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Flour Quality Characteristics of Korean Waxy Wheat Lines

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ABSTRACT : Flour physicochemical properties of six Korean waxy wheat lines and their parental plants, including Kanto 107 and BaiHuo, which have partially null in GBSS (granule bound starch synthase), were evaluated in this study. The very low amylose content (3.20%) of Korean waxy wheat lines, which had been influenced by the null in all three GBSS isoforms encoded by three *Wx* loci, could result in the higher starch swelling power (25.15%), lower starch and flour pasting temperature (61.37°C; 65.85°C), and higher starch pasting peak viscosity and breakdown (246.60 RVU; 161.50 RVU) than those of their parental plants. In addition to high swelling and pasting properties, Korean waxy wheat lines had the higher protein content (12.80%), alkaline water retention capacity (97.39%), SDS sedimentation volume (80.33 ml) and damaged starch content (4.35%) than those of their parental plants.

Keywords : waxy wheat, flour, granule-bound starch synthase (GBSS), swelling and pasting properties

Starch, mainly composed of amylose and amylopectin, is the major component of wheat flours. In general, it is well known that genetic variations of amylose content exist among varieties, and that lower amylose content is significantly related to good eating quality of noodles. Noodles is one of the major manufactured food products processed from soft wheat in Asia (Toyokawa *et al.*, 1989). Flours with swelling volume, swelling power and high peak viscosity and low breakdown in pasting properties can produce high eating quality of cooked noodles. These swelling and pasting properties are significantly related to amylose content. Improvement of noodle quality could be achieved by the genetic manipulation of the amylose content.

Granule-bound starch synthase (GBSS; EC 2.4.1.21), known as waxy (Wx) protein, is responsible for the synthesis of amylose in wheat (Yamamori *et al.*, 1994). The identification of GBSS has been proposed as a means of selecting lines for noodle-making purposes in wheat breeding programs. In common wheat the GBSS comprises three products encoded by three *Wx* loci and the 60KDa protein (Nakamura *et al.*, 1993; Yamamori *et al.*, 1992). Three loci, *Wx-A1, Wx-B1* and *Wx-D1*, located on the chromosome arms 7AS, 4AL and 7DS, respectively, encode GBSS (Nakamura *et al*, 1993; Yamamori *et al.*, 1994). Recently, Japanese scientists have discovered the presence of naturally occurring mutants that are absent of one or two of GBSS in *Wx* loci (Nakamura *et al.*, 1993). Wheat with each lacking one or two *Wx* proteins is frequently termed "partial waxy mutant" (Miura & Tanii, 1994).

The worlds first waxy wheat lines were produced by genetically eliminating the GBSS isoforms, using Kanto107 (null in *Wx-A1* and *Wx-B1)* as the pollen parent and BaiHuo (null in *Wx-D1)* as the seed parent, and selecting F_2 progenies lacking the three GBSS isoforms (Nakamura *et al.*, 1995; Yamamori *et al.*, 1995). Waxy wheat lines were also produced by treating seeds of the double null genotypes with the mutagen ethyl methane sulphonate (Oda *et al.*, 1992). Waxy wheat, which lacks all three GBSS, produces amylose-free starch. Wheat flours from partial waxy mutants exhibit excellent starch characteristics suitable for production of white salted noodles. Most Canadian and U.S. wheat cultivars lack the null alleles for GBSS isoforms, and wheat accessions with mutations at the *Wx* loci are being used in a breeding program to develop partially waxy wheats (Demeke *et al.*, 1997; Graybosch *et al.*, 1998).

Wheat cultivars with low amylose content are more suitable for noodle making, because the eating quality of noodles is negatively correlated with amylose content of starch. Waxy wheat lines have been shown to have lower amylose content and higher peak viscosity than those of other wheat flours (Kiribuchi-Otobe *et al.*, 1997). Food producers, wheat breeders and numerous researchers have recently focused on the availability of waxy wheat lines for improvement of end-use quality of wheat, since waxy wheat could have potential commercial utilities. In Korea, waxy wheat lines with genetic recombinations have recently been developed through crosses Kanto107, BaiHuo and Korean wheat cultivars. There is no available information about the physico-chemical properties of waxy wheat lines. Therefore, this study was conducted to determine the physical, chemical and functional properties

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of Korean waxy wheat lines.

MATERIALS AND METHODS

Six Korean waxy wheat lines, SW97108-B-WV-18-B (Urimil//Kanto107/BaiHuo), SW97129-B-WV-23-B (Alchanmil//Kanto107/BaiHuo), SW97132-B-WV-17-B (Alchanmil//Kanto107/BaiHuo), SW97110-B-WV-9-B (Geurumil// Kanto107/BaiHuo), SW97111-B-WV-3-B (Geurumil// Kanto107 /BaiHuo) and SW97112-B-WV-21-B (Geurumil// Kanto107/BaiHuo), were obtained from the National Crop Experiment Station, Suwon, Korea. Waxy lines and their parental cultivars and lines, Kanto 107, BaiHuo, Alchanmil, Geurumil and Urimil, were harvested in Suwon (Upland Crop Experimental Farm of National Crop Experimental Station) in 1998.

Wheat was milled to about 60% extraction on a Bühler experimental mill. Protein content (N $\times$ 5.7) was determined by boric acid modification of the micro-Kjeldahal method. Ash content was determined by AACC approved methods 08-01 (AACC, 1983). Amylose content was determined by the procedure of Morrison and Bernard (1983) with a primary starch. Primary starch was prepared by the procedure of South and Morrison (1990). SDS sedimentation test and micro-alkaline water retention capacity (AWRC) were performed according to the procedure of Axford *et al.* (1979) and Kitterman and Rubenthaler (1971), respectively. Swelling power of starch (SSP) and damaged starch content was measured according to the procedure of Crosbie (1991) and Gibson *et al.* (1992), respectively. Falling number values were measured by the procedure described by Bason *et al.* (1995) using a Rapid-Visco Analyser (RVA)-3 (Newport Scientific Pty. Ltd., Warriewood, Australia). The flour mixing characteristics were determined by 10-g mixograph (National Mfg., Lincoln, NE, U.S.A.), AACC approved methods 54-40A (AACC, 1983).

Water-washed prime starch of Korean winter wheat cultivars and Korean commercial flours was isolated by AACC approved methods 38-10 (AACC, 1983). Pasting properties of flour and starch were measured by RVA-3, using 4.0 g of flour and 3.0 g of starch suspended in 25.0 ml of water. The temperature profile of RVA was followed the procedure of Battey *et al.* (1997), with some modifications. The temperature profile is as follows: hold at 60°C for 2 min, heat to 95°C at 5.83°C/min over 6 min, hold at 95°C for 4 min, cool to 50°C at 11.25°C/min over 4 min, and hold at 50°C for 6 min for flour and 7 min for starch. Total analysis was taken 22 min for the flour sample and 23 min for the starch sample. The measured parameters of RVA were pasting temperature, peak viscosity, holding strength, final viscosity, breakdown, setback and total setback. The viscosities of RVA were expressed in Rapid-Visco Analyser units (RVU).

To determine granule-bound starch synthase (GBSS) isoforms, the purification of starch granules from a mature single kernel was performed as previously described for friabilin (Hong & Park, 1999). The preparation of GBSS from isolated starch granules was based on the procedure described by Seo *et al.* (1998).

Data analysis were performed by the SAS Package (SAS, 1995) using analysis of variance (ANOVA), and Fishers least significant difference procedure (LSD).

RESULTS AND DISCUSSION

Granule-Bound Starch Synthase (GBSS) of Korean waxy wheat lines

Fig. 1. shows electrophoration patterns of granule bound starch synthase (GBSS) of waxy wheat lines and their parental lines. Waxy wheat lines showed null in all three GBSS encoded by three loci *Wx-A1, Wx-B1* and *Wx-D1*. Kanto107 showed null in *Wx-A1* and *Wx-B1*, and BaiHuo showed null in *Wx-D1*, as previously reported (Seo *et al.*, 1998). Alchanmil, Geurumil and Urimil contained all three GBSS proteins. This single kernel analysis method could be utilized effectively for small sample selection in wheat quality breeding programs, because single kernel analysis requires only 3/4 of a single kernel and showed a good resolution in SDS-PAGE.

Takaoka *et al.* (1997) reported minor proteins with high molecular weight of starch granule-bound proteins (HMW-SGPs), SGP-1, -2 and -3. They also reported that the electro-

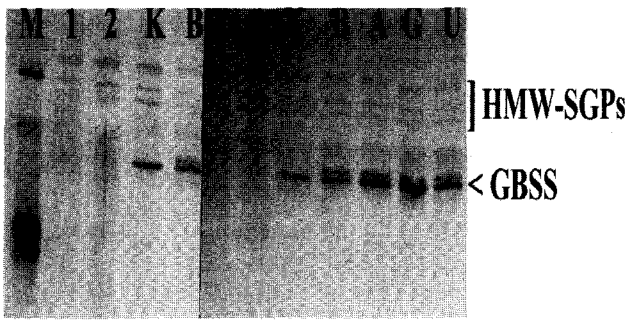


Fig. 1. One-dimensional SDS-PAGE (17%) patterns of GBSS isolated from a single kernel of Korean waxy wheat lines and their parental lines. Waxy lines corresponding to each lane were: lane 1, SW97108-B-WV-18-B; lane 2, SW97129-B-WV-23-B; lane 3, SW97132-B-WV-17-B; lane 4, SW97110-B-WV-9-B; lane K, Kanto107; lane B, BaiHuo; lane A, Alchanmil; lane G, Geurumil; lane U, Urimil. M contains molecular weight markers of 97.4, 66.2, and 45 kDa. Arrow indicates GBSS band.I. Pasting Patterns of Starch II. Pasting Patterns of Flour.

phoresis patterns of the SGP-2 and -3 (92 and 80 KDa) of wheat were similar to those of rice and barley, but that the SGP-1 (115-100 KDa) of wheat were not found in rice and barley. There were no differences of HMW-SGPs between waxy wheat lines and their parental lines in this study. Therefore, further study of HMW-SGPs should be considered to elucidate the relationships between the starch properties and starch synthases of waxy wheat lines and their parental lines.

Physicochemical characteristics of Korean waxy wheat lines

Physicochemical characteristics of Korean waxy wheat lines and their parental lines are summarized in Table 1. Ash content of Korean waxy wheat lines showed no difference from that of their parental plants. Waxy wheat lines showed much lower amylose content, which formed 2.24% for SW97129-B-WV-23-B to 4.20% for SW97110-B-WV-9-B, than that of their parental plants. Protein content of waxy wheat lines ranged from 12.10% for SW97110-B-WV-9-B to 13.45% for SW97108-B-WV-18-B, and was lower than 10.72% in parental plants except in BaiHuo (12.56%). Damaged starch content of Korean waxy wheat lines ranged from 3.11% for SW97108-B-WV-18-B to 5.59% for SW97112-B-WV-21-B, and that of their parental plants ranged from 2.18% for Kanto107 to 4.64% for Alchanmil. SDS-sedimentation volume of waxy wheat lines was higher than 75.67 ml and their parental plants showed lower than 73.33 ml except in Alchanmil (88.00 ml). Micro-alkaline water retention capacity (AWRC) of waxy wheat lines was also higher than 91.30% and their parental plants ranged from 65.45% for Urimil to 75.08% for BaiHuo. However, the falling number of waxy wheat lines was lower than 397.58 SN but their parental plants showed higher than 466.48 SN. Starch swelling power of waxy wheat lines ranged from 24.40% for SW97132-B-WV-17-B to 26.02% for SW97108-B-WV-18-B, and was lower than 15.36% in their parental plants. Mixograms of dough used in this study are depicted in Fig. 2. Korean waxy wheat lines showed higher water absorption of mixograph than that of their parental plants, except for BaiHuo. Alchanmil and Urimil showed higher mixing tolerance than that of other parental lines and waxy wheat lines. Korean waxy wheat lines, except for SW97129-B-WV-23-B and SW97132-B-WV-17-B, showed lower mixing time than that of their parental plants.

Yasui *et al.* (1996) reported that the apparent amylose content (1.2-2.0%) of waxy wheat was significantly lower (26.0-28.4%) than that of their non-waxy parents. They also reported that waxy wheat lines had much lower lipid content compared with their non-waxy parents. However, amylopectin of waxy wheat lines was structurally identical to that of the parents. Fujita *et al.* (1998) reported no difference in the mean granule diameter of each of the waxy wheat samples when these were compared with non-waxy wheat. Very low amylose content of Korean waxy wheat lines in this study is in agreement with the result of Yasui *et al.* (1996). Kanto107, *Wx-A1* and *Wx-B1*-deficient cultivar showed lower amylose content (19.39%) than that of Korean winter wheat cultivars. However, no difference was found between BaiHuo, *Wx-D1*-deficient cultivar and Korean parental plants.

Table 1. Flour characteristics of Korean waxy wheat lines and their parental plants.

Cultivar/Line	Ash (%)	Protein (%)	Amylose (%)	SDSS[†] (ml)	SD (%)	AWRC (%)	FN (SN)[‡]	SSP (%)	MAB (%)	MTO (Cm)	MTM (Sec)
SW97108-B-WV-18-B	0.43	13.45	3.39	81.67	3.11	96.99	368.87	26.02	62.60	7.00	150.00
SW97129-B-WV-23-B	0.43	12.44	2.24	78.67	5.54	102.68	253.08	24.55	61.50	6.50	240.00
SW97132-B-WV-17-B	0.55	12.96	3.61	87.00	3.59	95.02	342.07	24.40	62.10	6.50	270.00
SW97110-B-WV-9-B	0.43	12.10	4.20	75.67	3.87	91.30	233.94	24.52	61.20	7.00	120.00
SW97111-B-WV-3-B	0.45	12.99	2.32	81.00	4.38	99.67	383.23	25.69	62.10	6.00	135.00
SW97112-B-WV-21-B	0.48	12.87	3.42	78.00	5.59	98.66	397.58	25.73	62.00	6.00	135.00
Kanto107	0.44	10.72	19.39	73.33	2.18	67.56	585.15	14.25	59.90	7.00	150.00
BaiHuo	0.47	12.56	30.62	68.00	4.21	75.08	700.95	15.36	61.70	7.00	142.50
Urimil	0.52	8.62	28.71	62.67	2.31	65.45	559.31	11.42	58.00	11.50	270.00
Geurumil	0.53	10.42	31.50	62.67	4.13	74.08	466.48	12.60	59.60	6.25	130.00
Alchanmil	0.46	10.34	29.80	88.00	4.64	72.24	534.43	13.35	59.50	11.00	270.00
LSD[§]	0.05	0.31	1.21	0.01	0.10	1.11	27.15	0.41	0.00	0.51	7.10

[†]SDSS=SDS sedimentation volume; SD=damaged starch content; AWRC=alkaline water retention capacity; FN=falling number.; SSP= starch swelling power; MAB=water absorption of mixograph; MTO=mixing tolerance of mixograph; MTM=mixing time of mixograph.
[‡]Arbitrary unit described by Bason *et al.* (1995).
[§]Least significant difference (P = 0.05). Differences between two means exceeding this value are significant.

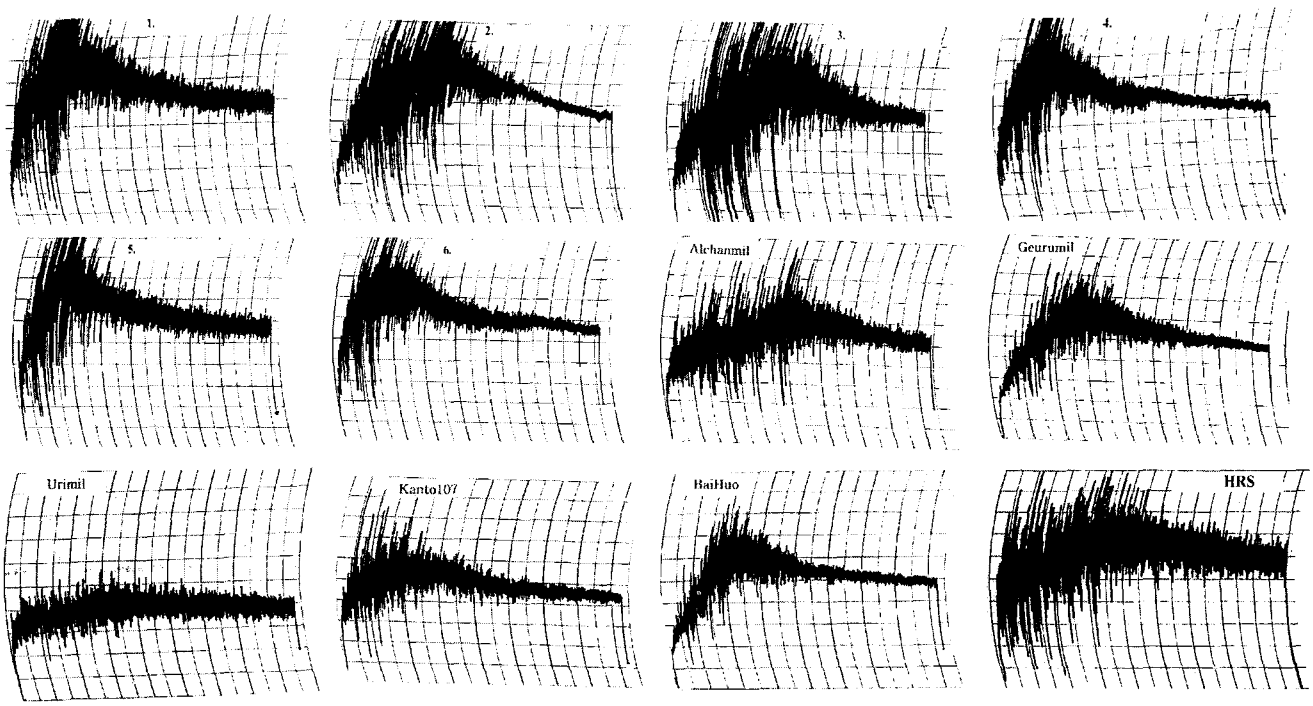


Fig. 2. Mixogram of Korean waxy wheat lines and their parental lines. Waxy lines corresponding to each number were: 1, SW97108-B-WV-18-B; 2, SW97129-B-WV-23-B; 3, SW97132-B-WV-17-B; 4, SW97110-B-WV-9-B; 5, SW97111-B-WV-3-B; 6, SW97112-B-WV-21-B; HRS, Hard Red Spring Wheat Standard Flours for Baking.

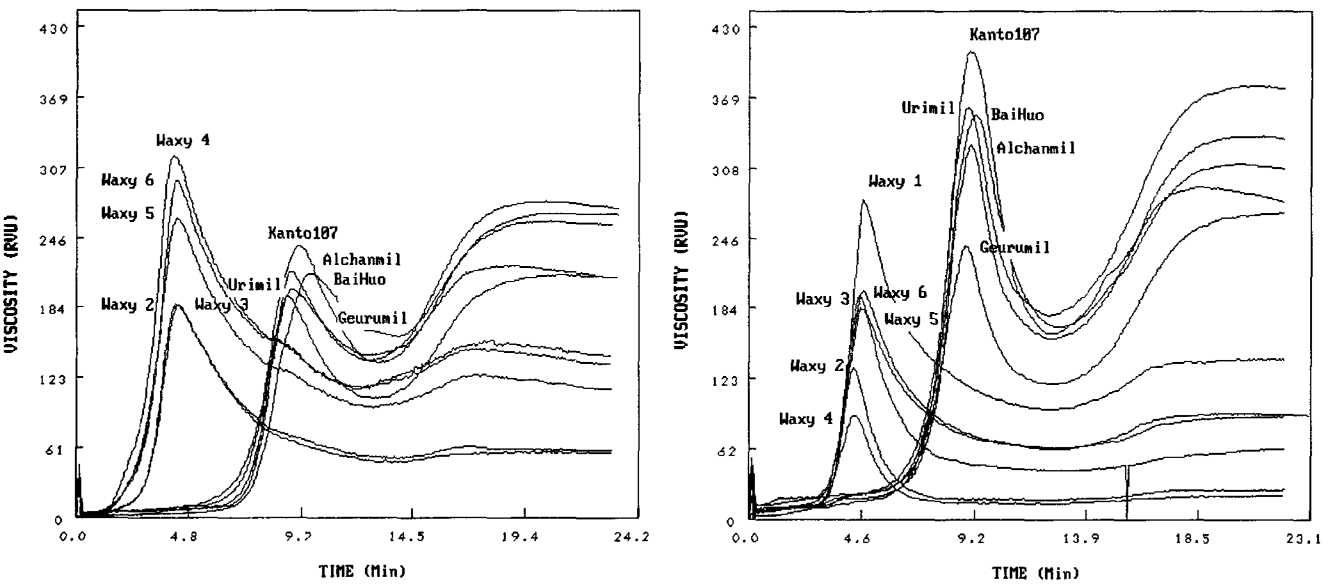
Yasui *et al.* (1999) reported that waxy wheat lines showed low starch content and higher β-glucans and particle size than those of non-waxy parental plants. They also reported that waxy wheat lines had higher swelling power than their parental plants, although no difference was found in protein content between waxy wheat lines and their parental plants (Yasui *et al.*, 1999). Korean waxy wheat lines showed higher starch swelling power (25.15%) than that of their parental plants, and Kanto107 and BaiHuo had higher starch swelling power (14.25% and 15.36%) than Korean winter wheat cultivars. Two Korean waxy wheat lines, SW97129-B-WV-23-B and SW97132-B-WV-17-B, had similar protein contents (12.44% and 12.96%) to hard red spring wheat standard flours for bread (12.50%). Compositions of high molecular weight glutenin subunits (HMW-GS) of SW97132-B-WV-17-B are 1Ax1, 1Bx7+1By8 and 1Dx5+1Dy10. SW97129-B-WV-23-B also had 1Dx5+1Dy10 of HMW-GS. The protein content and HMW-GS compositions of these two lines might influence higher mixing time of mixograph than those of other waxy lines.

Korean waxy wheat lines had higher protein content than their parental plants. SDS sedimentation volume of Korean waxy wheat lines may have been higher than their parental plants because SDS sedimentation volume is mainly influenced by their high protein content and/or high proportion of high molecular weight. Korean waxy wheat lines showed higher starch damage content, except in SW97108-B-WV-18-B, than that of their parental lines. Korean waxy wheat lines also showed higher AWRC value than their parental plants. Although many physico-chemical properties of Korean waxy wheat lines were not measured, Korean waxy wheat could also have different characteristics compared with their parental plants. These different physico-chemical characteristics of waxy wheat lines, along with high amylopectin of starch, could influence water binding capacity in dough mixing. Korean waxy wheat lines showed higher water absorption of mixograph compared with their parental plants. These characteristics could influence rheological properties of waxy wheat flours, and these rheological properties could eventually affect end-use properties.

The very low falling number of Korean waxy wheat lines was not influenced by sprout damage at the post-harvesting stage. Waxy wheat flours had low falling numbers compared with non-waxy wheat plants, albeit waxy wheats had less α-amylase activity than non-waxy plants (Graybosch *et al.*, 2000; Yasui *et al.*, 1999). Compared with non-waxy starch, since waxy starch swells greatly and would disintergrate at lower temperature, where α-amylase is still active, amylopectin molecules would be hydrolyzed by active α-amylase in swelled and disintegrated waxy starch granules (Yasui *et al.*, 1999). Waxy wheat flours had low falling numbers that were independent α-amylase activity (Graybosch *et al.*, 2000). This more α-amylase attractable property at lower temperature of waxy wheats could influence end-use quality, especially baking of bread and cooking of noodles.

Pasting properties of Korean waxy wheat flour and starch

Fig. 3 shows the typical patterns of pasting properties of starch and flour of Korean waxy wheat lines and their parental plants. Starch and flour pasting properties of Korean waxy wheat lines and their parental lines are summarized in Table 2. Pasting temperature of waxy wheat lines was lower than 63.58°C and higher than 86.78°C for parental plants. Peak viscosity of Korean waxy wheat lines was higher than 263.50 RVU, except in SW97132-B-WV-17-B and SW97129-B-WV-23-B (176.00 and 180.50 RVU, respectively). Peak viscosity of their parental plants was lower than 240.50 RVU. Breakdown of waxy wheat lines was



I. Pasting Patterns of Starch **II. Pasting Patterns of Flour**

Fig. 3. Pasting properties of starch (I) and flour (II) of Korean waxy wheat lines and their parental plants using Rapid Visco Analyser. Waxy lines corresponding to line were: Waxy1, SW97108-B-WV-18-B; Waxy2, SW97129-B-WV-23-B; Waxy3, SW97132-B-WV-17-B; Waxy4, SW97110-B-WV-9-B; Waxy5, SW97111-B-WV-3-B; Waxy6, SW97112-B-WV-21-B.

Table 2. Pasting properties of starch and flour of Korean waxy wheat lines and their parental plants.

Cultivar/Line	Pasting Properties of Starch							Pasting Properties of Flour						
	PT† (°C)	PV (RVU)	HS (RVU)	FV (RVU)	BD (RVU)	SB (RVU)	TS (RVU)	PT (°C)	PV (RVU)	HS (RVU)	FV (RVU)	BD (RVU)	SB (RVU)	TS (RVU)
SW97108-B-WV-18-B	-	-	-	-	-	-	-	66.08	278.50	94.50	137.50	184.00	-141.00	43.00
SW97129-B-WV-23-B	63.15	180.50	51.00	61.00	129.50	-119.50	10.00	65.83	131.50	17.50	27.50	114.00	-104.00	10.00
SW97132-B-WV-17-B	63.58	176.00	51.00	62.50	125.00	-113.50	11.50	66.35	193.00	41.00	57.00	152.00	-136.00	16.00
SW97110-B-WV-9-B	60.08	315.50	115.00	153.50	200.50	-162.00	38.50	65.58	91.00	12.50	19.50	78.50	-71.50	7.00
SW97111-B-WV-3-B	60.03	263.50	95.00	123.50	168.50	-140.00	28.50	65.38	186.00	61.00	88.00	125.00	-98.00	27.00
SW97112-B-WV-21-B	60.00	297.50	113.50	148.00	184.00	-149.50	34.50	65.93	164.00	54.50	78.50	109.50	-85.50	24.00
Kanto107	87.25	240.50	139.00	224.00	101.50	-16.50	85.00	84.35	408.50	163.00	289.00	245.50	-119.00	126.50
BaiHuo	89.68	217.50	163.00	264.00	54.50	46.50	101.00	85.73	315.50	148.00	283.00	167.50	-32.50	135.00
Urimil	86.78	197.50	136.00	262.00	61.50	64.50	126.00	85.28	362.00	181.00	383.00	181.00	21.00	202.00
Geurumil	87.95	188.50	104.00	222.50	84.50	34.00	118.50	86.45	241.00	123.00	277.00	118.00	36.00	154.00
Alchanmil	88.50	215.50	138.00	278.00	77.50	62.50	140.00	85.50	334.00	159.00	341.50	175.00	7.50	182.50
LSD‡	1.70	9.81	7.70	7.70	8.61	13.00	8.00	1.00	9.22	7.70	7.14	8.17	6.84	5.10

†PT=pasting temperature; PV=peak viscosity; HS=holding strength; FV=final viscosity; BD=breakdown; SB=setback; TS=total setback.
‡Least significant difference (P = 0.05). Differences between two means exceeding this value are significant.

higher than 125.00 RVU, and their parental plants showed a lower than 101.50 RVU. In pasting temperature of flour, waxy wheat lines also showed lower temperature than that of parental plants, like the pasting temperature of starch. Peak viscosity of waxy wheat lines was lower than that of parental plants, except in SW97108-B-WV-18-B (278.50 RVU). Breakdown of waxy wheat lines was also lower than that of parental plants except in SW97110-B-WV-9-B (184.00 RVU). Korean waxy wheat lines showed lower final viscosity in both starch and flour pastings than those of parental plants.

Starch pasting properties of waxy wheat lines showed higher peak viscosity, holding strength and final viscosity than those of their parental plants. SW97129-B-WV-23-B and SW97132-B-WV-17-B showed lower peak viscosity, holding strength and final viscosity than those of other waxy wheat lines. Further study about pasting properties of these lines should be considered for the elucidation of differences in starch pasting properties within waxy wheat lines. Flour pasting parameters of parental plants, except for Geurumil, were higher than those of waxy wheat lines. Kanto107 showed higher flour pasting parameters than those of others. Flour pasting parameters of Korean waxy wheat lines were lower than those of their parental lines and starch pasting parameters of waxy wheats.

In starch pasting properties, waxy wheat lines showed lower pasting temperature (61.37) than their parental plants, which is in agreement with a report by Hayakawa *et al.* (1997). Kiribuchi-Otobe *et al.* (1997) reported that pasting profiles of the starch of waxy wheats and waxy maize were similar, though the peak viscosity of the waxy wheat was much higher than that of waxy maize. Waxy wheat lines showed higher peak viscosity (246.60 RVU) and breakdown (161.50 RVU) than their parental plants, which is in agreement with a report by Reddy and Sieb (1999). In a flour pasting properties, Korean waxy wheat lines showed lower pasting temperature (65.85) than their parental plants. Kanto107 showed the highest peak viscosity among waxy wheat lines and their parent plants. Kanto107 also had higher flour and starch swelling volume (26.88 ml and 31.59 ml) than other parental plants (*data not shown*). These properties of Kanto107 might have resulted from the reduced amylose content by the null GBSS encoded by *Wx-A1* and *Wx-B1*, and also could influence end-use quality, especially noodle quality. Korean waxy wheat lines, however, showed lower peak viscosity (174.00 RVU) and holding strength (46.83 RVU) than their parental plants. Hayakawa *et al.* (1997) found that peak viscosity of waxy wheat samples to be lower than that of non-waxy wheat samples, while Kiribuchi-Otobe *et al.* (1997) reported that peak viscosity of waxy wheat samples to be higher than others. However, Yasui *et al* (1999) reported that the flour pasting tests of waxy wheat demonstrated lower peak temperature and viscosity in water, but viscosity parameters in silver nitrate solution, which acted as inhibitors amylase, increased significantly, while temperature parameters were unaffected. They also proposed that the difference in viscosity parameters in the different pasting media probably arises from the hydrolysis of amylopectin molecules in starch granules by active α-amylase at lower temperatures. Although limited sample numbers of waxy wheat lines were assessed in this study and further study on pasting properties of waxy wheat lines is required, waxy wheat starch and flours could be used as new materials to improve starch pasting properties in other foods and to reduce energy in food processing because of their high peak viscosity and rapid increase in peak viscosity in low temperature. In addition to pasting properties of waxy wheat lines, study on flour composition effect on end-use quality should be considered for the improvement and utilization of waxy wheat lines.

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