Agronomy Research **10** (1–2), 311–318, 2012

Starch properties of native proso millet (*Panicum miliaceum* L.)

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Abstract. Proso millet (*Panicum miliaceum* L.) starches extracted from three varieties ('Andongjaerae', 'Danyangjaerae' and 'Andong 48ho') have been studied. Amylose content ranged between 1.2 and 21.5%. Starch content was from 84.4 to 85.67%. The onset temperature (T*o*) of all starches ranged from 73.1 to 76.4 °C, peak temperature (T*p*) ranged 78.0 to 81.5 °C, and their gelatinization enthalpy varied from -0.81 to -4.48 J g^{-1}, respectively. The highest peak, highest breakdown, very high final viscosity, and very low setback were noted in 'Danyangjaerae'. X-ray diffraction angles showed in all starch granules possessing a typical A-type crystallinity, exhibiting strong deflections at about 15° 2θ, an unresolved double at 17°, 18° 2θ and separated angle 23° 2θ. The degree of crystallinity was higher in 'Andong 48ho' than in 'Andongjaerae' and 'Danyangjaerae'. A highly negative correlation was obtained between setback viscosity and relative crystallinity ($r = 0.987$, $p < 0.01$) with the amylose content and relative crystallinity ($r = 0.964$, $p < 0.01$). Positive correlation was observed between amylose content and setback viscosity ($r = 0.869$, $p < 0.01$).

Key words: proso millet, *Panicum miliaceum* L., starch, amylose, DSC, RVA

INTRODUCTION

Paniceae is the largest tribe of the *Poaceae* (Gramineae), with more than 1400 species. Economically important species of this tribe include proso millet (*Panicum miliaceum* L.), foxtail millet (*Setaria italica* (L.). P. Beauv.), and pearl millet (*Pennisetum glaucum* (L.) R. Br.). The term "millet" is used for several small seeded annual grasses that are of minor importance in the Western world but a staple in the diets or additives of Asian and African people. Among several millet crops, proso millet, known as hershey, broom corn, or hog millet, is planted in some African countries as a food crop (Yañez et al. 1991). Interestingly, in the United States, dehulled proso millet is consumed as a puffed or cooked breakfast cereal or replacement for up to 30% of wheat flour in certain baked products and other household recipes (Hinze, 1972). In Korea, proso millet is generally used in feeding rations, as bird seed and human foods, and its flours are also used for additives to make rice cake and cloudy liquor. Although it was one of the main foods grown here until the 1960s, now it is cultivated mainly in the provinces of Gyeongsangbuk-do and Kangwon-do, which raise nearly 55, and 37%, respectively. Starches comprised of two components, amylose and amylopectin, from different sources such as wheat, corn, potato, foxtail millet, rice, and proso millet are utilized in the food and pharmaceutical industries. In this study, we attempted to isolate the starch from domestically cultivated waxy and glutinous proso millets and compare the starches based on physicochemical properties to ascertain the differences in waxy and non-waxy starches purified from native proso millet.

MATERIALS AND METHODS

General procedures. Three local varieties (‘Andongjaerae’, ‘Danyangjaerae’ and ‘Andong48ho’) of proso millet (*Panicum miliaceum* L.) were grown and harvested in the Institute for Bioresources Research, Gyeongbuk Provincial Agricultural Technology Administration, Andong, Korea. Two of the varieties were the non-glutinous phenotype and one was the waxy phenotype.

Starch isolation. The alkaline steeping procedure to isolate proso millet starch was followed by the method (Wang & Wang, 2001). Proso millet flour (20 g) was steeped in 40ml of 0.1% NaOH for 18 hr. The slurry was blended using a Waring blender at a high speed for 2 min, passed through 100 mesh sifter and centrifuged at 1,300 x *g* for 10 min. The top layer was carefully removed and bottom layer was re-slurried and washed three times with 0.1% NaOH. The top layer was removed, then the starch layer was washed with deionized water and centrifuged. The combined starch was then re-slurried and neutralized with 0.1 N HCl to pH 6.5, and washed with deionized water four times, centrifuged, dried in an oven at 45 °C for 48 hr.

Spectrophotometry of iodine-starch complexes and iodine affinity. The absorption curves of starch and iodine complexes were measured by a UV/VIS spectrophotometer (Evolution 300, Thermo Electron Corp., 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, to make 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.

Characterization of starch by RVA and DSC. 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). Thermal properties of the starches were analyzed using differential scanning calorimetry (DSC, DSC-SP, Rheometric Scientific, New Castle, DE, USA) as described by Fujita et al. (2003). Starch samples and distilled water (1:3, w/w) were hermetically sealed in aluminum pans, held overnight, and heated from 30 to 120 °C with 10 °C min^{-1} heating speed. An empty aluminum pan was used as reference.

Scanning electron microscopy and X-ray diffractometry. Purified starch granules were sputter coated with gold and examined with a scanning electron microscope (Model JSM-56000LV, JEOL) at 10 or 20 kV. 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.

Statistical analysis

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

Spectrophotometry of iodine-starch complexes and iodine affinity

The absorbance at 680 nm (blue value), starch and amylose contents for three proso millets, 'Andongjaerae', 'Danyangjaerae' and 'Andong 48ho' are presented in Table 1. 'Andongjaerae' and 'Danyangjaerae' in blue value were about 0.17 to 0.20 higher than that of 'Andong 48ho'. The highest blue value was only observed in 'Andongjaerae'.

Among these proso millets, the apparent starch content was higher in 'Andong 48ho', waxy type than that in 'Andongjaerae' and 'Danyangjaerae', non-glutinous proso millets. Amylose content was significantly different in analyzed proso millets and ranged from1.2 to 21.5%. The amylose content was highest in 'Andongjaerae' and lowest in 'Andong 48ho'. It is assumed that the higher amylose content from 'Andongjaerae' starch may be 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). Starch content in three proso millet was between 84.4 and 88.3%. Starch content was higher in waxy-type, 'Andong 48ho' than in those in non-glutinous types, 'Andongjaerae' and 'Danyangjaerae'.

Characteristics of DSC and RVA

Thermal properties of starches separated from different varieties are summarized in Table 2. The transition temperatures (T*o*, T*p*, and T*c*), the range of gelatinization temperature (T*c*-T*o*), enthalpies of gelatinization (ΔH_{gel}), and peak height indices (PHI) of starches differed significantly among the varieties.

Thermal properties of three isolated starches of proso millets showed that the onset temperature was different. The variations in initial 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. Transition temperatures are influenced by the molecular architecture of the crystalline region, which correspond to the relative ratio of amylose and amylopectin (Noda et al., 1998). DSC parameters showed that the onset temperature (T*o*) of all starches ranged from 73.1 to 76.4 °C, peak temperature (T*p*) ranged from 78.0 to 81.5 °C, and their gelatinization enthalpy varied from -0.81 to -4.48 J g^{-1}, respectively (Table 2).

The highest enthalpy of the gelatinization ($\Delta\ H_{gel}$) was in 'Andong 48ho'. The higher ΔH_{gel} of starches suggests 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 highest gelatinization temperature was obtained for ‘Andongjaerae’ (76.4 °C) compared to two proso millets (‘Danyangjaerae’ and ‘Andong 48ho’). The gelatinization enthalpy also reflects the overall measure of crystallinity (quality and quantity of crystallites against amorphous regions) of amylopectin, and is also an indicator of the loss of molecular order within the granules (Tester & Morrison 1990). The peak height index (PHI) refers to the ratio of ΔH_{gel} for gelatinization to the gelatinization temperature range and is a measure of uniformity in gelatinization. The PHI in two proso millet starches, ‘Andongjaerae’ and ‘Danyangjaerae’ showed the highest value, indicating that the higher PHI value is attributed to the presence of large-size granules (Aggarwal et al., 2004). ‘Danyangjaerae’ starch also showed the highest R value of 9.8 among these proso millet starches. Physicochemical properties from three proso millet flours were also examined.

The pasting properties, peak viscosity, breakdown viscosity, final viscosity, setback, and pasting temperature of starches of three proso millet varieties are shown in Table 3. The highest peak, highest breakdown, very high final viscosity, and very low setback were noted in ‘Danyangjaerae’. In contrast, the lowest peak viscosity, breakdown, and final viscosity were observed in ‘Andong 48ho’. Pasting temperature of proso millet starches ranged around 77.4 and 77.6 °C.

The lower peak viscosity was observed in the waxy proso millet starch, ‘Andong 48ho’. Paredes-Lopez (1994) reported that low peak viscosity is 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).

In our studies, it was found that the retrogradation of the two non-waxy starches, ‘Andongjaerae’ and ‘Danyangjaerae’, was much higher than those of glutinous starches, ‘Andong 48ho’.

Scanning electron microscopy and X-ray diffractometry

Scanning electron photographs of proso millet starch granules, X-ray diffraction angles and degree of crystallinity are presented in Figure 1, Table 4 and Table 5.

The starch granules of three proso millets presented as mostly polygonal and rarely elliptical in shape with round edges and some pores at the surface. Among these proso millet starches, the starch granules in both ‘Andongjaerae’ and ‘Danyangjaerae’ had more pores at the surface than that of ‘Andong 48ho’; these starch granule sizes ranged from 4.3 to 8.9 μm (Fig. 1). X-ray diffraction angles presented in all starch granules possessing a typical A-type crystallinity, exhibiting strong deflections at about 15° 2θ, an unresolved double at 17°, 18° 2θ and separated angle 23° 2θ . The degree of crystallinity was higher in ‘Andong 48ho’ than in ‘Andongjaerae’ and ‘Danyangjaerae’.

The variation in size and shape of starch granules may be due to their biological origin (Svegmark & Hermansson, 1993). The morphology of starch granules also depends on the biochemistry of the chloroplast or amyloplast as well as physiology of the plant (Badenhuizen, 1969).

Table 1. Wavelength at maximum absorption (λmax), absorbance at 680 nm (blue value, BV), starch and amylose contents in proso millet starches. Data are means of four determinations. Different letters within each column indicate significant differences ($p < 0.05$).

Varieties	λmax (nm)	Blue value (at 680 nm)	Starch content (%)	Amylose content (%)
'Andongjaerae'	595	0.267a	85.6±0.1b	21.5±0.1a
'Danyangjaerae'	591	0.235b	84.4±0.2c	18.3±0.2b
'Andong 48ho'	537	0.067c	88.3±0.1a	1.2±0.0c

Values are means ± standard deviations. Starch and amylose content were calculated by dry weight basis.

Table 2. Thermal properties of proso millet starches determined by Differential Scanning Calorimeter (DSC). Data are means of four determinations. Different letters within each column indicate significant differences ($p < 0.05$).

Varieties	Gelatinization parameters					
	T*o* (°C)	T*p* (°C)	T*c* (°C)	ΔH gel (J/g)	PHI	*R*
'Andongjaerae'	76.4±0.2a	81.5±0.1a	85.5±0.3a	-4.48a	0.872b	9.1b
'Danyangjaerae'	76.2±0.3a	81.2±0.1a	86.0±0.2a	-4.45a	0.890a	9.8a
'Andong 48ho'	73.1±0.1b	78.0±0.2b	79.3±0.1b	-0.81b	0.166c	6.2c

All values shown are means ± standard deviations. 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*).

Correlation between amylose content and setback viscosity with relative crystallinity

Correlation coefficients were determined to examine the relationships between relative crystallinity, amylose content and setback viscosity (SBV) in three proso millet starches examined in this study (Fig. 2). A highly negative correlation was obtained between setback viscosity and relative crystallinity (r =0.987, $p < 0.01$) with the amylose content and relative crystallinity (r =0.964, $p < 0.01$). A negative correlation coefficient was observed between amylase content and setback viscosity (r =0.869, $p < 0.01$) in this study.

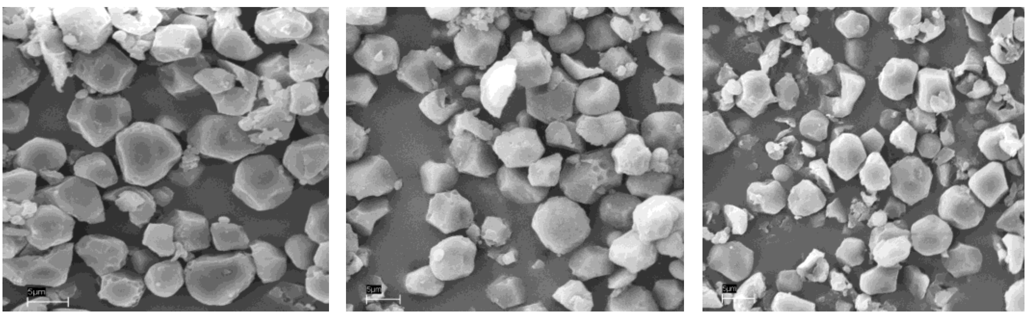

Figure 1. Scanning electron micrograph of three proso millet starch granules (2,000X). From the left, 'Andongjaerae', 'Danyangjaerae', and 'Andong 48ho'.

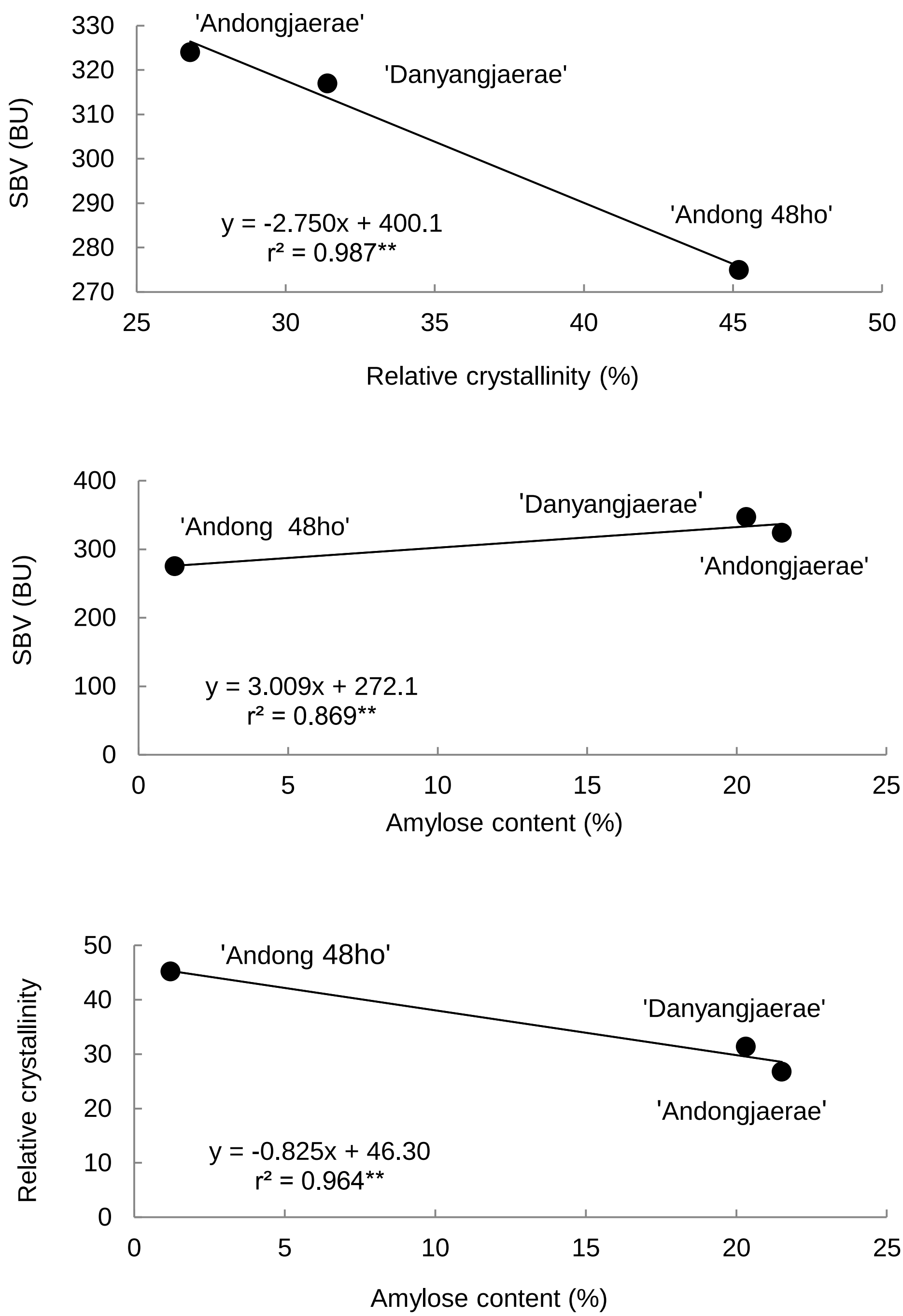

Figure 2. Correlation coefficients between relative crystallinity, amylose content and setback viscosity (SBV) of three proso millet varieties. **, $p < 0.01$.

Table 3. Pasting properties of proso millet flours determined by Rapid Visco Analyser. Data are means of four determinations. Different letters within each column indicate significant differences ($p < 0.05$).

Varieties	Pasting time (min.)	Pasting temp. (°C)	Viscosity (RVU)				
			Peak	Holding strength	Final	Breakdown	Setback
‘Andongjaerae’	3.33	77.6	1,380b	379b	703b	1,001b	324a
‘Danyangjaerae’	3.33	77.6	1,445a	396a	713a	1,049a	317b
‘Andong 48ho’	3.33	77.4	988c	316c	591c	672c	275c

Table 4. X-ray diffraction data of starches from proso millets.

Varieties	Diffraction peaks at 2θ values			
	15°	17°	18°	23°
‘Andongjaerae’	15.4	17.2	18.1	22.7
‘Danyangjaerae’	14.8	17.1	18.0	23.1
‘Andong 48ho’	14.9	16.9	18.2	22.8

Table 5. Crystal pattern and degree of crystallinity from proso millet starches.

Varieties	Degree of crystallinity (%)	Crystal pattern of starch
‘Andongjaerae’	26.8	A
‘Danyangjaerae’	31.4	A
‘Andong 48ho’	45.2	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.

CONCLUSIONS

Amylose contents in proso millet starches ranged between 1.2 and 21.5%. Starch content was from 84.4 to 85.67%. X-ray diffraction angles showed in all starch granules possessing a typical A-type crystallinity. The degree of crystallinity was higher in ‘Andong 48ho’ than in ‘Andongjaerae’ and ‘Danyangjaerae’. A highly negative correlation was displayed between setback viscosity and relative crystallinity with the amylase content and relative crystallinity. It showed that positive correlation was observed between amylose content and setback viscosity

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