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Physical and Structural Characteristics of Endosperm Starch of Four Local Barnyardgrass (*Echinochloa crus-galli* L.) Collections in Korea

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ABSTRACT Physical and structural characteristics of four local barnyardgrass flour and starch were investigated. Amylose content ranged from 6.9 to 12.5%. The BP 3563 and BP 3606 were low amylose lines, and the BP 3592 and BP 3610 showed very low amylose lines. Peak viscosities of four barnyardgrass collections BP-3563, BP-3592, BP-3606, and BP-3610 ranged 264 to 2397. All barnyard grass lines displayed an A-type X-ray diffraction pattern showing the strong diffraction peak at around 2θ values 15°, 17°, 18° and 23°, and a small peak at 20° 2θ. Scanning electron microscopy revealed that the starch granule sizes ranged 5.5-12.5 μm in diameter showing polygonal and rarely spherical in shape with round edges and some pores at the surface. Starch crystallinity of BP 3563, BP 3592, BP 3606, and BP 3610 was 23.9, 25.0, 24.7, and 25.5%, respectively.

Keywords : barnyardgrass, *Echinochloa crus-galli* L., starch, amylose

Barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] has been recognized the most significant biological constraint to rice production and also one of the major weeds in paddy fields. Varieties of the *E. crus-galli* complex are among the world's most serious agricultural weeds (Holm, 1977). Barnyardgrass is particularly abundant in flooded rice fields where it reduces yields by up to 40%. Since early 1998, the crop rotation like paddy-upland rotation cropping system in Korea has been widely examined using corn, soybean, sesame, barley and wheat before and after rice transplanting in order to reduce barnyard grass occurrence in Korea. Barnyardgrass has been increasingly cultivated by cattle ranchers as a billion-dollar grass and recommended for forage with some forage value. Furthermore, at present, the flours of barnyardgrass seed has been used to the additives like gruel, cake, liquor with rice flours. The seeds of barnyard grass contain about 13.2% protein, 2.6% fat, 9.5% ash, 7.2% lignin, and 19.6% amylose (Fujimoto *et al.*, 1986). The flours derived from barnyardgrass have been used as food additive or replacement in Korea. Until now, no an attempt to evaluate starch properties of the barnyardgrass was for edible use. There is very little information on starch separated from barnyardgrass. Thus, the objective of the present study is to investigate the starch viscosity and physicochemical properties of barnyard grass grown in Korea.

MATERIALS AND METHODS

Four local collections of barnyardgrass (*Echinochloa crus-galli* L.) were grown and harvested in the experimental fields of Gyeongsangbuk-do Agricultural Research and Extension Services, Daegu, Korea.

For the starch analysis, the alkaline steeping procedure to isolate barnyardgrass starch was followed by the method (Wang and Wang, 2001). Barnyardgrass flour (20 g) was steeped in 40 ml of 0.1% NaOH for 18 hr. The slurry was blended with a Waring blender at a high seed for 2 min, passed through 100 mesh sifter and centrifuged at 1,300×*g* for 10 min. The top layer was carefully removed and bottom layer was reslurried and washed with three times with 0.1% NaOH. The top layer was removed and then the starch layer was washed with deionized water and

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centrifuged. The combined starch was then reslurried and neutralized with 0.1 N HCl to pH 6.5, and washed with deionized water four times, centrifuged, dried in an oven 45℃ for 48 hr.

The absorption curves of starch and iodine complexes were measured by a UV/VIS spectrophotometer (Model Evolution 300, Thermo Electron Corporation, USA) at 700 to 500 nm. A solution containing 2 mg iodine and 20 mg potassium iodate was added to 1 mg NaOH-gelatinized and HCl-neutralized starch, and made up to 25 ml. The wavelength at maximum absorption (λmax) and blue value (BV), absorbance at 680 nm, were determined (Fujimoto *et al.* 1972). According to the method of Kainuma (1977), amperometric iodine titration of defatted starch was carried out at 1A and 50 mV.

The pasting properties of the starches (3 g, 14% moisture basis) in water (25 ml) were determined using the Rapid Visco Analyzer (RVA, Newport Scientific Pty. Ltd., Narrabeen, Australia). DSC was performed on a Differential Scanning Calorimeter (DSC-SP, Rheometric Scientific, New Castle, DE, USA) and the instrument was calibrated with indium. Starch samples and distilled water (1:3, w/w) were hermetically sealed in aluminum pans, held overnight, and heated from 30 to 120℃ with 10℃ min^{-1} heating speed. An empty aluminum pan was used as reference. Purified starch granules were sputter coated with gold and examined with scanning electron microscope (Model JSM-56000LV, JEOL) at 10 or 20 kV. DSC of starches was measured as described by Fujita *et al.* (2003). X-ray diffraction pattern of starches was obtained with copper, nickel foil-filtered, Kα-radiation using a diffractometer RINT 2000 at 50 kV and 27 mA. The data were analyzed using the ANOVA procedure. Significant differences ($P<0.05$) between means were further determined by Duncan's multiple range test.

RESULTS AND DISCUSSION

The absorbance at 680 nm (blue value), starch and amylose contents for four local barnyardgrasses, BP 3610, BP 3592, BP 3606, and BP 3563 are presented in Table 1. Blue values were ranged from 0.13 to 0.27 at 680 nm. The starch content was ranged to 83.4 to 88.6%. Among these barnyardgrasses, the BP 3610 had highest starch content as 88.6%. Apparent amylose content in four barnyardgrasses ranged from 6.9 to 12.5%. Among these barnyardgrasses, two barnyardgrass both BP 3563 and BP 3606 were classified to low amylose lines, and two barnyardgrass of BP 3592 and BP 3610 were also classified as very low amylose lines, respectively. It assumed that the higher amylose content from BP 3563 starch may due to the presence of large-size granules. It is also known that the amylose content is responsible for some factors affecting swelling powder, solubility, and gel forming property of starch. These differences might be explained by the different growing conditions, the methods used for lipid extraction, and amylose determination (Mali *et al.* 2003).

Paste properties of barnyardgrass flours were determined by using a Rapid Visco Analyser (Fig. 1). All barnyardgrass samples displayed a type-B pasting curve, as characterized by a low to medium peak viscosity and low shear thinning during cooking (Schoch and Maywald 1968). Two different curve patterns showed that high peak viscosity was observed at BP 3610 and BP 3592, and low peak viscosity was observed at BP 3606 and BP 3563.

Parades-Lopez (1994) reported that low peak viscosity is

Table 1. Wavelength at maximum absorption (λmax), absorbance at 680 nm (blue value, BV), starch and amylose content in barnyardgrass starches.

Local varieties	λmax (nm)	Blue value (at 680 nm)	Starch content (%)	Amylose content (%)
BP 3563	594	0.27a	84.8b	12.5a
BP 3592	573	0.15c	84.0b	8.4c
BP 3606	590	0.24b	83.4c	11.0b
BP 3610	539	0.13d	88.6a	6.9d

Starch and amylose content were calculated by dry weight basis. Data are means of four determinations. Different letters within each column indicate significant differences ($p<0.01$)

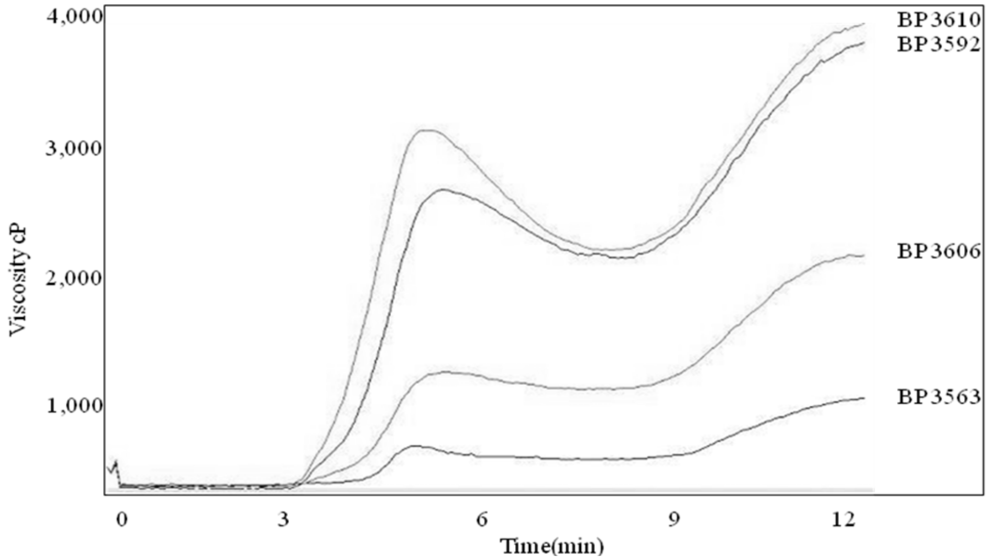

Fig. 1. Pasting properties of viscosity of barnyardgrass flours determined by Rapid Visco Analyzer.

Table 2. Pasting properties of barnyardgrass flours determined by Rapid Visco Analyser.

Local varieties	Pasting time (min.)	Pasting temp. (℃)	Viscosity (RVU)				
			PKV	HPV	CPV	Breakdown	Setback
BP 3563	4.53	91.7	264d	176	590d	88	414d
BP 3592	3.33	77.4	2000b	1515	2988b	465	1473b
BP 3606	4.20	87.7	764c	646	1551c	118	905c
BP 3610	3.27	76.7	2397a	1587	3123a	810	1536a

Different letters within each column indicate significant differences (p<0.01)

due to short chain length and to irreversible damage treated with alkaline media. Setback values in non-glutinous proso millet starches were higher than the waxy proso millet starches. It is generally recognized that if viscosity of setback is high, the retrogradation of starch paste would progress rapidly (Leeiarathi *et al.* 1987).

Table 2 presents the data of pasting properties of barnyardgrass flours determined by Rapid Visco Analyser. Pasting properties are dependent on the rigidity of starch granules, which in turn affect the granule swelling potential (Sandhya Rani and Bhattacharya, 1989) and the amount of amylose leaching out in the solution (Morris, 1990).

Two local barnyardgrass, BP 3592 and BP 3610, had faster pasting time than that of BP 3563 and BP 3606. The peak viscosity ranged from 264 in BP 3563 to 2397 in BP 3610. The cooling peak viscosity (CPV) varied from 590 in BP 3563 to 3123 in BP 3610. Hot paste viscosity is influenced by the rate of amylose exudation, amylose-lipid complex formation, granule swelling, and completion between exudated amylose and remaining granules for free water, while the cold paste viscosity is largely determined by the retrogradation tendency of the soluble amylose upon cooling (Olkku and Rha, 1978).

DSC data of starches separated from barnyardgrass seed are summarized in Table 3. The transition temperatures (T_o, T_p, and T_c), range (T_c-T_o), enthalpies of gelatinization (Δ Hgel), and peak height indices (PHI) of starches from barnyardgrass germplasms differed significantly. DSC parameters showed that the onset temperature (T_o) of all samples ranged from 68.5 to 86.9℃, peak temperature (T_p) ranged 73.4 to 91.3℃, and their gelatinization enthalpy varied from -6.31 to -6.72 J/g. A highest gelatinization temperature was obtained for BP 3606 compared to other

Table 3. Thermal properties of barnyardgrass starches determined by Differential Scanning Calorimeter.

Local varieties	Gelatinization parameters					
	T*o* (℃)	T*p* (℃)	T*c* (℃)	ΔH_{gel} (J/g)	PHI	*R*
BP 3563	83.7b	88.2b	94.5b	-6.72	1.68	10.8
BP 3592	76.5c	81.1d	86.2c	-6.45	1.40	9.7
BP 3606	86.9a	91.3a	96.1a	-6.58	1.50	9.2
BP 3610	68.5d	73.4c	79.3d	-6.31	1.29	10.8

T*o*, onset temperature; T*p*, peak temperature; T*c*, conclusion temperature; R, gelatinization range (T*c*-T*o*); ΔH, enthalpy of gelatinization (based on starch dry weight); PHI, peak height index ΔH gel/(T*p*-T*o*). Data are means of three or four determinations. Different letters within each column indicate significant differences ($p<0.01$)

Table 4. X-ray diffraction data of barnyardgrass starches.

Local varieties	Diffraction peaks at 2θ values			
	15°	17°	18°	23°
BP 3563	15.3	16.9	18.0	22.9
BP 3592	15.1	17.2	18.2	23.1
BP 3606	15.2	17.0	18.2	23.0
BP 3610	15.3	17.1	18.1	23.1

Table 5. Crystal pattern and degree of crystallinity of barnyard-grass starches.

Local vrieties	Degree of crystallinity (%)	Crystal pattern
BP 3563	23.9	A
BP 3592	25.0	A
BP 3606	24.7	A
BP 3610	25.5	A

Degree of crystallinity was determined following equation as Xc=Ac/(Ac+Aa); Ac: the crystallized area; Aa: the amorphous area on the X-ray diffractogram

barnyardgrass germplasms, BP 3563, BP 3592, and BP 3610. The differences in gelatinization temperature may be attributed to the differences in amylose content, size, shape, and distribution of starch granules, and to the internal arrangement of starch fractions within the granules (Grelda *et al.* 1997). Transition temperatures are influenced by the molecular architecture of the crystalline region, which correspond to the amylose and amylopectin ration (Noda *et al.* 1998). The high Δ Hgel of starches suggested that the double helices (formed by the outer branches of adjacent amylopectin chains) that unravel and melt during gelatinization are strongly associated within the native granule. The gelatinization enthalpy reflects the overall measure of crystallinity (quality and quantity of crystallites) of amylopectin and is an indicator of the loss of molecular order within the granules (Tester and Morrison 1990).

PHI is the ratio of Δ *Hgel* for gelatinization to the gelatinization temperatures range and is a measure of uniformity in gelatinization. PHI of BP 3563 starch was the highest which indicates that the higher PHI value of starch may be attributed to the presence of large-size granules (Aggarwal *et al.* 2004).

X-ray diffraction angles, crystallinity and scanning electron photographs of barnyardgrass starches are presented in Table 4, Table 5, Figure 2, and Figure 3, respectively. All barnyard grass lines displayed an A-type X-ray diffraction pattern showing the strong diffraction peak at around 2θ values 15°, 17°, 18° and 23°, and a small peak at 20° 2θ. X-ray diffraction angles showed a typical A-type crystallinity (Table 3). Crystallinity of starch granule in four barnyard grasses, of BP 3563, BP 3592, BP 3606, and BP 3610 was 23.9, 25.0, 24.7, and 25.5%, respectively.

The degree of crystallinity was higher in BP 3610 than in BP 3563, BP 3592, and BP 3606. The increased chrystalline region is strongly dependent on amylose content. It is generally held that A-type and B-type starches reflect the presence of parallel stranded double helices. These are packed quite closely in A-type structures but are more loosely associated for B-type starches (Ratnayake *et al.* 2001). These results suggest that these crystalline regions of amylopectin against amorphous regions had similar microstructure. Scanning electron microscopy revealed that the starch granule sizes ranged 5.5-12.5 μm in diameter. The starch granules of barnyardgrass showed mostly polygonal and rarely elliptical in shape with round edges and some pores at the surface. Among these barnyardgrass

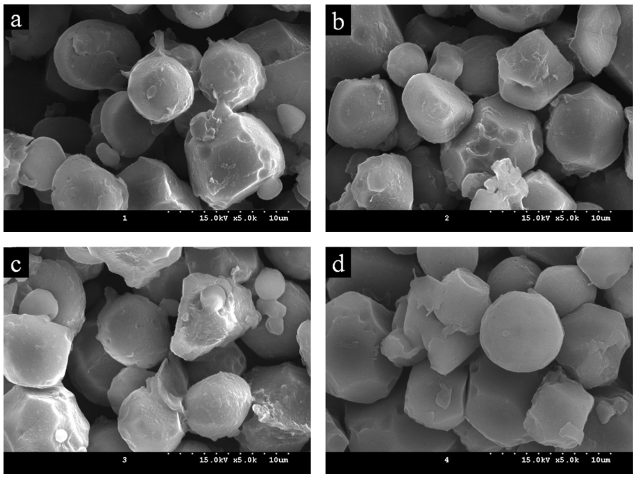

Fig. 2. Scanning electron micrograph of barnyardgrass starch granules (5,000X). a, BP3563; b,BP3592; c, BP3606; d, BP3610.

germplasms, the starch granules in both BP 3563 and BP 3592 had more pores at the surface than that of BP 3606 and BP 3610 and these starch granule sizes ranged from 5.5 to 9.4 μm (Fig. 2).

In brief, the variation in size and shape off starch granules may be due to the biological origin (Svegmark and Hermansson 1993). The morphology of starch granules depends on the biochemistry of the chloroplast or amyloplast, as well as physiology of the plant (Badenhuizen 1969). These results lead to the conclusion that some gelatinization and pasting properties reflect the specific starch granules in starches in addition to amylose content.

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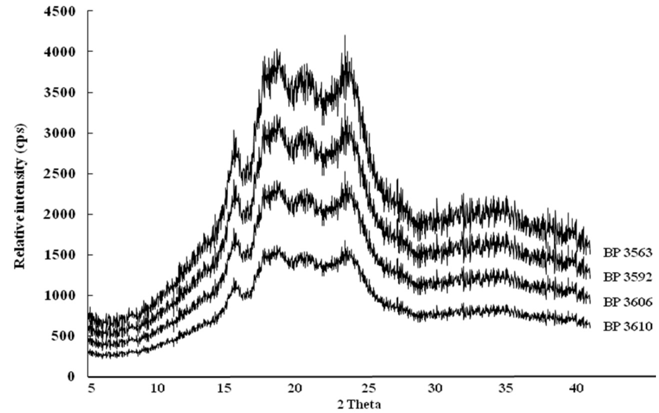

Fig. 3. X-ray diffraction patterns of barnyardgrass starches.

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