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RESEARCH ARTICLE

Characterization of Proso Millet Starches from Different Geographical Origins of China

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Abstract The influence of geographical origin (Lvliang, Baotou, Gulang, and Jilin) on the physicochemical properties of proso millet (*Panicum miliaceum* L.) starches from China, and starch chemical compositions were studied. Scanning electron microscopy showed that starch granules from millet starches were polygonal and spherical with smooth surfaces with sizes ranging from 2.5 to 12 μm. X-ray diffraction showed that millet starches were typical of A-type starch granules with a mean crystallinity of 35.81%. The transition temperatures (T_o, T_p, and T_c) and enthalpy of gelatinization (ΔH) of Lvliang, Baotou, Gulang, and Jilin proso millet starches were 66.81 to 70.01°C, 72.79 to 76.55°C, 78.30 to 82.44°C, and 10.4 to 14.46 J/g, respectively. Significant differences ($p<0.05$) were observed for the amylose content, granule size, peak temperature, gelatinization enthalpy, and peak viscosity temperature among the millet starches. Millet starches may have potential applications in production of puffed starch food products and other food items.

Keywords: proso millet (*panicum miliaceum* L.), environment, starch property, gelatinization

Introduction

Millet, also known as minor millet, refers to a number of different species of small-grained, annual cereal grasses (1). Minor millets include foxtail millet (*Sestaria italia*), barnyard millet (*Echinochola colona*), finger millet (*Eleusine coracana*), kodo millet (*Paspalum sorobiculatum*), little millet (*Panicum miliare*), and proso millet (*P. miliaceum*). Millet is nutritionally superior to rice and wheat because of 24 components that provide health benefits (2). The dietary fiber content of millet is higher than wheat and rice, and provides millet with hypocholesterolemic and hypoglycemic charateristics. Millet also contains a balanced protein content with a high biological value. A recent study reported antioxidant activities for phenolic compounds in millet.

Proso millet (*P. miliaceum* L.) or common millet is a cereal plant with a short seed-to-seed cycle (10 to 11 weeks). Proso millet has the lowest water requirement and the highest conversion rate of limited water supplies into grain of any crop. Proso millet contains starch as a major component and millet products are digested slowly (3,4). A previous study showed that a diet high in carbohydrates, rich in dietary fiber, and consisting largely of cereals allows withdrawal of oral hypoglycemic agents or a reduction in insulin dosages in diabetic subjects (5). Starch with higher amylose content have a lower hydrolysis index (HI) and higher amounts of resistant starch (RS) than starch with a low amylose content (6). Currently, renewed interest in proso millet starch used for human food is due to health benefits. In Japan, consumption of this cereal has increased because consumers believe that this cereal has a health benefit (7).

Starch is an important product with many industrial applications. Commercial starches are mainly obtained from seeds, tubers, and roots, particularly potato, sweet potato, and cassava (8,9). Developments in food and pharmaceutical industries have generated interest in new starches with distinctive properties (10). However, a lack of systematic documentation of the physicochemical properties of proso

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millet starch from different regions hinders the further development and use of this grain in diverse food and nonfood applications on large and industrial scales. Genetic variations and environmental conditions influence starch structure and properties (11). Freeman *et al.* investigated the influence of genetic and environmental factors on the gelatinization temperature of sorghum starches (12). Zhu *et al.* reported wide variations in the physicochemical properties of sweet potato starch from 11 representative genotypes from diverse geographic regions of China (13). Detailed knowledge of the characteristics of proso millet starch will facilitate application in food industries. Therefore, this study aimed to investigate the influence of growth conditions on the physicochemical properties of starches isolated from proso millet grown in the 4 regions of Lvliang, Baotou, Gulang, and Jilin in China.

Materials and Methods

Materials Proso millet samples were grown in (1) Lvliang City (latitude, 36-38ºN; longitude, 110-112ºE; rainfall, 521 mm; altitude, 1,400 m), (2) Gulang County (latitude, 37 ºN; longitude, 102-103ºE; rainfall, 300 mm; altitude, 2,500 m), (3) Baotou City (latitude 41-42ºN; longitude 109-111ºE; rainfall, 263 mm; altitude, 2,000 m), and Jilin City (latitude, 42-44ºN; longitude, 125º-127ºE; rainfall, 700 mm; altitude, 4,300 m). Amylose (A-9262) and potato amylopectin (A-8515) (Sigma Chemical Co., St. Louis, MO, USA) were used as standards. All other analytical grade reagents were purchased from a local company (Kelong, Chengdu, China).

Starch isolation Proso millet starches were isolated as described earlier (14). Proso millet was carefully washed with water and ground in a Waring blender (JJ-2; Jintan, Jiangsu, China) for 10 min to obtain Proso millet flour. Then, the Proso millet flour (100 g) was soaked in 200 mL 0.1% NaOH (w/v, AR; Kelong) and left to stand for 18 h. The resulting suspension was then blended in a blender (85-2A; Jintan) for 2 min, passed through a 63 μm screen (HLX5; Keyuan, Hebei, China) and centrifuged (5810; Eppendorf, Germany) at 1,400×*g* for 10 min. After careful removal of the top layer, the underlying starch layer was reslurried in 20 mL distilled water (Elix10; Millipore, Billerica, MA, USA) and washed with 0.1% NaOH (w/v, AR; Kelong). Then, the starch layer was washed using deionized water (Elix10; Millipore) and neutralized using 1.0 M hydrochloric acid (AR; Kelong) to pH 6.5. The neutralized starch was washed using 500 mL of deionized water (Elix10; Millipore) at least 3 times, then dried in an oven (DHG9070A; Keelrein, shanghai, China) at 45ºC for 48 h (15).

Chemical composition analysis The total starch content of samples was determined using the method proposed by Winton and Winton (16). The moisture content was calculated based on weight loss after samples were heated in an oven (DHG9070A; Keelrein) at 105ºC for 12 h (17). The ash content was determined by incineration in a muffle furnace (4-10; Baidian, Shanghai, China) 550ºC for 12 h. Fats were obtained by extraction using petroleum ether (AR; Kelong) in a Soxhlet apparatus (SXT-02; JOYN, Shanghai, China) (15). The protein content was estimated from the nitrogen content determined using the micro-Kjeldhal method (18) with a conversion factor of 6.25. The crude fiber content was determined gravimetrically after acid hydrolysis using 0.128 M sulphuric acid (AR; KeLong) and basic hydrolysis using 0.882 M sodium hydroxide (AR; KeLong). The amylose content was determined using the method reported by Williams *et al*. with minor modification (19). Starches were defatted using Soxhlet extraction (SXT-02; JOYN) with petroleum ether (AR; KeLong) for 12 h. Defatted starch samples (100 mg) were dispersed using 1 mL of ethanol (AR; KeLong) and suspended in 9 mL of a 0.5 M NaOH solution (AR; KeLong). Suspensions were heated at 95ºC for 30 min in a water bath, and left overnight. Solutions were diluted to a final volume of 100 mL with distilled water (Elix10; Millipore) and a 5 mL aliquot was pipetted into a 50 mL volumetric flask with 25 mL of distilled water (Elix10; Millipore), then 0.5 mL of 1 M acetic acid (AR; KeLong) and 1 mL of an iodine solution (0.2% iodine in 2% potassium iodide, AR; KeLong) were added and the mixture was diluted to 50 mL using distilled water (Elix10; Millipore). The solution was mixed and then left to stand for approximately 20 min in a dark room to develop color. The optical absorbance was recorded at 620 nm using a spectrophotometer (UV-1700; Shimadzu, Tokyo, Japan).

SEM Starch particle structure was observed using a SEM. Samples were coated (PE; Miaojie, Wuxi, China) with a very thin layer of gold powder to avoid charging under the electron beam. The starch granule surface and shape were observed using the Hitachi S-4800 scanning electron microscope (Tokyo, Japan).

Particle size analysis Particle size distribution analysis was performed using a Malvern Mastersizer 2000 laser diffraction particle-size analyzer (Malvern Instruments, Worcestershire, UK). An amount of 0.25 g of sample was suspended in 3 mL of distilled water (Elix10; Millipore). In order to disperse any existing agglomerates, the sample was subjected to an ultrasonic bath (500W, SB25-12DTS; Scientz, Ningbo, China) treatment for 10 min at ambient temperature. A total of 10 drops of prepared sample suspension was placed into the sample port of the instrument until an obscuration of 10-14% was recorded. The focal length was set at 300 mm and the measuring time was 30 s.

X-ray diffraction The X-ray diffraction pattern of samples were record using an X'Pert pro X-ray diffractometer equipped with X'celerator as a detetor (Panalytical; Kassel, Germany). Diffractograms were registered at a Bragg angle of (2θ)=3-60° at a scan rate of 5°/min, with a step width= 0.02°.

DSC Gelatinization temperatures and enthalpy changes were estimated using a 200 F3 Maia DSC (Netzsch Gerätebau GmbH, Selb, Germany). A total of 2.5 mg of sample sealed in an aluminum pan with 7.5 μL of deionized water (Elix10; Millipore) was scanned from 30 to 120°C at a rate of 10°C/min. Sealed empty pans were used as a reference. The onset temperature (*To*, °C), peak temperature (*Tp*, °C), conclusion temperature (*Tc*, °C), and gelatinization enthalpy (ΔHg, J/g) were determined. The range of the gelatinization temperature (*Rg*, °C), and the gelatinization peak height index (PHI, J/gK), $\Delta Hg/(Tp\text{-}To)$, were calculated as previously described (20).

Pasting properties A Brabender viscoamylograph (Viscograph VA-V; Brabender, OHG, Duisburg, Germany) equipped with a 300 cm·g_f cartridge was used to determine the pasting properties of samples at a concentration of 8% (w/v) (8 g of starch on a dry weight basis in 100 mL of water). Starch suspensions were heated from 30 to 93°C at a rate of 7.5°C/min and held at 93°C for 5 min. Then, samples were cooled to 50°C at a rate of −7.5 °C/min and held at 50°C for 2 min. The viscosity of the starch suspension or gel, in Brabender units (BU), was recorded as a function of time. Reported values are the means of duplicate measurements.

Starch solubility and swelling power The swelling power and solubility of starches were determined as described by Leach, McCowen, and Schoch (1959). Samples (1.0 g) were mixed with 50 mL of distilled water (Elix10; Millipore) in centrifuge tubes. The resulting suspensions were heated at a constant temperature (from 65 to 95°C) in a water bath for 30 min. The gelatinized samples were then cooled to room temperature and centrifuged (5810; Eppendorf) at 1,000×*g* for 20 min. The supernatant was dried presumably in an oven (DHG9070A; Keelrein) at 110°C to a constant weight in order to quantify the soluble fraction. The solubility was expressed as the percentage of dried solid weight based on the weight of the dry sample. The swelling power was represented as the ratio of the weight of the wet sediment to the weight of the initial dry sample less the amount of soluble starch (8).

Statistical analysis All measurements were performed in triplicate. Data were subjected to analysis of variance (ANOVA), and comparison of means was carried out using Duncan's multiple range test. Differences were considered to be significant at $p<0.05$. Statistical computations and analyses were conducted using SPSS (version 16.0; SPSS Inc., Chicago, IL, USA).

Results and Discussion

Chemical composition of proso millet starch The results of proximal analyses of starch samples are shown in Table 1. All 4 proso millet varieties contained comparable amounts of isolated starch (92.19 to 94.60%). The protein content was within the range of 0.27 to 0.67% for all proso millet starches. The average crude lipid content of proso millet starched was 0.18%. All starches contained the same crude fiber (0.02 to 0.03%) content. The ash contents of all proso millet starches were relatively high (0.22 to 0.68%). The amylose contents of proso millet starches ranged from 14.92 to 17.37%, which were lower than values for maize and potato starch (9). Statistical analysis confirmed the significant ($p<0.05$) influence of the 4 different geographic regions on the starch and amylose contents in the proso

Table 1. Proximate composition and amylose content of starches isolated from proso millets cultivated in different regions of China[1)]

Chemical composition (%)	Cultivation region			
	Lvliang	Gulang	Baotou	Jilin
Starch	92.19±0.11^a	92.71±0.31^d	94.60±0.62^b	93.54±0.02^c
Protein	0.67±0.01^a	0.27±0.03^b	0.58±0.01^a	0.29±0.02^b
Fat	0.13±0.03^c	0.23±0.04^{ab}	0.17±0.04^{bc}	0.25±0.02^a
Crude fiber	0.03±0.01^a	0.03±0.01^a	0.02±0.01^a	0.02±0.01^a
Ash	0.68±0.01^a	0.27±0.02^c	0.47±0.01^b	0.22±0.04^c
Amylose	17.07±0.12^a	14.92±0.07^d	17.37±0.06^b	16.27±0.03^c

[1)]Data are presented as mean±SD. Values in the same row followed by the same lowercase letter are not significantly different at the 5% level using Duncan's multiple range test.

millet starches (Table 1). Only the crude fiber content in proso millet starches was not significantly (p>0.05) affected by the geographic region.

The functional properties of starches are affected by the starch chemical composition (21). A significantly (p<0.05) lower moisture content in Lvliang starch than Gulang, Baotou, and Jilin starches was observed (Table 1). Both annealing and recrystallization are greatly influenced by the water and amylose contents (22). The level of starch and amylose were obtained as following: Lvliang was approximately equal to Baotou>Jilin>Gulang. Total starch values for all samples varied in a narrow range of 92.19 to 94.60%. The average amylose starch content of the 4 starches was 14.87%. When compared to pearl millet starch (24.6 to 27.2%) and nger millet starch (19.2 to 27.6%), proso millet (*P. miliaceum* L.) starch had a lower amylose content (23-25). Thus, the chemical compositions of proso millet starches varied among the different geographical regions and differed from other reported millet starches.

SEM The granule shape, size, and morphology of proso millet samples observed under SEM are shown in Fig. 1. The tested proso millet starches had a uniform polygonal shape for both small and large granules. However, small spherical, large spherical (rare), and large polygonal granules were reported in proso millet starch from India (26). A significant difference in granules was also reported between proso millet starch and pearl millet starch, the granules of which varied from polygonal to round. SEM photomicrographs of proso millet starch granules in this study showed a high percentage of large polygonal (2.5 to 12 μm) to small, spherical (0.3 to 1.2 μm) granules with smooth surfaces and no evidence of fissures. In previous studies, maize starch granules were polygonal with a diameter ranging from 5 to 30 μm with pores or channels in the surface (27). Potato starch granules varied from elliptical and oval to irregular with granule diameter sizes in the range of 10 to 90 μm and a smooth surface morphology. Differences in the morphology of starches may be attributed to variations in membranes, physical characteristics of plastids during granule development, and the biochemistry of the chloroplast (28).

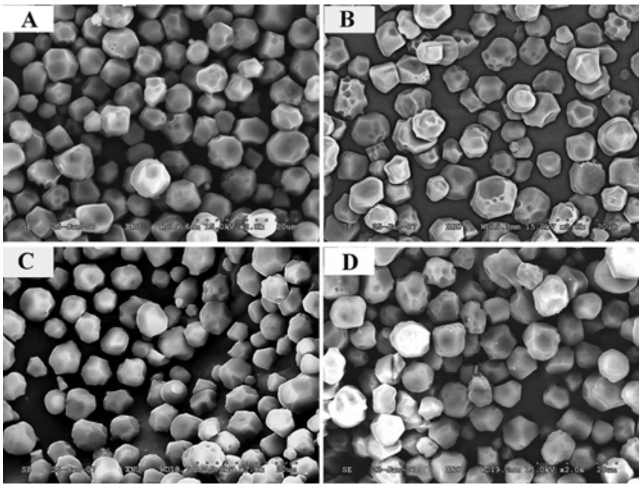

Fig. 1. SEM of proso millet starches, Lvliang (A), Gulang (B), Baotou (C), and Jilin (D). 2,000× magnification

Particle size analysis The full particle size distribution of the isolated proso millet starches is shown in Table 2. The particle size was a smooth bimodal distribution. The proso millet starches consisted of a minor population (<10%) of small granules (1.42 μm in diameter) and a major population (>90%) of large granules of up to 20.00 μm in diameter The average sizes of Lvliang, Baotou, Gulang, and Jilin starches were 6.47, 6.75, 6.53, and 6.95 μm, respectively. Differences in particle sizes of proso millet starches grown in different regions may be due to different environment factors. A higher altitude and a lower average temperature may cause a larger granule size. Other studies have also investigated the effects of environmental factors on starch granule size (29,30).

X-ray diffraction Tuber and root starches exhibited a B-type X-ray diffraction (XRD) pattern with a small peak at 5.6°, only one peak at 17°, and a doublet at 22° and 24°. By

Table 2. Particle size distribution of proso millets starches cultivated in different regions of China[1)]

Particle parameters	Cultivation region			
	Lvliang	Gulang	Baotou	Jilin
Size range (μm)	0.28-15.89	0.28-17.83	0.32-20.00	0.32-17.83
Average size (μm)	6.47±0.01^{c}	6.53±0.04b^{c}	6.75±0.28^{ab}	6.95±0.01^{a}
D0.1 (μm)	3.67±0.04^{a}	3.46±0.10^{b}	3.30±0.09^{c}	3.54±0.08^{ab}
D0.5 (μm)	6.41±0.02^{b}	6.44±0.01^{b}	6.78±0.22^{a}	6.91±0.03^{a}
D0.9 (μm)	9.75±0.07^{b}	10.11±0.24^{ab}	10.34±0.61^{ab}	10.72±0.06^{a}
Specific surface area (m^2/g)	1.64±0.02^{a}	1.67±0.01^{a}	1.77±0.14^{a}	1.63±0.01^{a}

[1)]Mean values±SD from triplicate analysis. D0.1, D0.5, and D0.9, respectively, represent 10, 50, and 90% of all particles finer than this size. Values in the same row followed by the same lowercase letter are not significantly different at the 5% level using Duncan's multiple range test.

Table 3. X-ray diffraction properties of proso millet starches

Region	Scattering angel (2θ)/interplanar distance (Å)				Relative crystallinity (%)	Diffraction pattern
Lvliang	15.20/5.82	17.30/5.12	18.04/4.91	23.22/3.83	36.33	A
Gulang	15.38/5.75	17.42/5.09	18.00/4.92	23.14/3.84	36.98	A
Baotou	15.14/5.85	16.98/5.22	17.98/4.91	23.44/3.79	36.57	A
Jilin	15.10/5.86	17.10/5.18	18.06/4.90	22.88/3.68	33.37	A

Table 4. Thermal properties of Lvliang, Gulang, Baotou, and Jilin starches analyzed using differential scanning calorimetry at a concentration of 25% (w/w) in aqueous suspension[1)]

Region	T_o (°C)	T_p (°C)	T_c (°C)	R_g (°C)	ΔH_g (J/g)	PHI (J/gK)
Lvliang	67.91±0.21[bc]	72.79±0.00[b]	78.30±0.11[c]	10.39±0.43[b]	10.89±0.19[b]	2.23±0.21[a]
Gulang	66.81±0.32[c]	73.68±0.11[b]	80.23±0.04[b]	13.42±0.12[a]	10.40±0.18[b]	1.51±0.32[a]
Baotou	70.01±0.11[a]	76.55±0.06[a]	82.44±0.33[a]	12.43±0.32[a]	14.46±0.15[a]	2.21±0.21[a]
Jilin	69.08±0.14[ab]	76.01±0.05[a]	81.42±0.17[a]	12.34±0.08[a]	13.97±0.13[a]	2.01±0.22[a]

[1)]Each value represents the mean of triplicate analysis. Values in the same column followed by the same lowercase letter are not significantly different at the 5% level using Duncan's multiple range test. T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; R_g, gelatinisation temperature range (T_c-T_o); ΔH_g, gelatinization enthalpy; PHI, gelatinisation peak height index, $\Delta H_g/(T_p-T_o)$

contrast, A-type starches showed double peaks at 17° and 18° and a single peak at 23°, typical of many cereal starches (31-33). Characteristic XRD results for proso millet starches are shown in Table 3. The proso millet starches exhibited double peaks at 17° and 18° and a single peak at 23°, a characteristic A-type crystallinity pattern. In previous studies, potato starch displayed a B-type pattern (34). The crystallinity values of Lvliang, Baotou, Gulang, and Jilin proso millet starches were 36.33, 36.57, 36.98, and 33.37%, respectively.

In the amylopectin molecule, the A cluster chain forms double helices with the adjacent A chain of another cluster, resulting in the crystalline order of starch granules (22,35). The crystalline level of starch granules is reportedly influenced by the amylopectin A chain length, the amount of double helices organized into the crystalline array, the crystallite size, the amylose content, and the degree of amylopectin phosphorylation. An increase in the moisture level can disrupt the crystalline order of starches, resulting in a weak intensity (36). Thus, the lower crystallinity value of the Jilin proso millet starch may be due to growth under the highest amount of rainfall among the tested starches.

DSC The gelatinization property of starch is of importance in food processing. The texture profiles of starch products are determined by the temperature and the degree of starch gelatinization. The thermal properties of Lvliang, Gulang, Baotou, and Jilin starches, as analyzed using DSC, are shown in Table 4. The gelatinization process of starch involves the uncoiling and melting of the external chains of amylopectin that clustered together as double helices (37). The Baotou and Jilin proso millet starches showed higher gelatinization temperatures than the other 2 proso millet starches. Thus, Baotou and Jilin proso millet starches required higher temperatures to ensure complete gelatinization and paste formation, suggesting potential use in products that require delayed pasting, such as retorted canned foods. Gulang proso millet starch showed the lowest T_o value (66.81°C) among the analyzed starches. The ΔH_g of Baotou and Jilin were 14.46 and 13.97 J/g, respectively, which were higher than the ΔH_g of other starches. Both the gelatinization temperature and the starch enthalpy of gelatinization depend on microstructures, the presence of crystalline regions, and differences in the granule size and the amylose/amylopectin ratio (30). Double helical and crystalline structures in starches are disrupted during gelatinization. A higher gelatinization temperature may be attributed to a high percentage of irregular large granules. Noda *et al*. postulated that the T_o, T_p, and T_c values were influenced by the molecular architecture of the crystalline region, which corresponds to the distribution of amylopectin shorter chains (35). Thus, further research should investigate the factors affecting gelatinization characteristics of starches.

Pasting properties The pasting properties of Lvliang, Gulang, Baotou, and Jilin starches are shown in Table 5. The pasting temperatures of starches were higher than the DSC peak temperature (Table 4), attributable to the melting of starch crystallites that occurred in the presence of excess water. Baotou and Jilin starches showed high peak viscosity values (457 and 426.5 BU, respectively), suggesting that the intermolecular forces within the Baotou and Jilin proso millet starches were weaker than in the other 2 cultivars. For viscosity values during heating, a significant ($p<0.05$) increment was observed at 50°C (330 and 307 BU for Lvliang and Gulang, respectively), compared to the peak viscosity for Lvliang and Gulang (219 and 264 BU, respectively) (Table 5). This result may be due to the

Table 5. Pasting parameters of proso millet starches[1)]

Region	PV (BU)	TV (BU)	FV (BU)	BV (BU)	SV (BU)
Lvliang	219.0±8.49[d]	139.5±4.95[d]	387.0±4.30[c]	79.5±3.54[d]	190.5±2.43[b]
Gulang	264.0±4.24[c]	156.0±4.23[c]	399.0±2.83[b]	108.0±0.05[c]	151.0±2.50[c]
Baotou	457.0±8.49[a]	217.5±2.11[a]	368.5±2.67[d]	239.5±6.36[b]	115.5±2.40[d]
Jilin	426.5±3.53[b]	186.5±2.02[b]	419.0±2.83[a]	240.0±1.41[a]	201.5±2.40[a]

[1)]Each value represents the mean of triplicate analysis. Values in the same column followed by the same lowercase letter are not significantly different at the 5% level using Duncan's multiple range test. PV, peak viscosity (BU); TV, trough viscosity; FV, final viscosity; BV, breakdown viscosity; SV, setback viscosity

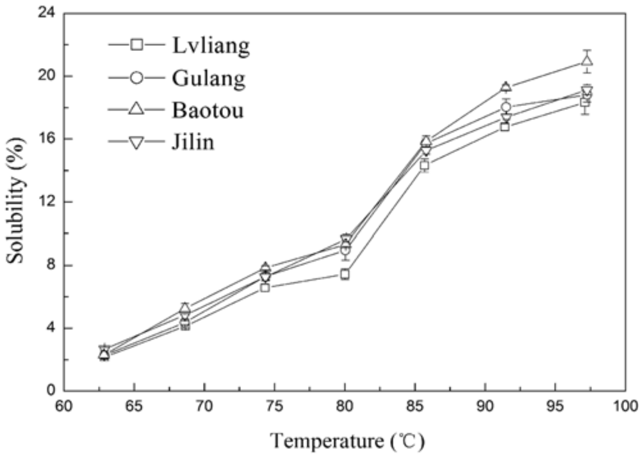

Fig. 2. Effect of temperature on solubility of proso millet starches (Lvliang, Gulang, Baotou, and Jilin).

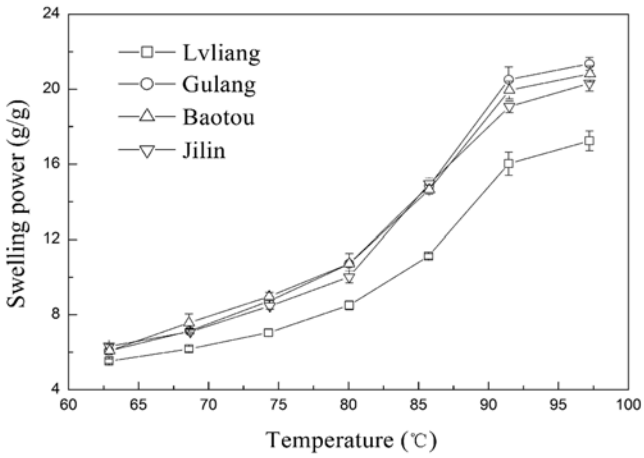

Fig. 3. Effect of temperature on the swelling power of proso millet starches (Lvliang, Gulang, Baotou, and Jilin).

tendency of the starch molecules to form aggregates of low solubility resulting in a retrogradation tendency. The opposite result was found for Baotou (333 BU) and Jilin (388 BU) starches in which the thinning effect may be due to weakening of bonding forces within granules. The lowest setback of Baotou (115.5 BU) proso millet starch measured with a continuous shearing force can be attributed to a low degree of amylose leaching.

Starch solubility and swelling power The solubility of the proso millet starches at different temperatures (Fig. 2) shows that the starches solubilized at different rates. The solubility order was Baotou>Gulang>Jilin>Lvliang. The lowest swelling power and starch solubility values observed in Lvliang were possibly caused by a low T_P value. Similar observations were reported for other studies on starches (38). The effect of temperature on the swelling power of starches (Fig. 3) revealed that the starches swelled as the temperature increased. The order of swelling power was Gulang>Baotou>Jilin>Lvliang. Different starches exhibited different swelling and solubilization patterns (23). Large variations in solubility and swelling properties were observed at 90 and 95°C. Compared with other millet starches (Fig. 2, 3), the lower solubility and swelling power of Lvliang proso millet starch can be attributed to the differences in varieties and environmental factors. Similarly, other properties of starch have been reported to be affected by different varieties and environmental factors (12).

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Disclosure The authors declare no conflict of interest.

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