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Changes in physicochemical properties of waxy corn starches at different stages of harvesting

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ABSTRACT

Starches were isolated from immature waxy corn kernels harvested at 0, 2, 4 and 6 days after optimum stage (DAO) and from mature kernels at 16 DAO. The starch contents showed varied according to genotypes and harvesting stages. The accumulation of starches showed an increasing trend in relation to delayed harvesting time, from the optimum stage until the physiological maturity stage. Among all harvesting stages, medium granules had the highest contribution to the total starch volume (60.8–81.5%), followed by large (5.7–30.1%), and small granules (9.1–15.3%). Average chain length distribution of amylopectin ranged from DP 14.7 to 16.9 for KKU–UB, DP 16.9 to 17.4 for KKU–JD, and DP 5.7 to 30.1 for Violet white. The pasting behaviors of starches were greatly affected by harvesting times. The peak viscosity of starches increased with delayed harvesting until physiological maturity and then decreased until dried kernels at 35 days after pollination.

1. Introduction

Waxy corn as the fresh vegetable has been improved and cultivated over East and Southeast Asia for more than a century. This corn is now appearing in the European and American markets in great quantities and is sold as canned corn kernels, frozen corn kernels, and frozen corn ears. The market share of waxy corn in seed companies has increased as well, because of its popularity and the wide distribution of suitable growing areas. Farmers in Thailand and Southeast Asia have grown waxy corn as a cash crop in addition to rice for fresh consumption, consumed similar to sweet corn as cooked green ears (Lertrat & Thongnarin, 2008). The corn is sold fresh or as cooked ears at roadside markets, while large-scale farmers grow corn for the food industry or for export (Lertrat & Pulam, 2007).

Texture is widely recognized as one of the most important determinants of the eating quality of waxy corn. It has been observed that Thai consumers prefer sticky and tender corn, whereas others prefer it mildly sweet. Numerous studies have shown that the basic components of starch can affect the textural properties of several staple food crops such as rice (Kang, Hwang, Kim, & Choi, 2006), potato (Singh, Kaur, Ezekiel, & Guraya, 2005) and sorghum (Cagampang & Kirleis, 1984). A previous report confirmed that physicochemical criteria, such as apparent amylose content, gelatinization temperature, and gel consistency, are key factors in evaluating the eating and cooking quality of rice; hence, the eating quality essentially reflects the starch properties (Juliano, 1998). Other important starch property parameters have been studied to precisely evaluate factors that determine the quality of cooked rice, such as past viscosity characteristics (Bao & Xia, 1999; Bao et al., 2000; Champagne et al., 1999; Lin, Shi, Wu, & Wu, 2005), and branch chain length distribution of amylopectin (He et al., 2010; Yang et al., 2012).

In waxy corn, the main chemical components of starch in the kernels are amylose and amylopectin, and some intermediate materials that retain characteristics of both fractions. Functional properties of starch are affected by the amylose content and branch chain length distribution of amylopectin (Hanashiro, Abe, & Hizukuri, 1996; Kuakpetoon & Wang, 2006). Amylose is linear or slightly branched molecules whereas amylopectin is highly branched molecules. Waxy corn starch contains essentially 100% amylopectin (Perera, Lu, Sell, & Jane, 2001). Therefore, for breeding

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strategies it is important to understand the changes in starch properties during kernel development in order to improve kernel quality.

Fresh or immature waxy corn is consumed after kernel development – about 20 days after pollination, depending on genetics and the environment (Lertrat & Pulam, 2007; Lertrat & Thongnarin, 2008). Eating qualities of some corn varieties rapidly decrease within a few days after a specific optimum harvest time. Several genotypes, however, retain their good eating qualities, such as softness and stickiness after cooking, and also have a longer harvesting time. To improve the quality of waxy corn, appropriate harvesting times should be determined in the wide range of varieties. Hence, both genotypes and harvesting stages can affect the starch structure and functional properties of waxy corn, and also cause loss of quality during maturation. Previous research reports have focused mainly on the physicochemical properties of waxy corn and modification of starch from mature kernels (Fiedorowicz & Rebilas, 2002; Jane et al., 1999; Ji et al., 2003; Tziotis, Seetharaman, Klucinec, Keeling, & White, 2005; Zhou & Lim, 2012). Also studied were the physicochemical and morphological properties of normal corn during kernel development (Li, Blanco, & Jane, 2007). However, few reports have evaluated the functional properties of the starches in fresh kernels during maturation. Therefore, the objective of this study was to determine the effect of waxy corn genotype on the physicochemical properties of starches and the changes at different harvesting stages. The results should establish some parameters, which could provide important information for plant breeders in selecting breeding lines with starch characteristics relating to eating quality, and also for growers to better manage harvesting time to ensure the desired properties for the consumer.

2. Materials and methods

2.1. Plant materials

Two inbred lines and one hybrid of waxy corn were used in this study. The lines included KKU–UB and KKU–JD from the Vegetable Corn Improvement Project, Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand. Seeds of the commercial F_1 hybrid variety "Violet white" were obtained from East-West Seed Co. Ltd. (Nonthaburi, Thailand). The seeds were grown at Khon Kaen University's vegetable research farm in Khon Kaen province, Thailand (16°28′N, 102°49′E) from November 2011 to January 2012. Standard agronomic practices were used to provide adequate nutrition and keep the plots disease free. Fifteen years of each genotype were produced by sib pollination. Fresh kernels were harvested at 0, 2, 4 and 6 days after optimum stage (DAO) and stored in sealed plastic bags at −20 °C until starch extraction. Kernels at the physiological maturity kernels were harvested at 16 DAO or 35 days after pollination (DAP) for seeds; these were dried at 40 °C for 3 days and then kept in plastic bags at 4 °C.

2.2. Isolation of native starches

Waxy corn starches were isolated using the method of Jiranuntakul, Puttanlek, Rungsardthong, Puncha-arnon, and Uttapap (2011) with slight modifications. Corn kernels, 400 g for each sample, were steeped in water containing 0.16% sodium hydrogen sulfite at 50 °C for 24 h to inactivate the enzymes. The steeped water was drained off, and the grains were ground in a blender. The ground slurry was screened through a 106 μm sieve. The cloudy supernatant was removed, and the sediment was re-slurried in 0.2% sodium hydroxide solution. Then the supernatant was discarded, and the starch cake was re-suspended in water. The starch slurry was then passed through a 63 μm sieve. The starch was given repeated washings with water until the pH of the starch slurry reached 7. The starch cake was then dried in an oven at 40 °C for 24 h.

2.3. Amylose and starch contents

Amylose content was determined using the iodine adsorption method of Hoover and Ratnayake (2001), and starch content was obtained using a Total Starch Assay Kit (Megazyme International, Wicklow, Ireland).

2.4. Starch granule morphology

Scanning electron micrographs were taken by a JSM-5300LV scanning electron microscope (JEOL, Tokyo, Japan) at the Department of Biology, Khon Kaen University, Thailand. Starch samples were fixed onto circular specimen stubs using double-sided sticky tape, and vacuum-coated with gold/palladium (60/40). An accelerating potential of 10 kV was used during micrography.

2.5. Particle size analysis

About 50 mg (db) of starch was weighed into glass tubes and suspended with 5 mL of distilled water. Particle size distribution of the starches was determined by laser scattering on triplicate samples using a Mastersizer S (Malvern Instruments Ltd., Malvern, UK).

2.6. Unit chain distribution of amylopectin

Unit chain distribution of amylopectin was determined following the procedure described by Jiranuntakul, Puttanlek, Rungsardthong, Puncha-arnon, and Uttapap (2012) with slight modifications. Starch (28 mg dry basis) was dispersed in 2.8 mL of distilled water; this was heated and allowed to boil until the sample was completely dissolved. Distilled water (3.85 mL) and 0.35 mL of 10 mM acetate buffer (pH 3.5) were added, then mixed with a vortex mixer and incubated at 45 °C for 10 min. A solution of isoamylase (0.03 unit/mg of substrate) from *Pseudomonas* sp. (1000 U/ml, EC 3.2.1.68; Megazyme) was added to each sample. Samples were debranched for 12 h at 45 °C in a water bath. After debranching, enzyme activity was stopped by heating in boiling water for 5 min. The debranched amylopectin solutions were dried using a freeze dryer at −80 °C and then kept in sealed plastic containers until analyzed. The unit chain distribution of debranched amylopectin was determined using a high performance size exclusion chromatograph (HPSEC). The HPSEC system included a binary pump (model 1525; Waters, Milford, MA, USA); an injector; two 6.2 mm × 250 mm columns connected in series (Zorbax PSM 60S; Agilent Technologies, Santa Clara, CA), packed with 5 mm porous silica microspheres; and a refractive index (RI) detector (model 2414; Waters). The temperature of the columns was maintained at 50 °C. The mobile phase was filtered 90% (v/v) DMSO (in H_2O), at a flow rate of 0.5 mL/min. A 40 mL aliquot was injected into the HPSEC system. A calibration curve for each chromatogram was constructed using maltoheptaose, pullulan 6000 and pullulan 12000 standards. Data analysis was performed using Breeze 2 software (Waters); each sample was analyzed in duplicate. The *y*-axis of the chromatogram was converted from the RI value to a molar response using the calibration curve.

2.7. Pasting properties

Pasting properties of isolated waxy corn starches were studied using a Rapid Visco Analyzer (Newport Scientific Pty. Ltd.,

Warriewood, NSW, Australia). Viscosity profiles of starches from different genotypes and stages were recorded using starch suspensions (6%, w/w). The temperature and time conditions included a heating step from 50 to 95 °C at 6 °C/min (after an equilibration time of 1 min at 50 °C), a holding phase at 95 °C for 1.5 min, a cooling step from 95 to 50 °C at 6 °C/min, and a holding phase at 50 °C for 2 min at the same rate.

2.8. Statistical analysis

Genotypes and harvest dates were determined as random effects and replication was determined as the fixed effect. Analysis of variance (ANOVA) was performed for each characteristic following a 3×5 factorial experiment in randomized complete block design (RCBD) with three replications; data were expressed as mean values ± standard deviation. The least significant difference (LSD) was used to compare mean differences at $p < 0.05$.

3. Results and discussion

3.1. Genotypic variability and genotype x stage interactions

Mean squares from ANOVA indicated that genotype, harvest stage, and genotype × stage effects were significant for all parameters (Table 1). Genotype × stage (G × S) interactions were significant ($p < 0.01$) for starch content, starch granule type, unit chain length distribution, and pasting properties. The genotype main effect (G) was the most important source of variation accounting for 75% of the total sum of squares for starch content. Stage main effect (S) also accounted for a large proportion of the variation, 56–75% for starch granule types. A large estimate of genetic variance indicates that selection and breeding initiatives can proceed immediately. G × S effects were greater than the genotypic effects on the sum of squares for average chain length (DP 6–12, DP 13–24, DP ≥37), peak viscosity, trough viscosity, breakdown viscosity, final viscosity and setback viscosity. G × S interactions must be considered when breeding for physicochemical property traits, because this interaction affects the magnitude of these genotypes. This information is very useful in the selection of parents and also in evaluating breeding criteria for eating quality of waxy corn. However, testing for multi-environment trails should be performed in order to identify superior genotypes (Falconer & Mackay, 1996; Hallauer, Carena, & Miranda Filho, 2010). Hence, using this information, recommendations for genotypes and harvest stages to target desired starch physicochemical properties and eating qualities can be attempted.

3.2. Accumulation of amylose and starch contents

Amylose content of starches in the three waxy corn genotypes were not significantly different at all harvesting stages (0.0%). In previous reports on normal corn, amylose content increased from 9.2% on 12 DAP to 24.2% on 30 DAP, and relative to kernel development (Li et al., 2007). Furthermore, amylose content has been shown to vary among botanical sources and growth stages, with 21% (immature stage) to 27% (mature stage) for kidney bean (Yoshida et al., 2003), 31.1% (immature stage) to 36.2% (mature stage) for canna (Puncha-arnon, Puttanlek, Rungsardthong, Pathipanawat, & Uttapap, 2007), and 36.5% (immature stage) to 39.0% (mature stage) for yam (Huang, Lin, & Wang, 2006). This indicated that amylose content increased relative to the enzyme activity of starch biosynthesis in maize endosperm development (Tsai, Salamini, & Nelson, 1970). Granule-bound starch synthase (GBSSI) is the key enzyme involved amylose synthesis in cereal endosperm, whereas the waxy mutant genes typically lack amylose and have starches comprised solely of amylopectin (James, Denyer, & Myers, 2003; Patron et al., 2002; Tsai, 1974).

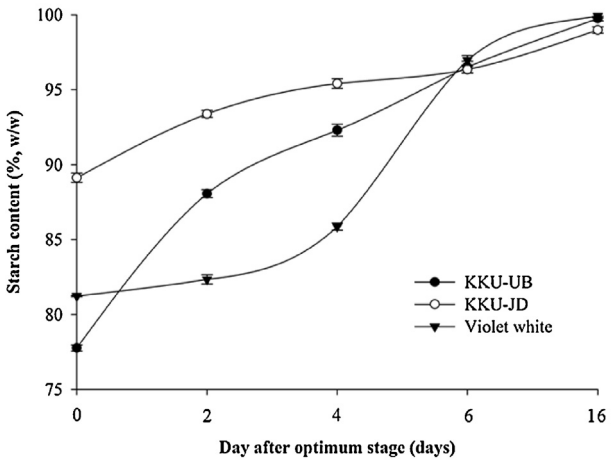

Fig. 1. Accumulation of starch contents (%, w/w) of three waxy corns during delayed harvesting at 0, 2, 4, 6 and 16 DAO (physiological maturity stage).

The total starch contents were significantly different at various stages of harvesting. KKU–UB exhibited starch content lower than KKU–JD at optimum stage (0 DAO), 77.8 and 89.1%, respectively, whereas Violet white showed a starch content of 81.2%. The starch contents in all three varieties increased relative to harvesting stage until the physiological maturity stage at 16 DAO (Fig. 1). For each harvesting time, KKU–JD showed a higher total starch content than KKU–UB and Violet white, respectively. Total starch contents for KKU–JD were 89.1, 93.3, 95.5, 96.3 and 98.9% at 0, 2, 4, 6 and 16 DAO, respectively. As determined in a previous study on agronomic traits, KKU–JD loses quality when harvesting delayed; hence increasing starch content may adversely affect the quality of these waxy corns (data not shown). The total starch content is affected by the activity of starch biosynthesis enzymes in the cereal endosperm (Dong, Chen, Xie, Qiao, & Yang, 2012; Jeon, Ryoo, Hahn, Walia, & Nakamura, 2010; Tsai et al., 1970). In wheat endosperm, the enzyme activities were different during kernel development, and were involved in starch synthesis and accumulation including sucrose synthase (SuSy), ADP-glucose pyrophosphorylase (AGPase), starch synthase (SSS), granule-bound starch synthase (GBSS), and starch branching enzyme (SBE) (Dai, Yin, & Wang, 2009). Furthermore, amylopectin and starch accumulation rate were positively correlated with GBSS, SSS, SBE and debranching enzyme (DBE), which are more important for the starch quality of wheat (Wang, Li, Qi, Shi, & Yin, 2011). However, a better understanding of the mechanism for starch accumulation and its component variations would be helpful both for improving the quality and promoting the physicochemical property traits related to the quality of waxy corn.

3.3. Morphology and granule distribution of starches

Scanning electron micrographs of waxy corn starches are shown in Fig. 2. Starch granules from different stages differ in structure, with diameters from 0.4 to 26 μm. Granules of every stage are oval, although around and polygonal shapes become more predominant at later stages. The surfaces of starch granules are smooth, without scratches, while the surfaces of starch granules from delayed harvesting were extensively eroded, with numerous cracks. Small spherical, shapes are exhibited at earlier development stages, becoming large, polygonal shapes at later stages of kernel development (Fig. 2). Various shapes of KKU–UB starch granules have been observed, from spherical to polygonal, during kernel development, while the presence of pinholes begins with a few

Table 1
Mean squares and proportion of sum of squares for starch content, starch granules, unit chain distribution and pasting properties of waxy corn starches harvested at different stages.

Source of variation	df				
	2 Genotype (G)	4 Stage (S)	8 G × S	28 Error	CV (%)
Starch content	114.88** (0.75)	407.48** (0.11)	37.64** (0.14)	0.24 (0.00)	0.53
Granule distribution					
Large-granules (>15 μm)	113.23** (0.12)	310.46** (0.68)	43.60** (0.19)	0.02 (0.00)	0.73
Medium-granules (5–15 μm)	86.57** (0.14)	117.45** (0.56)	48.52** (0.31)	0.00 (0.00)	0.01
Small granules (<5 μm)	2.22** (0.04)	19.81** (0.75)	2.71** (0.21)	0.00 (0.00)	0.23
Unit chain length					
Average chain length	7.01** (0.36)	1.80** (0.18)	2.25** (0.46)	0.01 (0.01)	0.51
DP 6–12	13.38** (0.22)	6.23** (0.20)	8.82** (0.57)	0.06 (0.01)	0.33
DP 13–24	3.99** (0.15)	2.44** (0.19)	4.02** (0.64)	0.04 (0.02)	1.32
DP 25–36	6.69** (0.47)	1.24** (0.17)	1.33** (0.37)	0.03 (0.03)	1.83
DP ≥37	1.02** (0.33)	0.17** (0.11)	0.34** (0.45)	0.02 (0.10)	8.68
Pasting properties					
Peak viscosity	813.99** (0.34)	409.35** (0.30)	105.31** (0.35)	2.28 (0.01)	0.96
Trough viscosity	727.16** (0.17)	466.38** (0.13)	341.21** (0.69)	5.18 (0.01)	2.42
Breakdown viscosity	21.33** (0.19)	141.41** (0.09)	429.85** (0.70)	3.02 (0.01)	2.83
Final viscosity	880.44** (0.17)	904.20** (0.20)	424.04** (0.63)	1.50 (0.01)	1.11
Setback viscosity	10.00* (0.04)	227.56** (0.36)	33.79** (0.53)	2.13 (0.06)	8.81
Pasting temperature	1.15** (0.49)	2.22** (0.15)	1.38** (0.31)	0.07 (0.04)	0.33

Numbers within parentheses are proportion of sum squares to total sum of squares.
df: degree of freedom.
* Significant difference at $p < 0.05$.
** Significant difference at $p < 0.01$.

at 6 DAO, progressing to many at 16 DAO (Fig. 2A–E). Whereas, KKU–JD showed spherical and polygonal granule shapes at the optimum stage, becoming polygonal and irregular shapes at 2 to 16 DAO; in addition, granules exhibited a number of pinholes at 2 DAO, which increased in relation to kernel development (Fig. 2F–J). Starch granules of Violet white showed oval and spherical shapes at the optimum stage, and then changed from spherical at 2–4 DAO to polygonal at 6–16 DAO; pinholes appeared at the physiological maturity stage (Fig. 2K–O). The changes in shape change could be caused by the pressure built up from the growth in the size of the endosperm, whereas the pericarp evolved from the ovary wall after pollination. The size increase in the endosperm was accompanied by cell division and enlargement during kernel development (Li et al., 2007; MacGregor, LaBerge, & Meredith, 1971). The large number of pinholes on the surface of starch granules resulted from endogenous enzyme activity (Hasjim, Srichuwong, Scott, & Jane, 2009). The porous structure formed could be an effect of enzyme hydrolysis during kernel development. In normal corn kernels, the number of pinholes is related to the activity of granule-bound starch synthase (GBSSI) and amylose accumulation (Gao, Fisher, Kim, Shannon, & Guiltinan, 1996; Li et al., 2007).

Starches of waxy corn genotypes from different harvesting stages showed significant differences in the size distribution as well as in the percent volume of the granules (Table 2). Granule sizes exhibited a bimodal distribution, with peak values within the groups, and also were classified into large (>15 μm), medium (5–15 μm), and small (<5 μm) granules. The size of starch granules in all genotypes increased relative to the days after optimum stage from 0 to 16. Average diameters of large, medium, and small granules ranged from 16.6 to 26.2, 5.7 to 14.2, and 0.4 to 4.8 μm, respectively. Among all the stages, medium granules had the highest contribution to the total volume (60.8–81.5%), followed by large (5.7–30.1%) and small granules (9.1–15.3%). The proportion of medium and small granules decreased relative to delayed harvest, while large granules showed an increased proportion (Fig. 3A–C). In KKU–UB, the proportion of large granules decreased from 17.4% (0 DAO) to 14.1% (2 DAO), and then increased to 26.7% at maturity stage (16 DAO); conversely, the proportion of medium granules increased at 2 DAO and then decreased until the maturity stage (Fig. 3A). The proportion of large-granules of KKU–JD starches decreased from 20.4% (0 DAO) to 18.8% (2 DAO) and then increased rapidly to 25.4, 26.6 and 29.1% at 4, 6 and 16 DAO, respectively, whereas the proportion of medium and small granules decreased until the physiological maturity stage (Fig. 3B). Violet white starch granules exhibited a small proportion of large granules (5.7%) at optimum stage; the proportion then increased rapidly, to 16.6% at 2 DAO and reaching 30.1% at 16 DAO (Fig. 3C). These results are in agreement with the starch granule development in normal corn endosperm (Li et al., 2007), and are similar to increases in the size of granules with kernel maturation of wheat (Bechtel, Zayas, Kaleikau, & Pomeranz, 1990), rice (Murugesan & Hizukuri, 1992) and barley (MacGregor et al., 1971). The results indicated that the development of individual starch granules was in parallel with development of the kernel. Furthermore, the starch granule numbers in kernel cells showed an increasing trend in relation to kernel maturity.

3.4. Chain length distribution of debranched waxy corn starches

The chain length distribution of amylopectin showed significant variation among the starches from different genotypes and stages (Table 3). The classification of amylopectin branched chain in the A, B1, B2, and B3 groups conform to chains with a DP of 6–12, 13–24, 25–36 and ≥37, respectively (Hanashiro et al., 1996). Average chain length (CL) ranged from DP 14.7 to 16.9 for KKU–UB, DP 16.9 to 17.4 for KKU–JD, and DP 16.5 to 18.7 for Violet white. Amylopectin of KKU–UB, KKU–JD and Violet white starches harvested on 2, 6 and 4 DAO, respectively, showed the longest CL. These results indicated that the structures of amylopectin molecules were not homogenous throughout the various starch granules but changed with the genotype and development of the granule (Li et al., 2007). The increase of CL can affect the physicochemical properties of starches, causing decreased gelatinization temperature and also peak viscosity (Jane et al., 1999). The three genotypes showed a larger proportion of short branched chains (DP 6–12) than of DP 13–24, DP 25–36 and DP ≥37, respectively. The A chains of KKU–UB decreased from

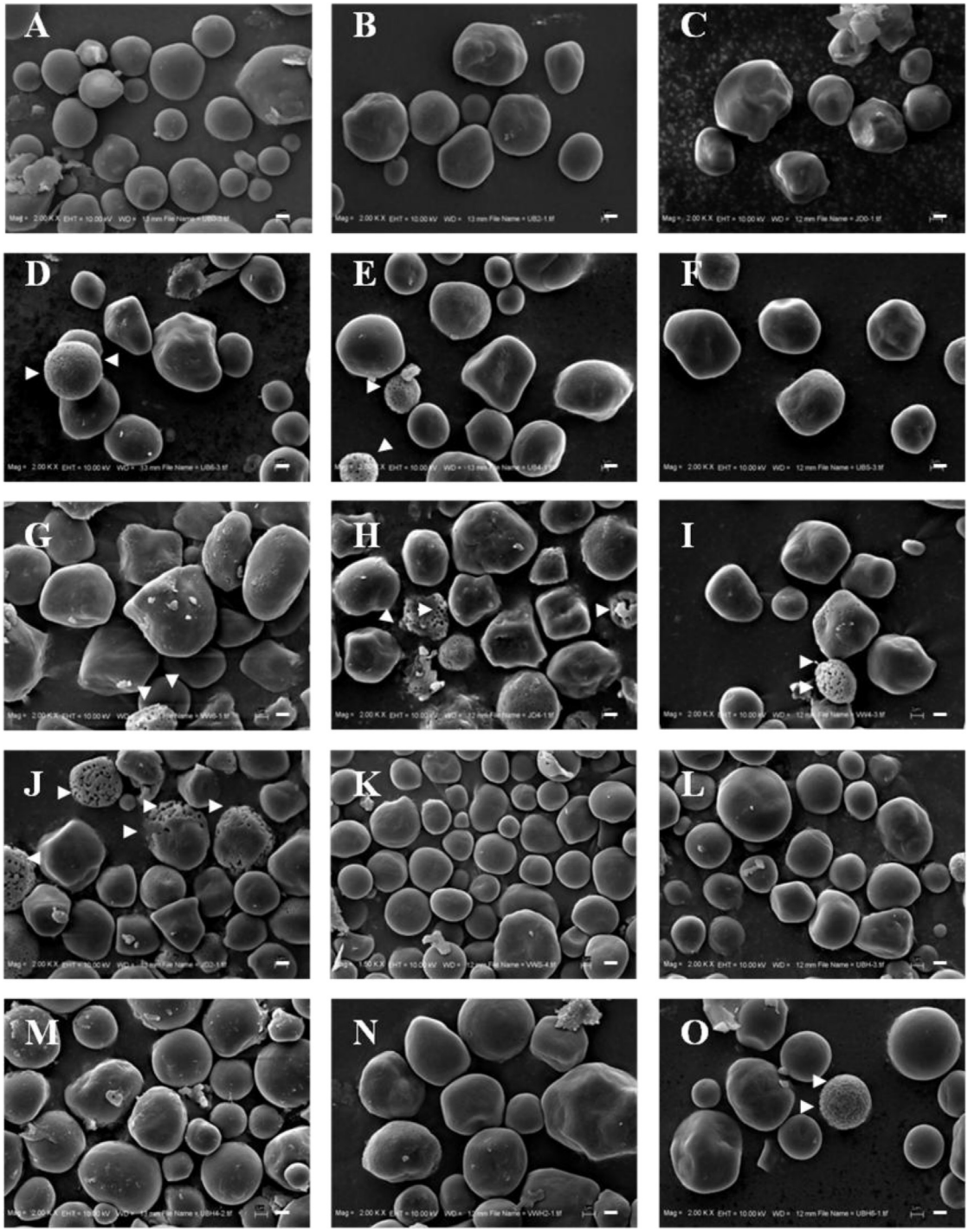

Fig. 2. Scanning electron micrographs of starch granules isolated from waxy corns at the different harvesting stages (2000×, scale bar 2 μm): (A–E) KKU–UB for 0, 2, 4, 6 and 16 DAO, respectively, (F–J) KKU–JD for 0, 2, 4, 6 and 16 DAO, respectively; and (K–O) Violet white for 0, 2, 4, 6 and 16 DAO, respectively. Arrowheads: pinholes on the surface starch granule.

74.9% (0 DAO) to 73.4% (2 DAO), then increased to 74.7% (4 DAO) and 77.7% (6 DAO), and finally decreased to 76.3% at 16 DAO; while B1 chains (DP 13–24) increased from 13.9% (0 DAO) to 15.4% (2 DAO) and then decreased to 11.6% at 6 DAO. KKU–JD, A chains increased from 74.0% (0 DAO) to 75.1% (2 DAO) and then decreased to 73.7 and 73.4% at 4 and 6 DAO, respectively, whereas DP 13–24 showed no variation but DP ≥37 increased from 1.5% (0 DAO) to 2.5% (4 DAO) and then decreased to 1.8% at physiological maturity. The same trend was also observed for DP 6–12 and DP 13–24 of Violet white. These results indicated that the long branched chains of amylopectin continually decreased after kernel development until becoming dried kernels (Li et al., 2007). The large proportion of short branched chains of amylopectin led to a defective crystalline structure, which resulted in a lower gelatinization temperature and a gelatinization change (Ai, Medic, Jiang, Wang, & Jane, 2011; Jane, 2007; Jane et al., 1999).

Table 2
Volume distribution of waxy corn starch granules harvested at different stages.

Sample	DAO	Volume distribution of starch granules (%)		
		Large-granules (>15 μm)	Medium-granules (5–15 μm)	Small-granules (<5 μm)
KKU–UB	0	17.4 ± 0.3 h	67.3 ± 0.1 h	15.3 ± 0.0 a
	2	14.1 ± 0.1 k	73.6 ± 0.2 b	12.4 ± 0.2 d
	4	16.2 ± 0.2 j	72.5 ± 0.0 c	11.3 ± 0.5 g
	6	26.5 ± 0.0 c	63.5 ± 0.2 l	9.9 ± 0.3 j
	16[a]	26.7 ± 0.1 c	63.6 ± 0.3 k	9.8 ± 0.2 k
KKU–JD	0	20.4 ± 0.1 f	67.8 ± 0.4 g	11.8 ± 0.0 f
	2	18.8 ± 0.2 g	68.9 ± 0.0 f	12.2 ± 0.0 e
	4	25.1 ± 0.0 d	64.3 ± 0.2 j	10.7 ± 0.1 i
	6	26.6 ± 0.2 c	62.8 ± 0.2 m	10.7 ± 0.2 i
	16	29.1 ± 0.3 b	61.3 ± 0.4 n	9.6 ± 0.4 l
Violet white	0	5.7 ± 0.1 l	81.5 ± 0.1 a	12.7 ± 0.0 b
	2	16.6 ± 0.2 i	70.9 ± 0.3 d	12.5 ± 0.0 c
	4	18.7 ± 0.5 g	69.2 ± 0.2 e	12.2 ± 0.5 e
	6	22.5 ± 0.5 e	66.4 ± 0.2 i	11.1 ± 0.2 h
	16	30.1 ± 0.3 a	60.8 ± 0.3 o	9.1 ± 0.1 m

Means with different letters (a, b, etc.) in the same row are significantly different ($p < 0.05$). Mean ± standard deviation.
DAO: days after optimum stage.
[a] Physiological maturity stage (dried kernels).

Table 3
Average chain length and unit chain distribution of debranched amylopectins isolated from waxy corn starches at different harvesting stages.

Sample	DAO	CL	Unit chain distribution (%)[a]			
			DP 6–12	DP 13–24	DP 25–36	DP ≥37
KKU–UB	0	16.6 ± 0.1 hi	74.9 ± 0.2 de	13.9 ± 0.1 d	9.7 ± 0.0 cd	1.5 ± 0.0 e
	2	16.9 ± 0.0 f	73.4 ± 0.1 i	15.4 ± 0.1 a	9.6 ± 0.0 de	1.6 ± 0.1 de
	4	16.8 ± 0.1 fg	74.7 ± 0.1 de	14.2 ± 0.0 c	9.6 ± 0.1 de	1.4 ± 0.0 ef
	6	14.7 ± 0.1 k	77.7 ± 0.1 a	11.6 ± 0.0 g	9.5 ± 0.2 def	1.4 ± 0.2 f
	16	15.5 ± 0.2 j	76.3 ± 0.2 b	13.1 ± 0.4 f	9.2 ± 0.1 fg	1.2 ± 0.0 ef
KKU–JD	0	17.3 ± 0.0 cd	74.0 ± 0.1 gh	14.5 ± 0.1 bc	10.0 ± 0.5 bc	1.5 ± 0.1 e
	2	17.2 ± 0.0 de	75.1 ± 0.1 d	14.8 ± 0.3 b	8.6 ± 0.2 h	1.5 ± 0.0 e
	4	17.1 ± 0.0 e	73.7 ± 0.5 hi	14.7 ± 0.2 b	9.2 ± 0.1 g	2.5 ± 0.2 a
	6	17.4 ± 0.0 c	73.4 ± 0.3 i	14.8 ± 0.1 b	9.3 ± 0.0 efg	2.4 ± 0.1 a
	16	16.9 ± 0.0 fg	74.2 ± 0.1 fg	14.6 ± 0.0 b	9.4 ± 0.2 efg	1.8 ± 0.1 bcd
Violet white	0	16.8 ± 0.1 gh	75.0 ± 0.2 d	13.1 ± 0.2 f	9.9 ± 0.2 c	1.9 ± 0.3 b
	2	16.5 ± 0.0 i	74.6 ± 0.1 ef	13.5 ± 0.3 e	10.3 ± 0.0 b	1.6 ± 0.3 cde
	4	18.7 ± 0.1 a	70.9 ± 0.4 k	15.5 ± 0.1 a	11.6 ± 0.2 a	1.9 ± 0.1 b
	6	18.3 ± 0.3 b	71.6 ± 0.5 j	15.4 ± 0.1 a	11.5 ± 0.0 a	1.5 ± 0.5 e
	16	16.5 ± 0.1 i	75.8 ± 0.1 c	13.1 ± 0.4 f	9.3 ± 0.3 efg	1.9 ± 0.2 bc

DAO, days after optimum stage; CL, average chain length; DP, degree of polymerization.
[a] Means with different letters (a, b, etc.) in the same column are significantly different ($p < 0.05$). Mean ± standard deviation.

Table 4
Pasting properties of waxy corn starches at different harvesting stages.

Sample	DAO	PV (RVU)	TV (RVU)	BD (RVU)	FV (RVU)	SV (RVU)	PT (°C)
KKU–UB	0	161.7 ± 1.6 cd	110.8 ± 2.5 a	50.9 ± 1.9 g	124.1 ± 2.5 a	13.3 ± 2.1 fg	79.3 ± 0.5 d
	2	160.9 ± 1.7 cd	109.1 ± 1.0 ab	51.8 ± 2.4 g	121.7 ± 1.5 b	12.6 ± 2.3 g	78.7 ± 0.0 e
	4	164.6 ± 1.5 b	98.3 ± 1.1 d	66.3 ± 2.7 cd	119.3 ± 0.6 cd	20.9 ± 1.2 bc	79.3 ± 0.0 d
	6	171.2 ± 0.8 a	102.1 ± 1.3 c	69.1 ± 1.3 c	121.2 ± 0.5 bc	19.1 ± 0.8 cd	80.1 ± 0.6 c
	16	162.2 ± 0.4 bcd	90.0 ± 0.3 f	73.5 ± 0.4 b	111.2 ± 0.9 f	21.2 ± 0.6 bc	81.7 ± 0.0 a
KKU–JD	0	136.9 ± 0.9 h	69.2 ± 0.5 h	67.8 ± 0.6 c	76.5 ± 0.5 h	7.4 ± 0.9 h	80.7 ± 0.1 b
	2	159.9 ± 0.5 d	92.5 ± 1.9 ef	67.4 ± 2.5 c	111.7 ± 0.6 ef	19.3 ± 1.0 bcd	80.1 ± 0.0 c
	4	150.8 ± 1.6 f	94.9 ± 0.5 de	55.9 ± 1.4 f	113.7 ± 0.5 e	18.8 ± 0.8 cd	80.1 ± 0.4 c
	6	156.8 ± 1.7 e	92.5 ± 0.4 ef	64.4 ± 1.5 d	108.1 ± 0.4 g	15.6 ± 1.2 ef	80.1 ± 0.8 c
	16	142.7 ± 0.4 g	97.2 ± 0.2 d	45.5 ± 1.6 h	118.7 ± 1.2 d	21.5 ± 1.1 b	80.1 ± 0.4 c
Violet white	0	140.2 ± 1.5 g	71.3 ± 2.1 h	68.9 ± 2.1 c	78.4 ± 1.0 h	7.1 ± 1.3 h	80.1 ± 0.5 c
	2	160.9 ± 0.3 cd	105.6 ± 1.5 bc	55.4 ± 0.5 f	119.3 ± 1.1 cd	13.8 ± 2.1 fg	79.3 ± 0.2 d
	4	162.7 ± 0.7 bc	95.9 ± 1.6 de	47.8 ± 0.6 h	103.2 ± 0.5 g	14.5 ± 0.7 efg	79.3 ± 0.0 d
	6	163.4 ± 2.1 bc	98.1 ± 0.9 d	60.8 ± 1.1 e	118.4 ± 0.4 d	16.8 ± 0.8 de	79.3 ± 0.4 d
	16	161.6 ± 1.9 cd	83.8 ± 1.3 g	77.8 ± 1.0 a	110.5 ± 2.1 f	26.8 ± 1.5 a	80.2 ± 0.1 c

Means with different letters in the same column are significantly different ($p < 0.05$). Mean ± standard deviation.
PV, peak viscosity; TV, trough viscosity; BV, breakdown viscosity; FV, final viscosity; SV, setback viscosity, and PT, pasting temperature.
DAO, days after optimum stage.

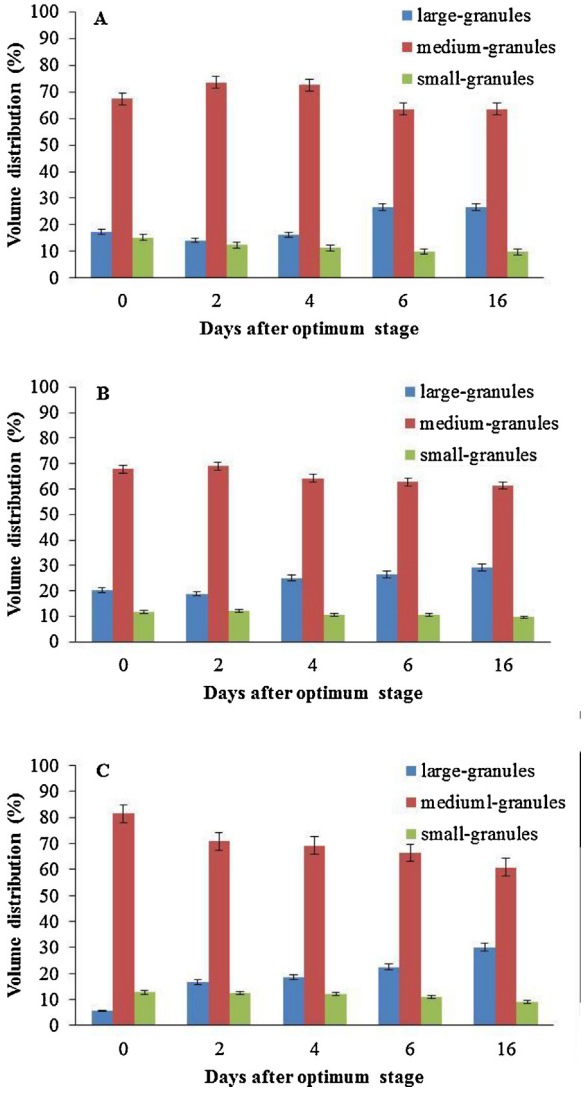

Fig. 3. Volume distribution (%) of waxy corn starches at different harvested stages: (A) KKU–UB, (B) KKU–JD and (C) Violet white.

3.5. Pasting properties of starches

The pasting properties of waxy corn starches from different harvesting stages showed significant differences in all genotypes (Table 4). According to rapid visco analysis patterns, the pasting behaviors of waxy corn starches were greatly affected by harvesting times. The starches at optimum stage (0 DAO) had the lowest peak viscosity; 161.7, 136.9 and 140.2 RVU for KKU–UB, KKU–JD and Violet white, respectively. The results also showed that the peak viscosity of starches increased with delayed harvesting times, and then decreased until becoming dried kernels at physiological maturity stage. The peak viscosity of starch is an important characteristic for discriminating a given starch from other genotypes. For KKU–UB starch, peak viscosity decreased from 161.7 (0 DAO) to 160.9 RVU (2 DAO), and then increased to 164.6 (4 DAO) and 171.2 RVU (16 DAO). Breakdown viscosity increased from 50.9 to 73.5 RVU at 0 and 16 DAO, respectively, but final and setback viscosity decreased relation to harvesting stages. In the case of KKU–JD and Violet white genotypes, the peak viscosity for the pasting behavior of the starches showed increased from 136.9 (0 DAO) to 142.7 RVU (16 DAO) and from 140.2 (0 DAO) to 161.6 RVU for KKU–JD and Violet white, respectively. The same trend was also observed for final and breakdown viscosity, which in KKU–JD increased from 76.5 (0 DAO) to 118.7 RVU (16 DAO) and from 7.4 (0 DAO) to 21.5 RVU (16 DAO), respectively; while in Violet white, these increased from 78.4 (0 DAO) to 110.5 RVU (16 DAO) and from 7.1 to 26.8 RVU (16 DAO), respectively. Waxy starches consisted mainly of amylopectin, which resulted in high swelling of granules, low setback viscosity and also reflecting gel network formation when the starch paste cooled (Jiranuntakul et al., 2011; Li et al., 2007; Tester & Morrison, 1990). This data may be used as criteria for evaluation of changes in the physicochemical properties of starches, which affect the quality of waxy corn at the fresh ear stage.

4. Conclusions

In conclusion, starch contents showed significant differences in relation to various genotypes and stages of harvesting. The granule size increased in relation to kernel development. Among all genotypes and stages, medium granules (5–15 μm) had the highest contribution to the total volume, followed by large (>15 μm) and small (<5 μm) granules, respectively. Debranched of waxy corn starches showed the highest CL at 2 (KKU–UB), 6 (KKU–JD) and 4 (Violet white) DAO. However, the unit chain distribution of amylopectin showed variation among the starches from different harvesting stages. Starches at the optimum stage had the lowest peak viscosity; 161.7 for KKU–UB, 136.9 RVU for KKU–JD and 140.2 RVU for Violet white genotypes. Furthermore, peak viscosity of starches increased with delayed harvesting times, and then increased until the dried kernel at the physiological maturity stage. These physicochemical properties can be used as selection criteria for evaluation of genotypes and provide useful information for plant breeders to improve eating quality in fresh waxy corn.

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References

Ai, Y., Medic, J., Jiang, H., Wang, D., & Jane, J. (2011). Starch characterization and ethanol production of sorghum. *Journal of Agricultural and Food Chemistry*, *59*, 7385–7392.

Bao, J. S., & Xia, Y. W. (1999). Genetic control of paste viscosity characteristics in *indica* rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, *98*, 1120–1124.

Bao, J. S., Zheng, X. W., Xia, Y. W., He, P., Shu, Q. Y., Lu, X., et al. (2000). QTL mapping for the paste viscosity characteristics in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, *100*, 280–284.

Bechtel, D. B., Zayas, I., Kaleikau, L., & Pomeranz, Y. (1990). Size-distribution of wheat starch granules during endosperm development. *Cereal Chemistry*, *67*, 59–63.

Cagampang, G. B., & Kirleis, A. W. (1984). Relationship of sorghum grain hardness to selected physical and chemical measurements of grain quality. *Cereal Chemistry*, *61*, 100–105.

Champagne, E. T., Bett, K. L., Vinyard, B. T., McClung, A. M., Barton, F. E., II., Moldenhauer, K., et al. (1999). Correlation between cooked rice texture and rapid visco analyser measurements. *Cereal Chemistry*, *76*, 764–771.

Dai, Z., Yin, Y., & Wang, Z. (2009). Comparison of starch accumulation and enzyme activity in grains of wheat cultivars differing in kernel type. *Plant Growth Regulation*, *57*, 153–162.

Dong, M. H., Chen, P. F., Xie, Y. L., Qiao, Z. Y., & Yang, J. C. (2012). Variation in carbohydrate and protein accumulation between spikelets at different positions within a panicle during rice grain filling. *Rice Science*, *19*, 223–232.

Falconer, S. D., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics* (4th ed.). England: Longman.

Fiedorowicz, M., & Rebilas, K. (2002). Physicochemical properties of waxy corn starch and corn amylopectin illuminated with linearly polarised visible light. *Carbohydrate Polymers*, *50*, 315–319.

Gao, M., Fisher, D. K., Kim, K. N., Shannon, J. C., & Guiltinan, M. L. (1996). Evolutionary conservation and expression patterns of maize starch branching enzyme I and IIb genes suggests isoform specialization. *Plant Molecular Biology*, *30*, 1223–1232.

Hallauer, R. A., Carena, M. J., & Miranda, J. B. (2010). *Handbook of plant breeding. Quantitative genetics in maize breeding* (1st ed.). New York: Springer.
Hanashiro, I., Abe, J. I., & Hizukuri, S. (1996). A periodic distribution of the chain length of amylopectin as revealed by high-performance anion-exchange chromatography. *Carbohydrate Research*, *283*, 151–159.
Hasjim, J., Srichuwong, S., Scott, M. P., & Jane, J. (2009). Kernel composition, starch structure, and enzyme digestibility of opaque-2 maize and quality protein maize. *Journal of Agricultural and Food Chemistry*, *57*, 2049–2055.
He, X. P., Zhu, C. L., Liu, L. L., Wang, F., Fu, J. R., Jiang, L., et al. (2010). Difference of amylopectin structure among rice varieties differing in grain quality and its correlations with starch physicochemical properties. *Acta Agronomy Sinica*, *36*, 276–284.
Hoover, R., & Ratnayake, W. S. (2001). Determination of total amylose content of starch. In R. E. Wrolstad, T. E. Acree, H. An, E. A. Decker, M. A. Penner, D. S. Reid, S. J. Schwartz, C. F. Shoemaker, & P. Sporns (Eds.), *Current protocols in food analytical chemistry (unit E 2–3)*. USA: John Wiley and Sons Inc.
Huang, C. C., Lin, M. C., & Wang, C. C. R. (2006). Changes in morphological, thermal and pasting properties of yam (*Dioscorea alata*) starch during growth. *Carbohydrate Polymers*, *64*, 524–531.
James, M. G., Denyer, K., & Myers, A. M. (2003). Starch synthesis in the cereal endosperm. *Current Opinion in Plant Biology*, *6*, 215–222.
Jane, J. (2007). Structure of starch granules. *Journal of Apply Glycoscience*, *54*, 31–36.
Jane, J., Chen, Y. Y., Lee, L. F., McPherson, A. E., Wong, K. S., Radosavljevic, M., et al. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry*, *76*, 629–637.
Jeon, J. S., Ryoo, N., Hahn, T. R., Walia, H., & Nakamura, Y. (2010). Starch biosynthesis in cereal endosperm. *Plant Physiology and Biochemistry*, *48*, 383–392.
Ji, Y., Wong, K., Hasjim, J., Pollak, L. M., Duvick, S., Jane, J., et al. (2003). Structure and function of starch from advanced generations of new corn lines. *Carbohydrate Polymers*, *54*, 305–319.
Jiranuntakul, W., Puttanlek, C., Rungsardthong, V., Puncha-arnon, S., & Uttapap, D. (2011). Microstructural and physicochemical properties of heat-moisture treated waxy and normal starches. *Journal of Food Engineering*, *104*, 246–258.
Jiranuntakul, W., Puttanlek, C., Rungsardthong, V., Puncha-arnon, S., & Uttapap, D. (2012). Amylopectin structure of heat-moisture treated starches. *Starch/Stärke*, *64*, 470–480.
Juliano, B. O. (1998). Varietal impact on rice quality. *Cereal Foods World*, *43*(207-211), 214–216, 218-222.
Kang, H. J., Hwang, I. K., Kim, K. S., & Choi, H. C. (2006). Comparison of the physicochemical properties and ultrastructure of japonica and indica rice grains. *Journal of Agricultural and Food Chemistry*, *54*, 4833–4838.
Kuakpetoon, D., & Wang, Y. J. (2006). Structural characteristics and physicochemical properties of oxidized corn starches varying in amylose content. *Carbohydrate Research*, *341*, 1896–1915.
Lertrat, K., & Pulam, T. (2007). Breeding for increased sweetness in sweet corn. *International Journal of Plant Breeding*, *1*, 27–30.
Lertrat, K., & Thongnarin, N. (2008). Novel approach to eating quality improvement in local waxy corn: Improvement of sweet taste in local waxy corn variety with mixed kernels from super sweet corn. *Acta Horticulture*, *769*, 145–150.
Li, L., Blanco, M., & Jane, J. (2007). Physicochemical properties of endosperm and pericarp starches during maize development. *Carbohydrate Polymers*, *67*, 630–639.
Lin, J., Shi, C., Wu, M., & Wu, J. (2005). Analysis of genetic effects for cooking quality traits of *japonica* rice across environments. *Plant Science*, *168*, 1501–1506.
MacGregor, A. W., LaBerge, D. E., & Meredith, W. O. S. (1971). Changes in barley kernels during growth and maturation. *Cereal Chemistry*, *48*, 255–269.
Murugesan, G., & Hizukuri, S. (1992). Structure and properties of waxy-rice (IR29) starch during development of the grain. *Carbohydrate Research*, *223*, 235–242.
Patron, N. J., Smith, A. M., Fahy, B. F., Hylton, C. M., Naldrett, M. J., Rossnagel, B. G., et al. (2002). The altered pattern of amylose accumulation in the endosperm of low-amylose barley cultivars is attributable to a single mutant allele of granule-bound starch synthase I with a deletion in the 5′-non-coding region. *Plant Physiology*, *130*, 190–198.
Perera, C., Lu, Z., Sell, J., & Jane, J. (2001). Comparison of physicochemical properties and structures of sugary-2 cornstarch with normal and waxy corn cultivars. *Cereal Chemistry*, *78*, 249–256.
Puncha-arnon, S., Puttanlek, C., Rungsardthong, V., Pathipanawat, W., & Uttapap, D. (2007). Changes in physicochemical properties and morphology of canna starches during rhizomal development. *Carbohydrate Polymers*, *70*, 206–217.
Singh, N., Kaur, L., Ezekiel, R., & Guraya, H. S. (2005). Microstructural, cooking and textural characteristics of potato (*Solanum tuberosum* L.) tubers in relation to physicochemical and functional properties of their flours. *Journal of Science of Food and Agriculture*, *85*, 1275–1284.
Tester, R. F., & Morrison, W. R. (1990). Swelling and gelatinization of cereal starches. II. Waxy rice starches. *Cereal Chemistry*, *67*, 558–563.
Tsai, C. Y. (1974). The function of the waxy locus in starch synthesis in maize endosperm. *Biochemical Genetics*, *11*, 83–96.
Tsai, C. Y., Salamini, F., & Nelson, O. E. (1970). Enzymes of carbohydrate metabolism in the developing endosperm of maize. *Plant Physiology*, *46*, 299–306.
Tziotis, A., Seetharaman, K., Klucinec, J. D., Keeling, P., & White, P. J. (2005). Functional properties of starch from normal and mutant corn genotypes. *Carbohydrate Polymers*, *61*, 238–247.
Wang, Z., Li, W., Qi, J., Shi, P., & Yin, Y. (2011). Starch accumulation, activities of key enzyme and gene expression in starch synthesis of wheat endosperm with different starch contents. *Journal of Food Science and Technology*, http://dx.doi.org/10.1007/s13197-011-0520-z
Yang, Y. C., Ni, D. H., Song, F. S., Li, L., Lu, X. Z., Li, Z. F., et al. (2012). Identification of QTLs for rice starch RVA profile properties in different ecological sites. *Acta Agronomy Sinica*, *38*, 264–274.
Yoshida, H., Nozaki, K., Hanashiro, I., Yagi, F., Ito, H., Honma, M., et al. (2003). Structure and physicochemical properties of starches from kidney bean seeds at immature, premature and mature stages of development. *Carbohydrate Research*, *338*, 463–469.
Zhou, X., & Lim, S. T. (2012). Pasting viscosity and *in vitro* digestibility of retrograded waxy and normal corn starch powders. *Carbohydrate Polymers*, *87*, 235–239.