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Resistant-Starch Formation in High-Amylose Maize Starch during Kernel Development

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The objective of this study was to understand the resistant-starch (RS) formation during kernel development of a high-amylose maize, GEMS-0067 line. The RS content of the starch, determined using AOAC method 991.43 for total dietary fiber, increased with kernel maturation and increase in the amylose/intermediate component (IC) content of the starch. Gelatinization of the native starches showed a major thermal transition with peak temperature at 76.6−81.0 °C. An additional peak (∼97.1 °C) first appeared 20 days after pollination and then developed into a significant peak on later dates. After removal of lipids from the starch, this peak disappeared, but the conclusion gelatinization temperature remained the same. The proportion of the enthalpy change of the thermal transition above 95 °C, calculated from the thermogram of the defatted starch, increased with kernel maturation and was significantly correlated with the RS content of the starch ($r = 0.98$). These results showed that the increase in crystallites of amylose/IC long-chain double helices in the starch resulted in the increase in the RS content of the starch during kernel development.

KEYWORDS: Resistant starch; high-amylose maize; *ae* mutant; amylose double helices; amylose−lipid complex; kernel development

INTRODUCTION

Resistant starch (RS) is a portion of starch that cannot be hydrolyzed by enzymes in the human digestive tract and is fermented by bacteria in the large intestine. RS present in high-amylose maize starch has been reported to have many health benefits, including prevention of colon cancer, type II diabetes, obesity, and cardiovascular disease (*1*−*12*).

A new public high-amylose maize line, GEMS-0067 (PI 643420), was developed by Dr. Mark Campbell at Truman State University in Kirksville, MO, working cooperatively with the USDA-ARS Germplasm Enhancement of Maize (GEM) Project in Ames, IA. The GEMS-0067 maize line is a homozygous mutant of the amylose-extender (*ae*) gene and contains high-amylose modifier (HAM) gene(s) (*13*). GEMS-0067 starches consist of 83.1−85.6% apparent amylose, greater than the maize *ae* single-mutant starches of H99*ae*, OH43*ae*, B89*ae*, and B84*ae* (61.7−67.7%) (*14*) and normal maize starch (∼30%) (*15*). The RS contents of the GEMS-0067 starches, determined using thermally stable α-amylase at 95−100 °C (AOAC method 991.43) (*16*), are 39.4−43.2%, which are higher than that of the maize *ae* single-mutant starches (11.5−19.1%) (*14*) and normal maize starch (∼1.5%) (*17*). The RS content increases with the increase in the apparent amylose content (*14*).

The GEMS-0067 starches consist of 22.6−32.0% elongated starch granules. The proportions of elongated starch granules of the GEMS-0067 starches are much larger than that of the maize *ae* single-mutant starches (5.2−7.7%) (*18*) and normal maize starch (0%) (*19*). These elongated starch granules are formed by fusion of small granules through amylose interaction in the amyloplast at the early stage of granule development (*20*).

The RS in the maize *ae* mutant starch is attributed to long-chain double-helical crystallites of amylose/intermediate component (IC) present in the starch granules. These amylose/IC crystallites, having high gelatinization temperature (> 95 °C), maintain semicrystalline structures at 95−100 °C and, thus, are resistant to enzyme hydrolysis by thermally stable α-amylase at 95−100 °C (*14*, *18*, *21*). The objective of the current study was to understand RS formation during kernel development of the high-amylose maize GEMS-0067 line.

MATERIALS AND METHODS

Materials. GEMS-0067 maize plants were grown in the field of the USDA-ARS North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA, in the 2007 and 2009 crop years. Plants were self-pollinated by hand, and ears were harvested at 15, 20, 30, 40, and 54 (mature) days after pollination (DAP). Maize kernels were removed from ears and stored at −20 °C until analysis. All chemicals were of reagent grade and were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

Starch Isolation. Starch was isolated from maize endosperms using the method reported by Li et al. (*14*).

RS Content of Starch. The RS content of native starch was determined using AOAC method 991.43 for total dietary fiber (*16*) as described by Li et al. (*14*).

Amylose/IC Content of Starch. Amylose/IC content of the starch was determined using Sepharose CL-2B gel permeation chromatography

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Table 1. RS, Amylose/Intermediate Component (IC), and Lipid Contents of GEMS-0067 Starches Harvested at Different Kernel Developmental Stages

	RS[a] (%)		amylose/IC[b] (%)		lipid (%)	
sample	2007 crop year	2009 crop year	2007 crop year	2009 crop year	2007 crop year	2009 crop year
15 DAP[c]	9.0 ± 1.2	10.1 ± 0.1	55.2 ± 0.5	54.5 ± 0.3	not determined	not determined
20 DAP	26.4 ± 0.1	25.3 ± 0.7	78.4 ± 0.2	76.9 ± 0.6	0.43 ± 0.03	0.25[d]
30 DAP	29.6 ± 0.8	28.4 ± 0.3	81.9 ± 0.3	84.3 ± 0.8	0.42 ± 0.02	0.39
40 DAP	32.0 ± 0.1	32.9 ± 0.4	88.6 ± 1.3	89.5 ± 0.1	0.62 ± 0.03	0.45
54 DAP	32.1 ± 0.3	33.8 ± 0.2	87.6 ± 0.4	88.8 ± 0.4	0.64 ± 0.01	0.50
coefficient between RS and amylose/IC content			0.99[e]	0.99[e]		

[a] Resistant starch (RS) content was determined using AOAC method 991.43 for total dietary fiber. [b] Amylose/IC contents were determined using Sepharose CL-2B gel permeation chromatography followed by the total carbohydrate (phenol−sulfuric acid) determination. [c] DAP, days after pollination. [d] Values were analyzed one time because of limited material. [e] $p < 0.001$.

(GPC) (*22*) followed by total carbohydrate (phenol−sulfuric acid) (*23*) and blue value analyses (*24*). Amylopectin content of the starch was calculated by subtracting the amylose/IC content from 100%.

Lipid Content of Starch. The lipid content of the starch was determined using 85% methanol (v/v) extraction in a Soxhlet extractor for 24 h (*25*). The starch samples after removal of lipids were dried at 37 °C overnight.

Scanning Electron Microscopy (SEM). GEMS-0067 starches obtained at different kernel developmental stages were coated with gold−palladium (60:40). SEM images of starch granules were produced using a scanning electron microscope (JEOL 5800, www.jeol.com) at 10 kV in the Microscopy and NanoImaging Facility at Iowa State University, Ames, IA (*26*).

X-ray Diffractometry. Native GEMS-0067 starches were equilibrated in a chamber with 100% relative humidity at 25 °C for 24 h. X-ray diffraction patterns of the starches were determined using an X-ray diffractometer (D-500, Siemens, Madison, WI) with copper Kα radiation. The percentage crystallinity of starch was determined using the method described previously (*26*).

Thermal Properties of Starch. Thermal properties of GEMS-0067 starches isolated from kernels harvested at different developmental stages were analyzed using a differential scanning calorimeter (DSC) (DSC-7, Perkin-Elmer, Norwalk, CT) as reported previously (*14*, *27*). Starch (~6 mg, dry starch basis) was precisely weighed and mixed with 3 times (by weight) deionized−distilled water (~18 μL). The mixture was sealed in a stainless steel pan and equilibrated at 25 °C for 1 h. The starch sample was then heated from 10 to 180 °C at a rate of 10 °C/min. A sealed empty stainless steel pan was used as the reference. Onset (T_o), peak (T_p), and conclusion (T_c) temperatures and enthalpy changes (ΔH) of starch gelatinization were determined using Pyris software (Perkin-Elmer, Norwalk, CT).

RESULTS AND DISCUSSION

RS, Amylopectin, Amylose/IC, and Lipid Contents of Starch. The RS contents of the GEMS-0067 starches isolated from kernels harvested at different developmental stages in the 2007 and 2009 growing seasons are shown in **Table 1**. The RS contents of the GEMS-0067 starches grown in both the 2007 and 2009 crop years increased with kernel maturation and reached a plateau after 40 DAP.

The amylose/IC contents of the GEMS-0067 starches obtained at different kernel developmental stages are summarized in **Table 1**. The amylose/IC contents of the GEMS-0067 starches grown in both the 2007 and 2009 crop years increased with kernel maturation and reached a plateau on around 40 DAP. This trend agreed with the result previously reported for high-amylose maize (*28*) and was similar to the increase in amylose content of normal maize starch during kernel development (*29*).

The RS content of the starch at different kernel developmental stages increased with the increase in amylose/IC content, having correlation coefficients of 0.99 ($p < 0.001$) for both the 2007 and 2009 crop years (**Table 1**). These results indicated that amylose/IC molecules were principal components for RS formation during kernel development. It has been shown that amylose/IC molecules in high-amylose maize starch form long-chain double-helical crystallites (*18*), which were resistant to enzymatic hydrolysis at 95−100 °C.

The lipid contents of the starches isolated at different kernel developmental stages are summarized in **Table 1**. In general, the lipid contents of the starches grown in both the 2007 and 2009 crop years increased with kernel maturation. It is known that lipids present in starch granules reduce the enzymatic hydrolysis of the granules at 95−100 °C (*18*).

Morphology of Starch Granules. SEM images of the GEMS-0067 starches harvested at different kernel developmental stages in 2007 are shown in **Figure 1**. The SEM images of the 2009 starch samples were similar to that of the 2007 starch samples and, thus, are not shown. All of the GEMS-0067 starches obtained at different kernel developmental stages consisted of mainly two types of starch granules, spherical and elongated, as previously reported (*18*, *20*, *28*, *30*). Starch harvested at 15 DAP (**Figure 1A**) had smaller granule sizes and consisted of more spherical granules and fewer elongated granules than the starches harvested on later dates (**Figure 1B−E**). These morphological images showed that starch granules of 15 DAP were immature and were expected to further grow by apposition to increase the granule size. These small granules would eventually become the inner part of mature and large granules. The amylopectin content of the starch harvested at 15 DAP (44.8%) was much larger than those of the starches harvested on later dates (21.6−11.4%) (calculated from **Table 1**). These results indicated that amylopectin molecules were more concentrated at the core and inner part of the mature starch granule. The results agreed with previous reports of starch granule structures, showing that amylose molecules were more concentrated at the periphery of starch granules (*31*−*33*). Most starch granules harvested at 15 DAP (**Figure 1A′**) and a few granules in the starches harvested on later dates appeared to have a rough surface structure (**Figures 1B−E**). This feature was different from the smooth surface of the normal maize starch granules during kernel development (*29*). The rough surface of the GEMS-0067 starch granules could be attributed to amylose/IC crystallites of the starch, which lacked branched chains to fill the spherical surface (*26*).

Starch harvested at 15 DAP consisted of less amylose/IC content and a smaller proportion of elongated starch granules than the starches harvested at 20 DAP and later dates (**Table 1** and **Figure 1**). These results were consistent with previous reports showing that the proportion of the elongated starch granules increased with kernel maturation (*28*) and with the increase in the amylose content (*18*, *20*, *28*). It also has been reported that elongated starch granules consist of more RS (*18*).

Crystallinity of Starch Granules. X-ray diffraction patterns of the GEMS-0067 starches harvested at different developmental stages in the 2007 crop year are shown in **Figure 2**. Starch samples

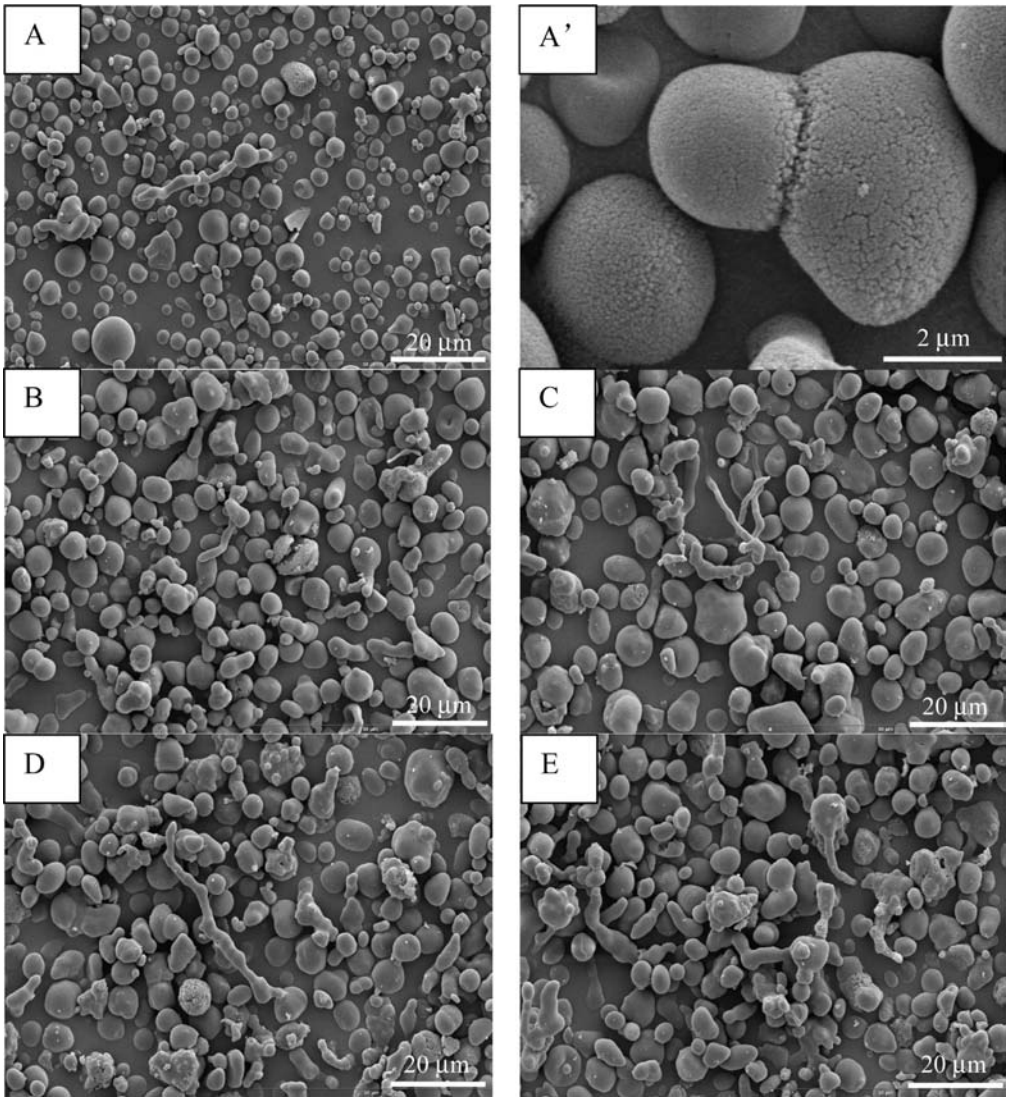

Figure 1. Scanning electron micrographs of GEMS-0067 starches harvested at different kernel developmental stages in 2007 crop year: **A** and **A′**, 15 days after pollination (DAP); **B**, 20 DAP; **C**, 30 DAP; **D**, 40 DAP; **E**, 54 DAP.

harvested in 2009 showed similar results (data not shown). All of the starches displayed the B-type polymorph. There was no V-type X-ray diffraction pattern of 2θ peaks at 8°, 13°, and 20° (*34*−*36*) observed for any of the starches, indicating that no crystalline amylose−lipid complex was present in the starch granule.

The percentage crystallinity of the starch (**Figure 2**) decreased with kernel maturation and increase in the amylose/IC content ($r = -0.90$, $p < 0.05$). The starch of 15 DAP had the greatest percentage crystallinity, resulting from its largest amylopectin content. Amylopectin molecules in normal starch granules are predominantly crystalline because of their clustered branched chains and abundant free ends, which are prompt to crystallize. Amylose molecules in normal starch granules are amorphous because of the low concentration and being interspersed among amylopectin (*37*, *38*). The amylose/IC molecules were dominant components in the maize *ae* mutant starch granules and were known to interact with one another to form double helices and contribute to the total starch crystallinity (*18*).

Thermal Properties of the Starch. DSC thermograms of the GEMS-0067 starches harvested at different kernel developmental stages in the 2007 crop year are shown in **Figure 3**, and the thermal properties are summarized in **Table 2**. Thermal properties of starches harvested in 2009 showed consistent patterns and, thus, are not shown. The onset gelatinization temperatures (T_o) were 68.8−69.9 °C for the starches harvested at 15, 20, and 30 DAP and then gradually decreased to 64.9 °C at 54 DAP (**Table 2**). These results showed a trend similar to that of the T_o of normal maize starch (B73) during kernel development. The normal maize starch reached the maximum T_o (69.0 °C) at 14 DAP and then decreased to 62.8 °C at kernel maturation (*29*).

All of the native starches showed a gelatinization thermal transition peak with the peak temperature (T_{p1}) increasing from 76.6 to 81.0 °C (**Table 2** and **Figure 3**). The intensity of the peak decreased with kernel maturation and with the increase in amylose/IC content (**Table 1** and **Figure 3**), indicating that this peak was likely due to the dissociation of short-chain double-helical crystallites of the amylopectin molecules (*14*, *18*, *27*). An additional peak (T_{p2}, ~97.1 °C) first appeared as a shoulder at 20 DAP, became a minor peak at 30 DAP, and then developed into a significant peak at 40 and 54 DAP (**Figure 3**). The size of this peak coincided with the lipid content of the starch (**Table 1**), and the peak shown in **Figure 3** disappeared after removal of lipids from the starch (**Figure 4**). These results suggested that the additional peak at ~97.1 °C (**Figure 3**) corresponded to the melting of the amorphous amylose−lipid complex (*39*, *40*), which

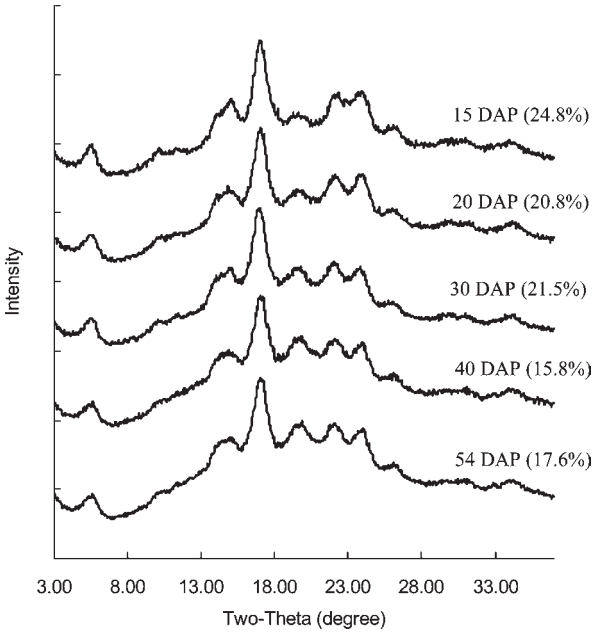

Figure 2. X-ray diffraction patterns of GEMS-0067 starches harvested at different kernel developmental stages in the 2007 crop year. Percentage crystallinity (%) is given in parentheses. DAP, days after pollination.

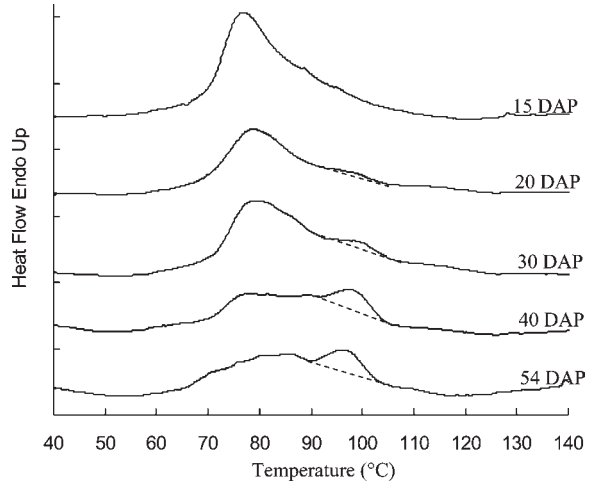

Figure 3. DSC thermograms of native GEMS-0067 starches harvested at different developmental stages in the 2007 crop year. The peak area above the dashed line indicates melting of the amylose−lipid complex. DAP, days after pollination.

Table 2. Thermal Properties of GEMS-0067 Starches Harvested at Different Kernel Developmental Stages in the 2007 Crop Year[a]

	thermal properties				
sample	T_o (°C)	T_{p1} (°C)	T_{p2} (°C)	T_c (°C)	ΔH (J/g)
15 DAP[b]	69.5 ± 0.1	76.6 ± 0.6	nd[c]	105.0 ± 0.7	16.9 ± 0.2
20 DAP	68.8 ± 1.0	78.5 ± 0.2	96.7 ± 0.2	119.3 ± 2.5	12.3 ± 0.6
30 DAP	69.9 ± 0.5	79.5 ± 0.2	98.3 ± 0.6	122.2 ± 0.2	14.2 ± 0.8
40 DAP	66.1 ± 0.7	80.2 ± 0.7	97.1 ± 0.4	122.0 ± 0.5	11.3 ± 0.5
54 DAP	64.9 ± 0.3	81.0 ± 0.6	96.2 ± 0.9	117.8 ± 1.9	11.8 ± 1.3

[a] Samples (~6.0 mg, db) and deionized−distilled water (~18.0 μL) were used for the analysis; T_o, T_{p1}, T_{p2}, T_c, and ΔH are onset, peak 1, peak 2, and conclusion temperatures and enthalpy change, respectively. [b] DAP, days after pollination. [c] nd, not detectable.

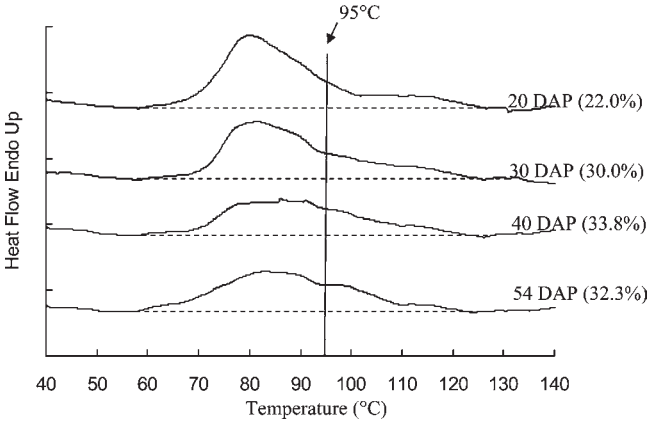

Figure 4. DSC thermograms of methanol-defatted GEMS-0067 starches harvested at different developmental stages in the 2007 crop year. The proportion of the enthalpy change of the thermal transition above 95 °C is given in parentheses. DAP, days after pollination.

had a lower thermal transition temperature than the crystalline amylose−lipid complex (T_p, ~120 °C) and showed no V-type crystalline pattern (**Figure 2**). It is known that the content of amylose−lipid complex in wheat, barely, and rye starches increases with kernel maturation (*41*). The presence of lipids in starch granules has been reported to reduce the enzyme digestibility of the granules at 95−100 °C (*18*).

The conclusion gelatinization temperatures of the native starches increased from 105.0 °C at 15 DAP to 117.8−122.2 °C on later dates (**Table 2**). After removal of the lipids from the starches, the conclusion gelatinization temperatures remained about the same. The proportion of the enthalpy change of the thermal transition above 95 °C (**Figure 4**) increased with kernel maturation and was significantly correlated with the RS content of the starch with a correlation coefficient of 0.98 ($p < 0.05$). These results confirmed that long-chain double-helical crystallites of amylose/IC present in the starch granule increased with kernel maturation of the GEMS-0067. The long-chain double-helical crystallites maintained semicrystalline structures at 95−100 °C and, thus, were resistant to enzymatic hydrolysis (*14*, *18*).

In conclusion, the RS content of GEMS-0067 starch increased with kernel maturation and with the increase in the amylose/IC content of the starch. Starch granules isolated from kernels harvested at 15 DAP had smaller granule sizes and consisted of more spherical granules and fewer elongated granules than those harvested on later dates. The amylopectin molecules were more concentrated in the starch granules harvested at 15 DAP and at the core and inner part of the mature granules. The increase in the amylose/IC content of the starch during kernel development led to the formation of long-chain double-helical crystallites that had gelatinization temperatures above 95 °C and resulted in the increase in the RS content of the starch. The increase in the lipid content of the starch could also reduce the enzyme digestibility of the starch.

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LITERATURE CITED

(1) Englyst, H. N.; Macfarlane, G. T. Breakdown of resistant and readily digestible starch by human gut bacteria. *J. Sci. Food Agric.* **1986**, *37* (7), 699–706.

(2) Englyst, H. N.; Cummings, J. H. Digestion of the polysaccharides of some cereal food in the human small intestine. *Am. J. Clin. Nutr.* **1985**, *42* (5), 778–787.
(3) Robertson, M. D.; Bickerton, A. S.; Dennis, A. L.; Vidal, H.; Frayn, K. N. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *Am. J. Clin. Nutr.* **2005**, *82* (3), 559–567.
(4) Robertson, M. D.; Currie, J. M.; Morgan, L. M.; Jewell, D. P.; Frayn, K. N. Prior short-term consumption of resistant starch enhances postprandial insulin sensitivity in healthy subjects. *Diabetologia* **2003**, *46* (5), 659–665.
(5) Behall, K. M.; Scholfield, D. J.; Hallfrisch, J. G.; Liljeberg-Elmstaahl, H. G. M. Consumption of both resistant starch and β-glucan improves postprandial plasma glucose and insulin in women. *Diabetes Care* **2006**, *29* (5), 976–981.
(6) Behall, K. M.; Scholfield, D. J.; Hallfrisch, J. G. Barley β-glucan reduces plasma glucose and insulin responses compared with resistant starch in men. *Nutr. Res.* (*N.Y.*) **2006**, *26* (12), 644–650.
(7) Zhang, W.; Wang, H.; Zhang, Y.; Yang, Y. Effects of resistant starch on insulin resistance of type 2 mellitus patients. *Chinese J. Prev. Med.* **2007**, *41* (2), 101–104.
(8) Higgins, J. A.; Higbee, D. R.; Donahoo, W. T.; Brown, I. L.; Bell, M. L.; Bessesen, D. H. Resistant starch consumption promotes lipid oxidation. *Nutr. Metab.* **2004**, *1*, 8.
(9) Pawlak, D. B.; Kushner, J. A.; Ludwig, D. S. Effects of dietary glycaemic index on adiposity, glucose homoeostasis, and plasma lipids in animals. *Lancet* **2004**, *364* (9436), 778–785.
(10) Dronamraju, S. S.; Coxhead, J. M.; Kelly, S. B.; Burn, J.; Mathers, J. C. Cell kinetics and gene expression changes in colorectal cancer patients given resistant starch: a randomised controlled trial. *Gut* **2009**, *58* (3), 413–420.
(11) Van Munster, I. P.; Tangerman, A.; Nagengast, F. M. Effect of resistant starch on colonic fermentation, bile acid metabolism, and mucosal proliferation. *Dig. Dis. Sci.* **1994**, *39* (4), 834–842.
(12) Sajilata, M. G.; Singhal, R. S.; Kulkarni, P. R. Resistant starch − a review. *Compr. Rev. Food Sci. Food Saf.* **2006**, *5* (1), 1–17.
(13) Campbell, M. R.; Jane, J.; Pollak, L.; Blanco, M.; O'Brien, A. Registration of maize germplasm line GEMS-0067. *J. Plant Regist.* **2007**, *1*, 60–61.
(14) Li, L.; Jiang, H.; Campbell, M.; Blanco, M.; Jane, J. Characterization of maize amylose-extender (*ae*) mutant starches: part I. Relationship between resistant starch contents and molecular structures. *Carbohydr. Polym.* **2008**, *74* (3), 396–404.
(15) Hasjim, J.; Srichuwong, S.; Scott, M. P.; Jane, J. Kernel composition, starch structure, and enzyme digestibility of *opaque-2* maize and quality protein maize. *J. Agric. Food Chem.* **2009**, *57* (5), 2049–2055.
(16) AOAC. Association of Official Analytical Chemists (AOAC) Official Method 991.43. Total, soluble, and insoluble dietary fiber in foods. In *Official Methods of Analysis of the AOAC International*, 17th ed., rev. 2 ed.; Horwitz, W., Ed.; AOAC International: Gaithersburg, MD, 2003.
(17) Hasjim, J.; Jane, J. Production of resistant starch by extrusion cooking of acid-modified normal-maize starch. *J. Food Sci.* **2009**, *74* (7), C556–C562.
(18) Jiang, H.; Campbell, M.; Blanco, M.; Jane, J. Characterization of maize amylose-extender (*ae*) mutant starches. Part II: Structures and properties of starch residues remaining after enzymatic hydrolysis at boiling-water temperature. *Carbohydr. Polym.* **2010**, *80* (1), 1–12.
(19) Perera, C.; Lu, Z.; Sell, J.; Jane, J. Comparison of physicochemical properties and structures of sugary-2 cornstarch with normal and waxy cultivars. *Cereal Chem.* **2001**, *78* (3), 249–256.
(20) Jiang, H.; Horner, H. T.; Pepper, T. M.; Blanco, M.; Campbell, M.; Jane, J. Formation of elongated starch granules in high-amylose maize. *Carbohydr. Polym.* **2010**, *80* (2), 534–539.
(21) Jiang, H.; Srichuwong, S.; Campbell, M.; Jane, J. Characterization of maize amylose-extender (ae) mutant starches. Part III: Structures and properties of the Naegeli dextrins. *Carbohydr. Polym.* **2010**, *81* (4), 885−891.
(22) Jane, J.; Chen, J. F. Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chem.* **1992**, *69* (1), 60–65.
(23) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356.
(24) Juliano, B. O. A simplified assay for milled-rice amylose. *Cereal Sci. Today* **1971**, *16*, 334–340.
(25) Schoch, T. J. Noncarbohydrate substances in the cereal starches. *J. Am. Chem. Soc.* **1942**, *64*, 2954–2956.
(26) Ao, Z.; Jane, J. Characterization and modeling of the A- and B-granule starches of wheat, triticale, and barley. *Carbohydr. Polym.* **2007**, *67* (1), 46–55.
(27) Kasemsuwan, T.; Jane, J.; Schnable, P.; Stinard, P.; Robertson, D. Characterization of the dominant mutant amylose-extender (Ae1-5180) maize starch. *Cereal Chem.* **1995**, *72* (5), 457–464.
(28) Mercier, C.; Charbonniere, R.; Gallant, D.; Guilbot, A. Development of some characteristics of starches extracted from normal corn and amylomaize grains during their formation. *Starch/Staerke* **1970**, *22* (1), 9–16.
(29) Li, L.; Blanco, M.; Jane, J.-l. Physicochemical properties of endosperm and pericarp starches during maize development. *Carbohydr. Polym.* **2007**, *67* (4), 630–639.
(30) Wolf, M. J.; Seckinger, H. L.; Dimler, R. J. Microscopic characteristics of high-amylose corn starches. *Starch/Staerke* **1964**, *16* (12), 377–382.
(31) Jane, J.; Shen, J. J. Internal structure of the potato starch granule revealed by chemical gelatinization. *Carbohydr. Res.* **1993**, *247*, 279–290.
(32) Pan, D. D.; Jane, J. Internal structure of normal maize starch granules revealed by chemical surface gelatinization. *Biomacromolecules* **2000**, *1* (1), 126–132.
(33) Jane, J.; Ao, Z.; Duvick, S. A.; Wiklund, M.; Yoo, S.-H.; Wong, K.-S.; Gardner, C. Structures of amylopectin and starch granules: how are they synthesized? *J. Appl. Glycosci.* **2003**, *50* (2), 167–172.
(34) Zobel, H. F.; French, A. D.; Hinkle, M. E. X-ray diffraction of oriented amylose fibers. II. Structure of V-amyloses. *Biopolymers* **1967**, *5* (9), 837–845.
(35) Zobel, H. F. Starch crystal transformations and their industrial importance. *Starch/Staerke* **1988**, *40* (1), 1–7.
(36) Czuchajowska, Z.; Sievert, D.; Pomeranz, Y. Enzyme-resistant starch. IV. Effects of complexing lipids. *Cereal Chem.* **1991**, *68* (5), 537–542.
(37) Jane, J.; Xu, A.; Radosavljevic, M.; Seib, P. A. Location of amylose in normal starch granules. I. Susceptibility of amylose and amylopectin to cross-linking reagents. *Cereal Chem.* **1992**, *69* (4), 405–409.
(38) French, D. Organization of starch granules. In *Starch: Chemistry and Technology*, 2nd ed.; Whistler, R. L., Bemiller, J. N., Paschall, E. F., Eds.; Academic Press: New York, 1984; pp 183−247.
(39) Tufvesson, F.; Wahlgren, M.; Eliasson, A.-C. Formation of amylose−lipid complexes and effects of temperature treatment. Part 1. Monoglycerides. *Starch/Staerke* **2003**, *55* (2), 61–71.
(40) Tufvesson, F.; Wahlgren, M.; Eliasson, A.-C. Formation of amylose−lipid complexes and effects of temperature treatment. Part 2. Fatty acids. *Starch/Staerke* **2003**, *55* (3−4), 138–149.
(41) Karlsson, R.; Olered, R.; Eliasson, A. C. Changes in starch granule size distribution and starch gelatinization properties during development and maturation of wheat, barley and rye. *Starch/Staerke* **1983**, *35* (10), 335–340.

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