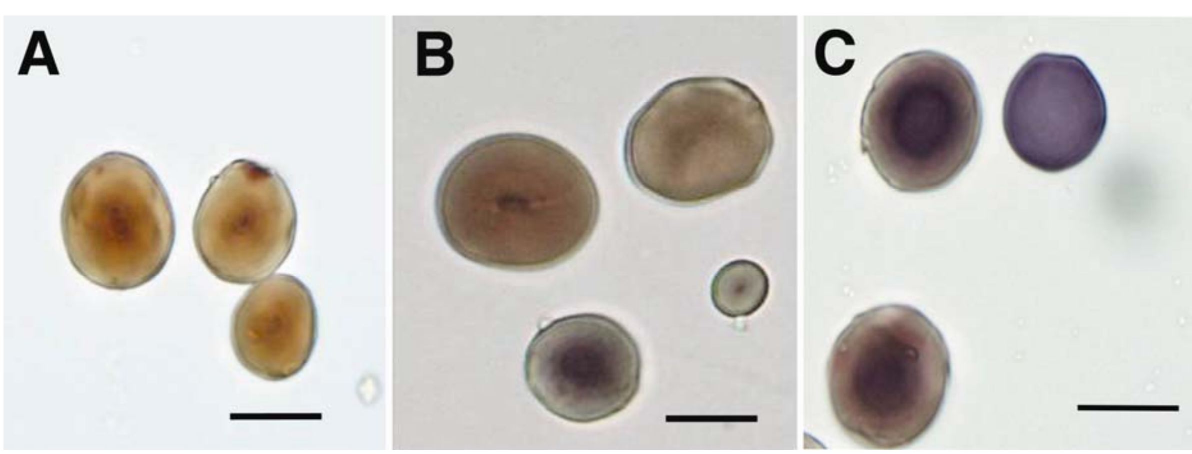

Fig. 3. Photographs of starch-iodine stain of three waxy barley lines. **A,** Shikoku-hadaka 97; **B,** Shikoku-hadaka 46; **C,** Shikoku-hadaka 96. Bar = 20 µm.

and 16.3–29.4% in Yamamori et al (2000). In our study, the values were –0.11 (PV), 0.32 (MV), 0.64 (FV), –0.18 (BD), and 0.61 (SB). Our study reported values that were lower than those in the earlier studies, which may be due to the difference in the range of amylose content. In earlier studies, amylose contents varied 16.3–29.4%, whereas amylose content varied only 0–9.5% in this work. We previously reported that RVA profiles and parameters changed according to the increased amylose content using mutant waxy wheat (≈5–11% amylose) (Yanagisawa et al 2004). Correlation coefficients between RVA parameters and amylose contents were –0.80 (PT), 0.87 (temperature at PV), 0.31 (PV), 0.11 (MV), 0.64 (FV), 0.31 (BD), 0.58 (SB), and –0.79 (HPS) in mutant wheat. The results for both studies showed that the correlation was observed between amylose content and temperature at PV, FV, or SB, while no correlation was observed between amylose content and PV, MV, or BD. HPS and amylose content were negatively correlated in mutant wheat.

Analyses of barley lines with higher (10–20%) amylose content are required to understand the relationship between RVA parameters and amylose content. Table II shows the outer parts of the grains, which contain higher amounts of amylose than the inner parts and showed higher temperature at PV than the inner parts. This, together with high correlation coefficient (0.72) between amylose content and temperature at PV, suggests that amylose inhibits the swelling of starch granules. Other factors such as amylopectin structure may influence HPS in RVA.

Figure 3A indicated the stained starch granules of Shikoku-hadaka 97. No Wx protein (amylose synthase) is expressed in the artificial mutant waxy gene (Ishikawa et al 1994). All grains are homogeneously stained reddish-brown in agreement with the results of Andersson et al (1999). The starch granules of normal barley were evenly stained with blue (data not shown). The finding that starch granules of Shikoku-hadaka 46 and 96 were heterogeneously stained from reddish brown to blue suggests that starch granules in these lines are heterogeneous in amylose content. The difference in amylose content in the outer and inner parts of waxy barley may be due to the difference in amylose synthase activity in these parts.

Applications

Our results showed that native waxy barley starches from some indigenous and introgressed waxy lines have stable hot paste viscosity, suggesting that these starches could be more widely used than modified starches because most consumers prefer natural ingredients over chemically modified forms. These results should prove to be useful to barley breeders and food chemists.

CONCLUSIONS

Amylose content of waxy barley starch was obtained by measuring the absorbance in starch-iodine complex. Starch pasting properties were measured using an RVA and eight parameters were calculated. FV (0.64) and SB (0.61) significantly correlated with amylose content. High (0.72) correlation was observed between amylose content and temperature at PV. There was the highest correlation (0.79) between HPS and amylose content. No correlation was observed between PT, PV, MV, or BD and amylose content. Amylose content in the outer parts is higher than that in the inner parts of indigenous and introgressed waxy lines.

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