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GRANULE SIZE DISTRIBUTION AND PASTING PROPERTIES OF STARCH IN NORMAL, WAXY AND SWEET MAIZE KERNELS

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Abstract

The granule size and pasting properties of starch and their relationship with quality of maize (*Zea mays* L.) was investigated. A clear bimodal distribution of granule sizes was found in six cultivars of maize. A number of starch granules were made up of small starch granules in kernels. Normal maize had a smaller proportion of granules <12 μm and a greater proportion of granules >12 μm, whereas sweet maize had a greater proportion of granules <12 μm and a smaller proportion of granules >12 μm. The peak, trough and breakdown viscosities were found higher in waxy maize and lower in sweet maize. The final and setback viscosities were found higher in normal maize and lower in sweet maize. The peak, trough, final and setback viscosities were significantly and negatively correlated to volume percentage of granules <12 μm and significantly positively correlated with the volume percentage of granules >12 μm.

Introduction

Maize (*Zea mays* L.) starch supplies over 80% of the world starch market, followed by potato starch (Shang *et al.* 2016). Starch constitutes the major carbohydrate in the endosperm of cereal grains and is deposited as discrete semi-crystalline aggregates known as starch granules in cereal endosperms (Matsushima *et al.* 2014, Makowska *et al.* 2015). Starch granules in the starch endosperm of maize, wheat and barley are in the form of single granules (Campbell *et al.* 1996, Ao and Jane 2007). The mean and mode of the diameter of starch granules in maize, usually discoid or lenticular in shape, are in the ranges of 16.2 - 18.2 μm and 14.2 - 16.8 μm (Campbell *et al.* 1996). Starch granules can be divided into large A-type and small B-type starch according to their size and shape in wheat grains (Shinde *et al.* 2003). The large and small starch granules have different physicochemical and functional properties (Cai *et al.* 2014). An increase in the rate of the small granules (<10 μm) increased the farinograph water absorption and improved pasta quality in wheat (Soh *et al.* 2006). The differences between large and small starch granules result in the two starch granule types being used differently in industrial food and nonfood applications in cereal crops (Chen *et al.* 2003, Hung *et al.* 2006, Cui *et al.* 2014).

The size distribution of starch granules has a significant influence on swelling (Zhang *et al.* 2017), pasting (Peterson and Fulcher 2017), thermal (Ji *et al.* 2003), and rheological properties (Sandhu *et al.* 2004) in maize. Starch pasting is a process of breaking the intermolecular bonds of starch molecules in starch granules in the presence of water and heat (Zobel 1988). The pasting properties of flour are closely related to the granule size distribution of starch: large starch granules have a lower peak viscosity than small ones in wheat grains (Peterson and Fulcher 2017). The amylose content of large starch granules was significantly higher than that of small starch granules, resulting in a significant difference in starch gelatinization between the large and small starch granules in wheat (Shinde *et al.* 2003).

There are a few reports about the effects of cultivars on the size distribution of starch granules in normal maize (Paterson *et al.* 2001, Cui *et al.* 2014). However, little is known about the starch granule size distribution and its relationship with pasting properties in different maize cultivars

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with different quality types. Therefore, the objective of this study was to investigate the starch granule size distribution and its relationship to pasting properties in maize kernels from six cultivars of normal, waxy and sweet maize.

Materials and Methods

The field experiments were carried out at the farm of Anhui Science and Technology University, Fengyang, China (32°51’N, 117°33’E) during the maize growing seasons from June to October, 2014 and June to October, 2015. The soil was sandy loam. The average organic matter content in the tillage layer was 14.5 g/kg and the available N, phosphorus (P) and potassium (K) were 88.7, 31.6 and 68.3 g/kg, respectively. The basal fertilizer was applied at the rates of 135 kg/hm^2 N, 100 kg/hm^2 P_2O_5, 100 kg/hm^2 K_2O before planting. A total of 135 kg/hm^2 N was top-dressed at the booting stage of maize. The experiment was conducted as a randomized complete design with three replicates for each cultivar. The plot size was 4.8 × 6.7 m with 8 rows (60 cm between rows). Seeds were sown on 17 June, 2014 and 22 June, 2015 with a density of 67500 plants per hm^2.

Six maize cultivars currently used in local maize production, normal maize (Zhongdan901, Longping206), waxy maize (Jingkenuo609, Fengnuo2146) and sweet maize (Wantian150, Zhongtian5), were chosen in this study. At maturity, maize kernels were dried at 60°C for 48 hrs for starch granule size analysis and determination of pasting properties.

Starch was extracted from the maize cultivars according to the methods of Peng *et al.* (1999) with some modifications. Maize kernels (5 g) were steeped in 30 ml double distilled water at 4°C for 24 hrs. The softened seeds were sterilized and ground with a mortar and pestle in double distilled water until essentially all the starch granules were released. The slurry was filtered through a 74 µm screen and centrifuged at 1700 × g for 15 min to extract the crude starch. The crude starch was purified three times using 25 ml of 2 mol/l NaCl, 0.2% NaOH, 2% SDS and double distilled water. In addition, the starch was washed once with acetone to remove the water. Then, it was air-dried at room temperature and stored at – 40°C.

The particle size distribution of starch was determined by using an LS13320 Laser Diffraction Particle Size Analyzer (Beckman Coulter, USA). Approximately 50 mg of starch was suspended with 5 ml of double distilled water in 10-ml Eppendorf tubes. After aligning the instrument and making background measurements, the starch suspension was transferred into the laser diffraction particle size analyzer’s dispersion tank containing double distilled water. The starch granule size was evaluated when sufficient dispersion was added to obtain an obscuration of 8 - 12%.

The starch pasting properties (3.5 g total weight, 14% dry basis) were measured using a rapid viscosity analyzer (RVA-Starch Master 2, Perten Instruments, Sweden) according to the methods of Lim and Seib (1993) with some modifications. A sample suspension was equilibrated at 50°C for 1 min, heated to 95°C at 12°C/min, maintained at 95°C for 2.5 min, cooled to 50°C at 12°C/min, and then maintained at 50°C for 1 min. The paddle speed was set at 160 rpm for starch pasting property analysis.

One-way ANOVA was performed with the SPSS statistical analysis package (SPSS Incorporated, USA). When significant treatment effects were detected, multiple comparisons among the treatments were carried out using the LSD test. Pearson's correlation coefficients were calculated to determine the relationship between the pasting properties and volume distribution of starch granules in maize kernels. The experimental data from 2015 are reported in this paper.

Results and Discussion

The volume distribution (Fig. 1a) of starch granules showed two populations of starch granules with peak values ranging from 1.593 - 1.748 and 18 μm (normal maize), 1.593 and 16.4 μm (waxy maize), and 1.451 - 1.593 and 8.537 - 9.371 μm (sweet maize). The limit between the two populations occurred at approximately 2.8 μm. Fig. 1b shows a typical population of number distribution of starch granules with peak values ranging from 0.52 - 0.829 μm (normal maize), 0.92 - 0.999 μm (waxy maize), and 0.52 - 0.755 μm (sweet maize). As with the volume distribution, the surface area distribution of granules had bimodal trend with peak values ranging from 1.204 - 1.451 and 16.4 - 18 μm (normal maize), 1.322 - 1.451 and 14.94 - 16.4 μm (waxy maize) and 1.204 - 1.451 and 6.453 - 7.776 μm (sweet maize) (Fig. 1c). The limit between the populations occurred at approximately 2.8 μm among the six maize cultivars.

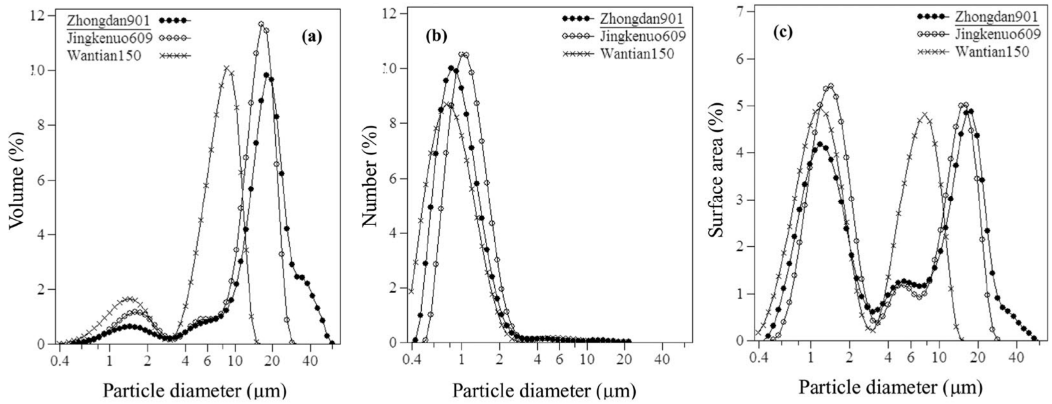

Fig. 1. Typical volume (a), number (b) and surface area (c) distribution of starch granules in maize kernels.

There are two types of starch granules in wheat grain at maturity: the large granules (generally larger than 10 μm in diameter) and the small granules (smaller than 10 μm in diameter) (Peng *et al.* 1999, Li *et al.* 2016). In this study, all of the maize cultivars confirmed a clearly bimodal distribution in the volume distribution, and the cutoff point for differentiating size classes of granules was approximately 2.8 μm. However, Ji *et al.* (2003) concluded that cutoff points were at 9 and 17 μm. This difference could be explained by different methods of calculating starch granule size or by the different maize cultivars used in each study. Starch granules were assigned to three groups according to their equivalent diameters in maize: <2.8 μm (small), 2.8 - 12 μm (midsize), >12 μm (large) in this study.

The means of the volume percentage of starch granules <30 μm were approximately 93.17% (normal maize), 99.25% (waxy maize), and 100% (sweet maize) of the total volume (Table 1). Contributions of the small granule population (<2.8 μm) to the total volume were 7.34% (normal maize), 10.38% (waxy maize), and 16.47% (sweet maize). Variability within the starch granule population of >2.8 μm from the six cultivars was appraised by dividing the group into two regions of 2.8 - 12 and >12 μm. The volume percentages of granules of 2.8 - 12 μm (midsize) were in the ranges of 15.28 (normal maize), 20.42 (waxy maize), and 66.65 (sweet maize). In addition, contributions from the starch granules >12 μm to the total volume were 77.38% (normal maize), 69.58% (waxy maize), and 16.88% (sweet maize). It showed that the proportion of granules <12 μm was higher in sweet maize and lower in normal maize. In addition, the proportion of granules >12 μm was higher in normal maize and lower in sweet maize. The means of the D (4, 3) (weighted average volume of smaller and larger granules with weighted value of 4 : 3) were higher in normal maize and lower in sweet maize.

The proportions of granules <2.8 and <12 μm were in the range of 97 - 99.2 and 99.73 - 99.95% of the total number, respectively (Table 2), showing that a number of granules were made up of small starch granules in maize kernels. The contribution of the starch granules <1 μm to the total number was 39.97 - 75.37% in six maize cultivars. The variability in the starch granules among cultivars was highly significant. The percentage of the number of granules <12 μm was higher in sweet maize and lower in normal and waxy maize. In addition, the proportion of granules >12 μm was higher in normal and waxy maize and lower in sweet maize.

Table 1. Volume distribution of starch granules in maize kernels (%).

Types	Cultivars	Particle diameter of starch granule (μm)				D (4, 3) (μm)
		< 2.8	< 12	< 20	< 30	
Normal maize	Zhongdan901	6.6 ± 0.02f	22.03 ± 0.12f	63.33 ± 0.5d	92.57 ± 0.06d	17.19 ± 0.24a
	Longping206	8.07 ± 0.03e	23.2 ± 0.1e	61.8 ± 0.3e	93.77 ± 0.06c	16.98 ± 0.02a
	Average	7.34	22.62	62.57	93.17	17.09
Waxy maize	Jingkenuo609	10.8 ± 0.17c	31.47 ± 0.83c	85.57 ± 0.6c	100 ± 0a	13.77 ± 0.12c
	Fengnuo2146	9.96 ± 0.21d	29.37 ± 0.4d	86.13 ± 0.38c	98.5 ± 0.44b	14.14 ± 0.07b
	Average	1038	30.42	85.85	99.25	13.96
Sweet maize	Wantian150	18.2 ± 0.2a	84.57 ± 0.75a	96.5 ± 0.2b	100 ± 0a	7.82 ± 0.09e
	Zhongtian5	14.73 ± 0.15b	81.67 ± 0.35b	97.97 ± 0.21a	100 ± 0a	8.26 ± 0.05d
	Average	16.47	83.12	97.12	100	8.04
F value	Cultivars	2414.6**	9971.26**	4826.81**	1062.08**	3413.28**

Means within columns followed by different letters are significantly different at p = 0.05 according to the LSD test (n = 5). **p < 0.01. D (4, 3), weighted average volume of smaller and larger granules with weighted value of 4 : 3.

Table 2. Number distribution of starch granules in maize kernels (%).

Types	Cultivars	Particle diameter of starch granule (μm)			
		< 1	< 2.8	< 12	> 12
Normal maize	Zhongdan901	58.27 ± 0.06d	98.2 ± 0bc	99.73 ± 0.06b	0.27 ± 0.06a
	Longping206	75.37 ± 1.35a	99.2 ± 0a	99.77 ± 0.06b	0.23 ± 0.06a
	Average	66.82	98.7	99.75	0.25
Waxy maize	Jingkenuo609	39.97 ± 0.42f	98.2 ± 0bc	99.73 ± 0.06b	0.27 ± 0.06a
	Fengnuo2146	46.57 ± 1.42e	98.37 ± 0.29b	99.73 ± 0.06b	0.27 ± 0.06a
	Average	43.27	98.29	99.73	0.27
Sweet maize	Wantian150	70.47 ± 0.55b	98.07 ± 0.06c	99.95 ± 0.04a	0.05 ± 0.04b
	Zhongtian5	61.6 ± 2.17c	97.0 ± 0.17d	99.91 ± 0.01a	0.09 ± 0.01b
	Average	66.04	97.54	99.93	0.07
F value	Cultivars	368.51**	76.56**	11.3**	11.3**

Means within columns followed by different letters are significantly different at p = 0.05 according to the LSD test (n = 5). **p < 0.01.

Contribution of the small granules (<2.8 μm) was in the ranges of 97 - 99.2% the total number, which showed that a number of granules were made up of small starch granules in maize kernels in this study. And contribution of granules <2.8 μm to the total volume was in the ranges of 6.6 - 18.2%. These suggested that small starch granules possess most of the number, despite being fewer volume in compared with midsize and large starch granules in maize kernels.

The starch granules <2.8, 2.8 - 12 and >12 μm occupied 45.23 - 56.6, 17.7 - 44.77 and 3.85 - 35.7% of the total surface area among six cultivars, respectively (Table 3). The means of the D (3, 2) (weighted average surface area of smaller and larger granules with weighted value of 3 : 2) were higher in normal maize and lower in sweet maize.

The starch pasting properties were significantly affected by maize cultivars (Table 4). The peak, trough and breakdown viscosities were higher in waxy maize and lower in sweet maize. In addition, the final and setback viscosities were higher in normal maize and lower in sweet maize. In addition, the pasting temperature was higher in sweet maize and lower in normal maize.

Waxy maize had significantly higher peak, trough, final, breakdown and setback viscosities, compared with normal maize (Sandhu *et al.* 2004). Singh *et al.* (2006) found that the starch of sweet maize could not be gelatinized under the same conditions, so the pasting parameters such as peak viscosity were lower than those of normal maize. This study showed that the peak and trough viscosities were higher in waxy maize and lower in sweet maize. The high peak viscosities of waxy maize indicated that the starch granules were harder to paste compared to normal and sweet maize. A possible explanation for this observation is that starch with high crystallinity in waxy maize exhibits high peak and breakdown viscosities (Shang *et al.* 2016). The final and setback viscosities were higher in normal maize and lower in sweet maize in this study. The high final viscosities in normal maize may be because larger starch granules increase the rate of swelling and thus occupy more volume and enhance viscosity (Srichuwong *et al.* 2005). The pasting temperature was higher in sweet maize compared with normal and waxy maize. Peng *et al.* (1999) found that small starch granules had higher gelatinization peaks and completion temperatures compared to large starch granules in wheat grains.

Table 3. Surface area distribution of starch granules in maize kernels (%).

Types	Cultivars	Particle diameter of starch granule (μm)				D (3, 2) (μm)
		< 1	< 2.8	2.8-12	> 12	
Normal maize	Zhongdan901	14.0 ± 0b	45.23 ± 0.06d	19.07 ± 0.06c	35.7 ± 0.1a	8.95 ± 0.01a
	Longping206	20.15 ± 1.2a	51.55 ± 0.07b	15.7 ± 0e	32.75 ± 0.07b	8.03 ± 0.01b
	Average	17.08	48.39	17.39	34.23	8.49
Waxy maize	Jingkenuo609	9.66 ± 0.14c	52.4 ± 0.26b	18.73 ± 0.35c	28.87 ± 0.59d	7.01 ± 0.08d
	Fengnuo2146	13.23 ± 3.09b	51.8 ± 1.47b	17.7 ± 1.06d	30.5 ± 0.5c	7.15 ± 0.09c
	Average	11.45	52.1	18.22	29.69	7.08
Sweet maize	Wantian150	18.37 ± 1.25a	56.6 ± 0.3a	39.57 ± 0.06b	3.85 ± 0.35f	4.01 ± 0.06f
	Zhongtian5	14.43 ± 0.59b	49.77 ± 0.38c	44.77 ± 0.32a	5.47 ± 0.12e	4.58 ± 0.03e
	Average	16.4	53.19	42.17	4.66	4.3
F-value	Cultivars	20.92**	99.81**	2160.16**	3812.44**	3691.06**

Means within columns followed by different letters are significantly different at $p = 0.05$ according to the LSD test ($n = 5$). **$p < 0.01$. D (3, 2), weighted average surface area of smaller and larger granules with weighted value of 3 : 2.

The pasting properties of starch, particularly the ability of starch to take up water and form a paste in the presence of heat, are affected by variations in the size distribution of starch granules (Soh *et al.* 2006). Peterson and Fulcher (2001) reported that large starch granules have lower peak, breakdown and setback viscosities than small ones. However, the higher average granule sizes were associated with lower values of peak and breakdown viscosities in the cocoyam (Lu *et al.* 2005). Small granules have lower peak and breakdown viscosities than large granules in the potato (Noda *et al.* 2005). The volume percentage of granules is equal to the mass percentage of granules in the case of uniform density between large and small starch granules (Peng *et al.* 1999, Wilson *et al.* 2010). In this study, correlation analysis showed that the peak, trough, final and setback

Table 4. The pasting properties of starch in maize kernels.

Types	Cultivars	Peak viscosity (cP)	Trough viscosity (cP)	Final viscosity (cP)	Breakdown (cP)	Setback (cP)	Pasting temp. (°C)
Normal maize	Zhongdan 901	1424.5 ± 2.5c	865 ± 9c	2376 ± 78a	559.5 ± 11.5b	1511 ± 69a	74.8 ± 0.2e
	Longping 206	1192 ± 33d	848.3 ± 8.5c	2293.7 ± 27.5a	343.7 ± 40.8c	1445.3 ± 36a	75 ± 0.4e
	Average	1308.3	856.7	2334.9	451.6	1478.2	74.9
Waxy maize	Jingkenuo 609	1644 ± 120.2b	1128 ± 39.6b	1782 ± 158.4b	516 ± 80.6b	654 ± 118.8b	76.6 ± 0d
	Fengnuo 2146	2612.7 ± 24.9a	1431.3 ± 22a	1783.3 ± 14.6b	1181.3 ± 2.9a	352 ± 9.5c	78.7 ± 0.5c
	Average	2128.4	1279.7	1782.7	848.7	503	77.7
Sweet maize	Wantian 150	95 ± 26.3e	82.3 ± 21.5d	101.3 ± 19.7c	12.7 ± 5d	19 ± 2d	83 ± 0b
	Zhongtian5	45.5 ± 2.5e	38.5 ± 2.5e	42.5 ± 6.5c	7.3 ± 0.6d	4 ± 3.5d	84.5 ± 0.6a
	Average	70.3	60.4	71.9	10	11.5	83.8
F value	Cultivars	1612.8**	3010.9**	1001.9**	672.1**	619.9**	444.2**

Means within columns followed by different letter are significantly different at p = 0.05 according to the LSD test (n = 5). *p < 0.05, **p < 0.01.

Table 5. Correlation coefficients between the pasting properties and volume distribution of starch granules in maize kernels.

Items	Volume percentage of starch granule (%)				D (4, 3) (μm)
	< 2.8 μm	2.8 - 12 μm	< 12 μm	> 12 μm	
Peak viscosity (cP)	–0.6988	–0.8045*	–0.7962*	0.7962*	0.6983
Trough viscosity (cP)	–0.739	–0.8969**	–0.8818**	0.8818**	0.7589*
Final viscosity (cP)	–0.9528**	–0.9879**	–0.9916**	0.9916**	0.9921**
Breakdown (cP)	–0.6057	–0.7289	–0.7174	0.7174	0.5898
Setback (cP)	–0.875**	–0.7983*	–0.8168*	0.8168*	0.9199**
Pasting temp. (ºC)	0.9035**	0.9523**	0.9537**	–0.9537**	–0.9704**

*p < 0.05; **p < 0.01, D (4,3), weighted average volume of smaller and larger granules with weighted value of 4 : 3.

viscosities were significantly negatively correlated to the volume percentage of granule <12 μm and significantly positively correlated to the volume percentage of granules >12 μm in maize kernels (Table 5). The D (4, 3) had a positive correlation with pasting properties, except pasting temperature and had a significant negative correlation with pasting temperature. These suggested that large granules (larger than 12 μm in diameter) have higher peak, trough, final and setback viscosities compared with small granules (smaller than 12 μm in diameter) in maize starch.

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