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Characterization and Development Mechanism of *Apios americana* Tuber Starch

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Characterization and development mechanism of *Apios americana* tuber starch

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ABSTRACT

Apios americana is a wild legume-bearing plant with edible tubers. Domestication of *Apios* is in progress because of the superior nutritional value and health benefits of the tuber. Objectives of this study were to: (1) characterize physicochemical properties of the *Apios* tuber starch; and (2) understand differences in starch structures and properties between the mother (seed) and child (progeny) tubers and the mechanism of starch development. Granules of the *Apios* tuber starch displayed ellipsoidal, rod, or kidney shape with diameter ranges of 1–30 μm. The mother tuber starches displayed greater percentage crystallinity, larger gelatinization enthalpy-changes, longer branch-chain lengths of amylopectin, and lower pasting viscosity than their counterpart child tuber starches. The mother tuber starch of *Apios* 2127 displayed distinct two peaks of gelatinization, which were attributed to starch granules located at different regions of the tuber having different structures and properties. The mother tuber displayed more active starch biosynthesis in the periphery than in the center of the tuber.

1. Introduction

Apios americana, also known as American groundnut or potato bean, is a wild nitrogen-fixing, legume-bearing plant with edible tubers (Belamkar et al., 2015; Wilson, Pichardo, Liuzzo, Blackmon, & Reynolds, 1987). It is native to North America and widely distributed from Canada to southern Florida (Reynolds, Blackmon, Wickremesinhe, Wells, & Constantin, 1990). *Apios* was a food plant for American Indians, and the tubers were emergency food for the Europeans upon arriving at the American continent in the middle of the nineteenth century (Kinugasa & Watanabe, 1992). Because of the low yield of the wild *Apios* tubers, *Apios* has been cultivated primarily as a garden crop instead of a food crop in recent years (Reynolds et al., 1990).

Recent studies, however, have shown that *Apios* tubers are highly nutritious compared with major food crops, including potatoes, sweet potatoes, and taros. The protein content of *Apios* tubers on the dry-weight basis ranges 11.1–14.0%, which is more than twice of the protein content of potatoes (Wilson et al., 1987; Wilson, Pichardo, Blackmon, & Reynolds, 1990). Proteins of *Apios* tuber have excellent and balanced contents of essential amino-acids, including leucine, isoleucine, phenylalanine, threonine, and valine (Wilson et al., 1987). *Apios* tubers have 4.2–4.6% lipid on the dry-weight basis, and linoleic acid is the dominant (Wilson, Gorny, Blackmon, & Reynolds, 1986). *Apios* tubers are also rich in iron and phosphorus and have five times the calcium content of taros (Kinugasa & Watanabe, 1992). The *Apios* tuber has a significant content of isoflavones (Kazuhiro, Nihei, Ogsawara, Koga, & Kato, 2011). Animal-feeding studies have shown that ingestion of *Apios* tubers reduces blood pressure, suggesting its hypertension-preventive function (Iwai & Matsue, 2007).

Product developments using *Apios* tubers have also received increasing attention. The tubers are soft and smooth in texture and taste slightly sweet like potatoes, taros, and chestnuts (Kikuta et al., 2011). In Japan, *Apios* tubers are cooked in different ways, including steaming, boiling, and deep frying, and the *Apios*-tuber flour is used as an ingredient for bakery products, including cookies, donuts, and breads (Kikuta et al., 2011). Chips made from *Apios* tubers display a lighter color than potato chips because of the lower reducing-sugar content of the *Apios* tuber, which decreases the Maillard reaction during baking (Reynolds et al., 1990).

Starch is a major component of *Apios* tubers (~68% dry matters) (Ogasawara, Hidano, & Kato, 2006). Physicochemical properties of the *Apios* tuber starch, however, have not been fully understood (Kikuta et al., 2011). A better understanding of the structures and properties of the starch is important for product developments

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using *Apios* tubers. It is also important for the starch industry to develop new starch-based ingredients with unique functionalities and good processing properties using alternative crops (Ji et al., 2003).

Hoshikawa (1995) reported the growth pattern of *Apios* tubers growing in the field. The mother (seed) tuber shows a decrease in mass until the full growth of the shoot, followed by an increase in mass thereafter until the end of the plant growth. The growth pattern of the mother tuber of *Apios* is similar to that of the root of sweet potato (Kodama, Nomoto, & Watanabe, 1957), but is different from other tubers, such as potato and Chinese yam (Hoshikawa, 1995). The mother tubers of potato and Chinese yam continue to degenerate during the plant growth. These results suggest that both starch degradation and starch biosynthesis occur in *Apios* mother tuber. Because of the unique growth pattern of *Apios* tubers, characterization of the starch isolated from mother and child tubers would advance understandings of the mechanism of starch biosynthesis during the development of *Apios* tubers.

Objectives of this study were to (1) characterize physicochemical properties of *Apios* tuber starch; and (2) understand differences in starch structures and properties between the mother and child tubers and the mechanism of starch development in the mother tuber. Results obtained from this study will provide understandings of physicochemical properties of the *Apios* tuber starch and the mechanism of starch biosynthesis in the mother tuber. Understanding properties of the *Apios* tuber starch will be useful for the development of value-added applications of the starch.

2. Materials and methods

2.1. Materials

Three breeding lines (LA-898, LA-2127, and LA-2155; abbreviated hereafter as 898, 2127, and 2155) of *Apios* were grown at the North Central Regional Plant Introduction Station (NCRPIS), Ames, IA. The tubers of the three breeding lines harvested in 2012 were planted in 2013, and those harvested in 2013 were planted in 2014 as mother tubers to further grow and produce child tubers. Mother and child tubers harvested in 2013 and 2014 were used in this study. The mother tuber displayed different morphology from the child tuber. The mother tuber was connected to both the stolon and the above-ground stem of the plant. The end of the tuber where the above-ground stem emerged was designated as the bud end, and the opposite end was designated as the distal end. The mother tuber was much larger in size (8–9 cm in diameter) than the child tuber (2–4 cm in diameter) (Fig. S-1).

Pseudomonas isoamylase (EC 3.2.1.68, 280 U/mg) was purchased from Megazyme International Ireland (Wicklow, Ireland). All other chemicals were reagent grade and were purchased from either Sigma-Aldrich Co. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA) and used without further treatments.

2.2. Starch isolation by wet-milling

The tubers were peeled and cut into small pieces (1 cm × 1 cm × 0.5 cm). The cut samples were wet-milled using a blender, and starches were isolated following the procedures reported by Li, Jiang, Campbell, Blanco, and Jane (2008).

2.3. Morphology of starch granules

Scanning electron micrographs of isolated starch granules were taken using a scanning electron microscope (JEOL 5800, Tokyo, Japan) following the methods previously reported (Ao & Jane, 2007). The average starch granule size was determined by measuring ~200 granules using an Infinity Analyze software (version 6.1.0, Lumenera Corp., Canada).

2.4. Crystallinity of starch

Starch samples were equilibrated in a chamber with 100% relative humidity for 24 h. X-ray diffraction patterns of the starch samples were analyzed using a diffractometer (Rigaku Ultima IV, Rigaku Americas, TX, USA) with copper Kα emission. The copper tube was operated at 44 mA and 40 kV, and the scanning region of the two-theta angle (2θ) was from 3° to 35°, with a scanning speed of 1°/min. The crystallinity of the starch was calculated using a MDI JADE software (version 6.5, Materials Data Inc., Livermore, California, USA). Crystallinity of the starch was calculated using the following equation:

Crystallinity (%) = 100% × $A_c/(A_c + A_a)$, where A_c is the crystalline area on the X-ray diffractogram and A_a is the amorphous area.

2.5. Amylose content of starch

The amylose content of the *Apios* tuber starch was determined using an iodine potentiometric autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY) (Song & Jane, 2000). Starch was defatted using 85% methanol in a Soxhlet extractor for 16 h prior to the analysis. The iodine affinity of amylose used for the calculation was 0.2 (Takeda & Hizukuri, 1987). The amylose content of the starch was calculated using the equation:

Amylose (%) = 100% × IA_s/0.2, where IA_s was the iodine affinity of the starch.

2.6. Lipid content of starch

Lipids of the starch were extracted following the AOAC method 996.06 (2000). The lipid content of the starch was determined gravimetrically after removal of the solvent and calculated using the equation:

Lipid (%) = 100% × Weight of extracted lipids/Weight of the starch (db).

2.7. Branch-chain length distribution of amylopectin

Amylopectin of the starch was separated from amylose and collected using a gel-permeation chromatographic (GPC) column packed with Sepharose CL-2B gel. The isolated amylopectin was debranched using *Pseudomonas* isoamylase (Megazyme International Irelands, Wicklow, Ireland). The branch chains of the debranched amylopectin were labeled with 8-amino-1,3,6-pyrenetrisulfonic acid (APTS) (0.2 M APTS in 15% acetic acid), and the branch-chain length distribution was analyzed using a fluorophore-assisted capillary electrophoresis (P/ACE MDQ) (Beckman Coulter, Fullerton, CA) following the methods previously reported (Jiang, Campbell, Blanco, & Jane, 2010; Morell, Samuel, & O'shea, 1998).

2.8. Thermal properties of starch

Thermal properties of the isolated starch were analyzed using a differential scanning calorimeter (DSC, Diamond, Perkin-Elmer, Norwalk, CT) following the method of Song and Jane (2000). Starch gelatinization onset (T_o), peak (T_p), and conclusion temperatures (T_c), and enthalpy-change (ΔH) were obtained using a Pyris software (Perkin-Elmer).

2.9. Characterization of starches displaying two thermal transitions of gelatinization

To characterize starches displaying two peaks of gelatinization in the DSC thermogram, a starch sample was heated in a DSC to

65 °C (Step 1 heating), the temperature between the two thermal-transition peaks in the original thermogram. The sample was cooled down to 20 °C, and then reheated to 110 °C (Step 2 heating). The thermograms were obtained using a Pyris software (Perkin-Elmer).

2.10. Isolation of starch granules with different gelatinization temperatures

The mother tuber starch of *Apios* 2127 displaying two peaks of gelatinization were separated into two groups of starch granules: the Group 1 starch gelatinized below 65 °C and the Group 2 starch gelatinized at temperatures above 65 °C. The Group 2 starch was isolated by incubating a starch suspension (1%, w/v) at 65 °C for 20 min to gelatinize the Group 1 starch. The un-gelatinized Group 2 starch, which maintained a crystalline structure and had a greater density than the gelatinized Group 1 starch, was isolated by centrifugation at 1000 *g* after washing with warm water (65 °C). The process was repeated five times.

2.11. Pasting properties of starch

Pasting properties of the starch were analyzed using a Rapid Visco-Analyzer (Newport Scientific, Sydney, Australia) following the methods of Ao and Jane (2007), with minor modifications. A starch suspension (6%, dsb, w/w) was used for the RVA analysis. The pasting temperature, and the peak, breakdown, and final viscosities were determined using the Thermocline software (Newport Scientific).

2.12. Statistical analysis

Data were subjected to analysis of variance and Tukey's multiple comparison analysis using PROC ANOA procedure of SAS 9.2 (SAS Institute, Inc., Cary, NC).

3. Results and discussion

3.1. Granule morphology and crystalline structures of starch

Images of *Apios* tuber starch granules obtained using a scanning electron microscope are shown in Fig. 1. The granules displayed smooth surface with the long-axis diameters ranging 1–30 μm. Granules with diameters larger than 5 μm showed an ellipsoidal, rod, or kidney shape, whereas granules with diameters smaller than 5 μm showed a spherical or ellipsoidal shape (Fig. 1). Average granule diameters and number-percentages of large granules (>5 μm) are shown in Table 1. For the 2013 samples, the child tuber starches of *Apios* 2127 and 2155 showed significantly ($p < 0.05$) smaller granule size (8.0 and 8.6 μm in diameter, respectively) than their counterpart mother tuber starches (10.1 and 9.8 μm in diameter, respectively). Child tuber starches of all the varieties showed smaller number-percentages of large granules (73.0–77.6%) than their counterpart mother tuber starches (78.0–85.9%). For the 2014 samples, the child tuber starch of *Apios* 2155 showed smaller granule size (9.8 μm in diameter) and percentage of large granules (73.7%) than the mother tuber starch (10.9 μm in diameter and 77.9%, respectively), whereas *Apios* 898 and 2127 showed no significant differences in granule size and percentage of large granules between the child and mother tuber starches.

X-ray diffraction analyses of *Apios* tuber starches showed a typical C-type diffraction pattern with strong peaks at 2θ of 5.5°, 15.0°, 17.0°, and 23.0° (Fig. S-2). Percentage crystallinity of the child tuber starches ranged 23.4–3.9% for the 2013 samples and 23.0–24.4% for the 2014 samples, whereas that of the mother tuber starches ranged 25.6–26.4% for the 2013 samples and 25.9–26.2% for the 2014 samples (Table 1). All the child tuber starches showed less crystallinity than their counterpart mother tuber starches.

3.2. Amylose and lipid contents of starch

Amylose contents of *Apios* tuber starches are shown in Table 1. For the 2013 samples, the child tuber starches of *Apios* 898 and 2127 showed no significant difference in amylose contents (32.1 and 32.2%, respectively) from the mother tuber starches (31.9 and 32.5%, respectively), whereas the child tuber starch of *Apios* 2155 showed a significantly smaller ($p < 0.05$) amylose content (32.1%) than the mother tuber starch (33.1%). For the 2014 samples, the child tuber starch of *Apios* 898 showed no significant difference in the amylose content (32.5%) from the mother tuber starch (32.2%), whereas the child tuber starches of *Apios* 2127 and 2155 showed significantly smaller ($p < 0.05$) amylose contents (32.5 and 31.8%, respectively) than their counterpart mother tuber starches (34.4 and 33.2%, respectively). The mother tuber starches that had larger granule size and consisted of more large granules displayed greater amylose contents than their counterpart child tuber starches (Table 1). These results agreed with that previously reported for maize starch: the amylose content increased with the increase in starch granule size during the development of the starch (Li, Blanco, & Jane, 2007; Pan & Jane, 2000).

Lipid contents of the *Apios* tuber starches ranged from 0.23 to 0.31% for the 2013 samples and from 0.19 to 0.32% for the 2014 samples (Table 1). All the samples, except the *Apios* 2155 grown in 2014, displayed a trend that the mother tuber starch consisted of more lipid than the child tuber starch, although the differences in lipid contents were not significant.

3.3. Amylopectin branch-chain length

The child tuber starches showed significantly ($p < 0.05$) larger percentages of short branch-chains of amylopectin (DP < 12, 20.7–21.1% for 2013 samples and 21.1–21.6% for 2014 samples) than their counterpart mother tuber starches (19.6–20.0% for 2013 samples and 19.4–21.1% for 2014 samples) (Table 2). The child tuber starches also showed significantly smaller percentages of long branch-chains (DP > 37, 17.3–17.9% for 2013 samples and 16.8–18.5% for 2014 samples) than their counterpart mother tuber starches (17.8–18.8% for 2013 samples and 17.4–19.0% for 2014 samples). As a result, the child tuber starches showed shorter average branch-chain length (23.4–23.9 DP for 2013 samples and 23.0–24.1 DP for 2014 samples) than their counterpart mother tuber starches (24.0–24.5 DP for 2013 samples and 23.6–24.5 DP for 2014 samples).

3.4. Starch thermal properties

DSC thermograms of *Apios* child and mother tuber starches are shown in Fig. 2, and the data are summarized in Table 3. The child tuber starches showed significantly lower onset gelatinization temperatures and smaller enthalpy-changes (56.0–57.5 °C and 14.2–15.2 J/g for 2013 samples and 56.2–57.2 °C and 14.5–15.0 J/g for 2014 samples, respectively) than their counterpart mother tuber starches (57.0–59.4 °C and 14.6–15.7 J/g for 2013 samples and 57.6–58.3 °C and 15.3–15.9 J/g for 2014 samples, respectively). The onset gelatinization temperatures of the starches were negatively correlated ($r = -0.78$, $p < 0.01$) with the percentages of the short branch-chains (DP < 12, Table 2), consistent with that previously reported (Jane et al., 1999; Srichuwong, Sunati, Mishima, Isono, & Hisamatsu, 2005a). The enthalpy-change of starch gelatinization reflects the energy required to dissociate the double-helical crystalline structures of starch (Donovan, 1979). The smaller gelatinization enthalpy-changes of the child-tuber starches were

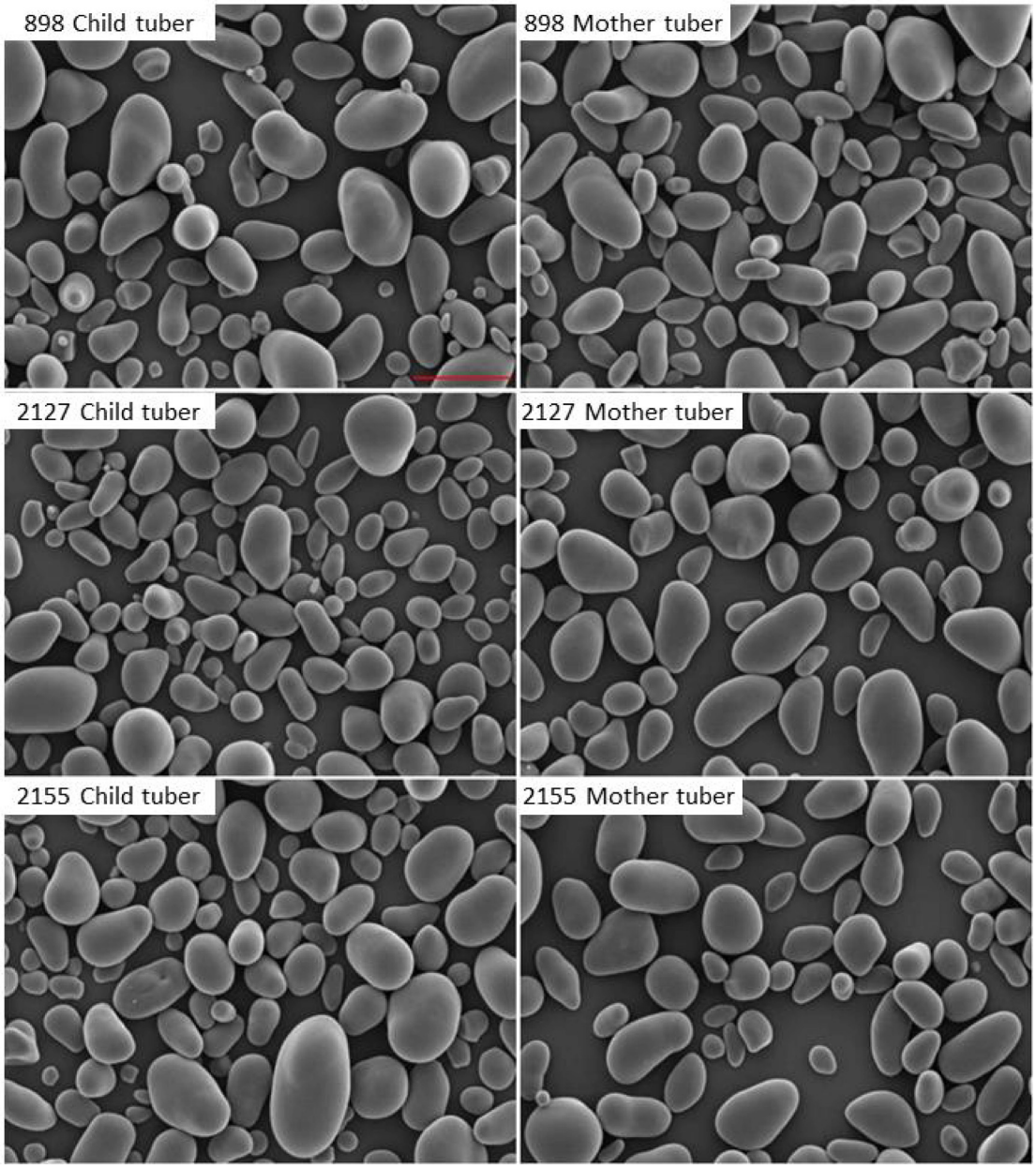

Fig. 1. Scanning electron micrographs of *Apios* tuber starch granules. Scale bar = 20 μm.

Table 1
Average granule size, percentage of large granules, amylose and lipid contents, and crystallinity (%) of *Apios* tuber starches.[a]

Samples			Average granule diameter (μm)	Large granules (%)[b]	Amylose (%)	Lipid (%)	Crystallinity(%)
2013	898	Child	9.8**a** ± 5.2	77.6	32.1**a** ± 0.4	0.23**a** ± 0.00	23.9
		Mother	9.4**a** ± 4.6	79.1	31.9**a** ± 0.0	0.28**a** ± 0.05	25.6
	2127	Child	8.0**b** ± 3.8	73.0	32.2**a** ± 0.1	0.24**a** ± 0.03	23.8
		Mother	10.1**a** ± 4.9	78.0	32.5**a** ± 0.1	0.26**a** ± 0.01	25.7
	2155	Child	8.6**b** ± 4.4	75.8	32.1**b** ± 0.3	0.26**a** ± 0.05	23.4
		Mother	9.8**a** ± 4.5	85.9	33.1**a** ± 0.2	0.31**a** ± 0.00	26.4
2014	898	Child	9.5**a** ± 5.4	75.7	32.5**a** ± 0.0	0.27**a** ± 0.01	24.4
		Mother	9.1**a** ± 4.9	74.2	32.2**a** ± 0.5	0.32**a** ± 0.06	26.2
	2127	Child	9.8**a** ± 5.8	74.3	32.5**b** ± 0.5	0.19**a** ± 0.00	24.4
		Mother	9.9**a** ± 5.5	75.1	34.4**a** ± 0.2	0.21**a** ± 0.00	25.9
	2155	Child	9.8**b** ± 5.8	73.7	31.8**b** ± 0.3	0.31**a** ± 0.02	23.0
		Mother	10.9**a** ± 6.5	77.9	33.2**a** ± 0.5	0.29**a** ± 0.01	26.1

[a] Different letters following the mean values within the same columns and same breeding lines indicate statistically different mean values between the child and mother tuber starches ($p < 0.05$).
[b] Number-percentage of the granules with a granule diameter >5 μm.

Table 2
Amylopectin branch chain length distribution[a] of *Apios* tuber starches.[b]

Sample			DP < 12	DP13-24	DP25-37	DP > 37	Ave.CL
2013	898	Child	20.7**a** ± 0.0	47.8 ± 0.2	13.9 ± 0.0	17.6**a** ± 0.1	23.8**a** ± 0.1
		Mother	19.8**b** ± 0.1	48.1 ± 0.4	14.2 ± 0.1	17.9**a** ± 0.2	24.0**a** ± 0.1
	2127	Child	20.9**a** ± 0.1	48.0 ± 0.1	13.8 ± 0.0	17.3**b** ± 0.0	23.4**b** ± 0.0
		Mother	20.0**b** ± 0.2	48.3 ± 0.0	13.9 ± 0.2	17.8**a** ± 0.3	24.0**a** ± 0.2
	2155	Child	21.1**a** ± 0.0	47.1 ± 0.0	13.9 ± 0.0	17.9**b** ± 0.0	23.9**b** ± 0.0
		Mother	19.6**b** ± 0.0	47.8 ± 0.2	14.0 ± 0.1	18.8**a** ± 0.3	24.5**a** ± 0.2
2014	898	Child	21.6**a** ± 0.1	46.0 ± 0.3	13.8 ± 0.0	18.5**b** ± 0.2	24.1**a** ± 0.2
		Mother	21.1**b** ± 0.0	46.3 ± 0.1	13.6 ± 0.2	19.0**a** ± 0.0	24.5**a** ± 0.2
	2127	Child	21.1**a** ± 0.1	48.2 ± 0.2	14.0 ± 0.1	16.8**b** ± 0.2	23.0**b** ± 0.1
		Mother	19.6**b** ± 0.2	48.8 ± 0.0	14.3 ± 0.0	17.4**a** ± 0.1	23.6**a** ± 0.1
	2155	Child	21.4**a** ± 0.0	47.6 ± 0.2	14.0 ± 0.2	17.0**b** ± 0.0	23.3**b** ± 0.2
		Mother	19.4**b** ± 0.0	48.1 ± 0.0	14.3 ± 0.0	18.2**a** ± 0.0	24.0**a** ± 0.0

[a] Molar basis.
[b] Different letters following the mean values within the same columns and same breeding lines indicate statistically different mean values between the child and mother tuber starches ($p < 0.05$).

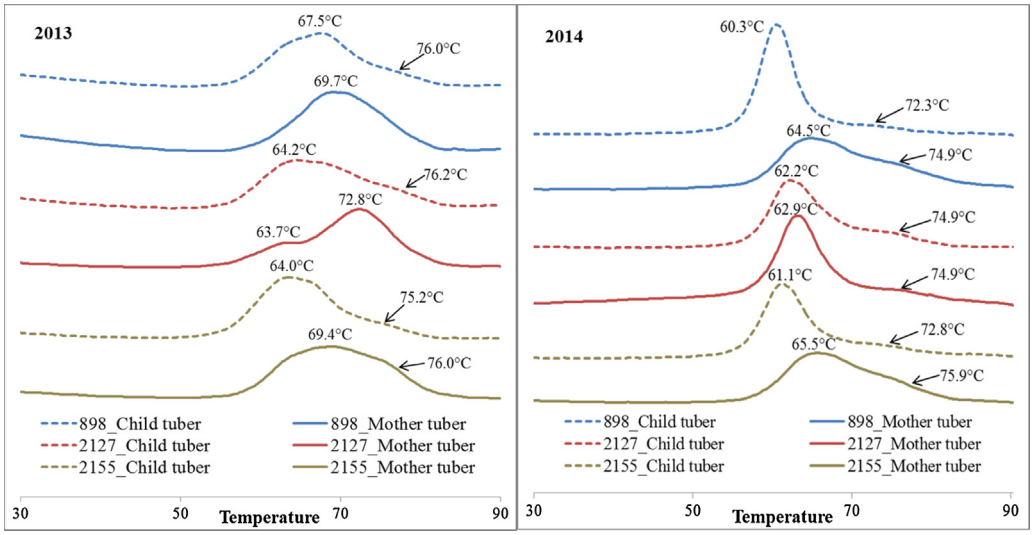

Fig. 2. Starch thermographs of *Apios* tubers grown in 2013 and 2014. Peak temperatures of starch gelatinization are labeled above the peak of gelatinization curves. Arrows indicate the shoulders of the gelatinization curves.

Table 3
Thermal properties of *Apios* tuber starches.[a]

Samples			T_o[b] (°C)	Peak 1(°C)	Peak 2(°C)	T_c(°C)	ΔH(J/g)
2013	898	Child	57.5**b** ± 0.3	67.5 ± 0.0	76.0 ± 0.2	79.9**b** ± 0.0	14.2**b** ± 0.0
		Mother	59.4**a** ± 0.2	69.7 ± 0.1	ND	80.6**a** ± 0.0	14.6**a** ± 0.1
	2127	Child	56.1**a** ± 0.5	64.2 ± 0.7	76.2 ± 0.3	81.0**a** ± 0.1	14.2**b** ± 0.1
		Mother	57.0**a** ± 0.2	63.7 ± 0.2	72.8 ± 0.6 *	80.6**a** ± 0.8	15.2**a** ± 0.2
	2155	Child	56.0**b** ± 0.3	64.0 ± 0.1	75.2 ± 0.0	81.1**a** ± 0.2	15.2**b** ± 0.0
		Mother	58.4**a** ± 0.3	69.4 ± 0.5	76.0 ± 0.1	81.8**a** ± 0.4	15.7**a** ± 0.3
2014	898	Child	56.3**b** ± 0.1	60.3 ± 0.4	72.3 ± 0.0	77.9**b** ± 0.5	15.0**b** ± 0.1
		Mother	57.6**a** ± 0.2	64.5 ± 0.3	74.9 ± 0.2	82.5**a** ± 0.5	15.7**a** ± 0.1
	2127	Child	57.2**b** ± 0.1	62.2 ± 0.5	74.9 ± 0.7	80.2**b** ± 0.2	14.9**b** ± 0.2
		Mother	58.1**a** ± 0.0	62.9 ± 0.1	74.9 ± 0.3	81.1**a** ± 0.0	15.9**a** ± 0.2
	2155	Child	56.2**b** ± 0.2	61.1 ± 0.0	72.8 ± 0.1	79.6**b** ± 0.3	14.5**b** ± 0.0
		Mother	58.3**a** ± 0.1	65.5 ± 0.0	75.9 ± 0.4	82.1**a** ± 0.0	15.3**a** ± 0.2

[a] Different letters following the mean values within the same columns and same breeding lines indicate statistically different mean values between the child and mother tuber starches ($p < 0.05$).
[b] T_o = onset gelatinization temperature, T_c = conclusion temperature, ΔH = enthalpy change. ND = Not detectable. Peak 1 is the major peak and Peak 2 is the minor peak for all the samples except for *Apios* 2127 mother tuber grown in 2013. *Major peak.

consistent with the lower percentage crystallinity of the child tuber starches than that of the mother tuber starches (Table 1).

Almost all the *Apios* samples grown in both years showed a second peak, a tail or a shoulder in the DSC thermogram (Table 3, Fig. 2), suggesting segregated thermal transitions of starch gelatinization. The shoulders, except *Apios* 2127 mother tuber starch, were present around 75.2–76.2 °C for the 2013 samples, and around 72.3–75.9 °C for the 2014 samples. Among all the samples, the mother tuber starch of *Apios* 2127 grown in 2013 displayed the most distinctly separated peaks in the DSC thermogram, with the first peak (minor peak) at 63.7 °C and the second peak (major peak) at 72.8 °C (Fig. 2). This pattern is rarely observed except for pea starch (C-type polymorph) heated in a salt solution (Bogracheva, Morris, Ring, & Hedley, 1997). The mechanism of the two

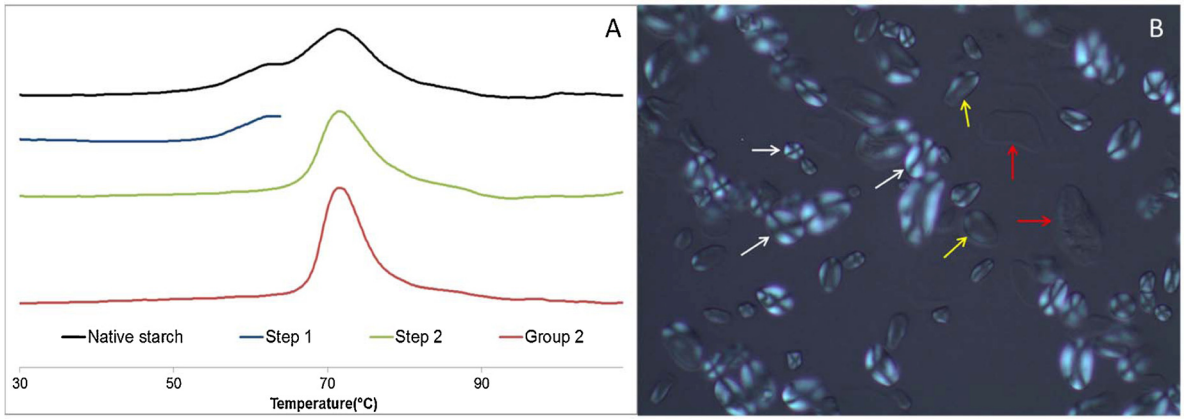

Fig. 3. (A) DSC thermograms of *Apios* 2127 mother tuber starches. The starch with three times of water (w/w) in a DSC pan was heated to 65 °C (Step 1), cooled down to 20 °C, and re-heated to 110 °C (Step 2). Group 2 granules: the group of starch granules of *Apios* 2127 mother tuber that gelatinized above 65 °C. (B) Micrograph of starch granules of *Apios* 2127 mother tuber under polarized light. The starch suspension (1%, w/v) was incubated at 65 °C for 20 min prior to the observation under the microscope. White arrows indicate granules that display intact Maltese crosses. Red arrows indicate completely gelatinized granules displaying no Maltese cross. Yellow arrows indicate partially gelatinized granules. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

gelatinization peaks of *Apios* 2127 mother tuber starch grown in 2013 was further investigated as follows.

3.5. The mechanism of two gelatinization peaks of starch

The two gelatinization peaks of starch could result from two independent thermal transitions of two groups of starch granules with different gelatinization temperature or two-stage thermal transition of the same starch granules (Bogracheva et al., 1997; Ji et al., 2003). Bogracheva et al. (1997) reported two gelatinization peaks in the DSC thermogram of pea starch (C-type polymorph) when the pea starch was heated in a salt solution. The authors demonstrated that the dissociation of crystalline structures during gelatinization of the pea starch began in the area around the hilum, where the starch had the B-type polymorph, and then propagated radially to the peripheral area, where the starch had the A-type polymorph. The pea starch, therefore, displayed a two-stage thermal transition of gelatinization.

A two-step heating experiment was conducted using the method described in Section 2.9 to understand the mechanism of the two-peak gelatinization of *Apios* 2127 mother tuber starch. After being heated to 65 °C and the sample was cooled down and reheated, there was only a single peak shown in the thermogram, and the temperature of the peak was consistent with that of the second peak in the original gelatinization thermogram (Fig. 3(A)). After being heated to 65 °C, some granules showed complete loss of Maltese cross, whereas others retained either intact or partial Maltese cross (Fig. 3(B)). These results indicated that the two gelatinization peaks corresponded to gelatinization of two different groups of starch granules: Group 1 starch gelatinized below 65 °C and Group 2 starch gelatinized at temperatures above 65 °C.

The Group 1 and Group 2 starches were separated by gelatinizing and removing the Group 1 starch using the method described in Section 2.10. The Group 2 starch was collected and characterized to understand differences in physicochemical properties between the Group 1 and Group 2 starch. The SEM images of the Group 2 starch granules are shown in Fig. S-3, which displayed intact granular structures. The Group 2 starch showed a substantially greater number-percentage of large granules (89.6%) and amylose content (34.5%) than the native starch (78.0% and 32.5%, respectively) (Table 4). The average branch-chain length and percentage of long branch-chains (DP > 37) of the Group 2 starch (DP 24.2 and 18.1%, respectively) were slightly greater than that of the native starch (DP 24.0 and 17.8%, respectively) (Table 4). These results indicated that the Group 2 starch had more large granules, consisted of more amylose, gelatinized at a higher temperature, and retained Maltese cross after heating at 65 °C.

3.6. Locations of the Group 1 and Group 2 starch in the Apios mother tuber

Starches at different locations of the *Apios* 2127 mother tuber was isolated and characterized to understand where the starch granules of the Group 1 and 2 were located in the tuber after the second year growth. Starch granules were isolated from the central region and two peripheral regions of the tuber, the bud end that was connected to the stolon and above-ground stem and the distal end that was on the opposite side of the bud end (Fig. S-1). Morphology of starch granules is shown in Fig. S-3, and the data of granule size are summarized in Table 4. The starch located at the central region of the mother tuber showed significantly ($p < 0.05$) larger granule size (average 10.0 μm in diameter) and greater number-percentage of large granules (79.7%) than the starch located at the bud end (8.8 μm in diameter and 74.0%, respectively) and the distal end (7.2 μm in diameter and 61.7%, respectively). The starch in the central region also consisted of significantly greater amylose content (33.8%) than that at the bud end (30.7%) and the distal end (30.5%) (Table 4). Amylopectin branch-chain length distributions of the starches are shown in Table 4. The starch in the central region showed a larger proportion of long branch-chains (DP > 37, 17.8%) and a longer average branch-chain length (DP 24.0) than the starch at the bud end (17.6% and DP 23.9, respectively) and the distal end (17.5% and DP 23.7, respectively), although the differences were not statistically significant. These results indicated that the Group 1 starch was mostly located in the peripheral regions, whereas the Group 2 starch was mostly in the central region of the tuber.

The Group 1 starch of *Apios* 2127 mother tuber showed similar gelatinization peak temperature (63.7 °C) (Fig. 2) to the counterpart child tuber starch (64.2 °C) (Table 3). The child tuber and its starch granules were initiated and developed in the second year of plant growth, displaying smaller granule size, less amylose content, and lower gelatinization temperature than the mother tuber starch that developed for two years (Tables 1 and 3). The similarity between the Group 1 starch and the child tuber starch suggested that the Group 1 starch was synthesized during the second year growth of the mother tuber in the bud end and distal end. On the contrary, the Group 2 starch, located in the central region of the tuber, was likely carried over from the previous growing season, further grew

Table 4
Granule size, percentage of large granules, amylose content, and amylopectin branch-chain length distribution of *Apios* 2127 mother tuber starches[a]

Sample	Average granule diameter (μm)	Large granules (%)[b]	Amylose (%)	DP < 12	DP13-24	DP25-37	DP > 37	Ave.CL[c]
Native	10.1**a** ± 4.9	78.0	32.5**b** ± 0.1	20.0**b** ± 0.2	48.3 ± 0.0	13.9 ± 0.2	17.8**a** ± 0.3	24.0**a** ± 0.2
Group 2 granules[d]	10.8**a** ± 4.5	89.6	34.5**a** ± 0.6	20.0**b** ± 0.1	47.9 ± 0.0	14.0 ± 0.0	18.1**a** ± 0.1	24.2**a** ± 0.1
Bud end[e]	8.8**b** ± 4.6	74.0	30.7**c** ± 0.4	20.1**ab** ± 0.3	48.1 ± 0.1	14.2 ± 0.0	17.6**a** ± 0.2	23.9**a** ± 0.1
Center	10.0**a** ± 4.9	79.7	33.8**a** ± 0.1	20.1**ab** ± 0.0	48.1 ± 0.4	14.0 ± 0.3	17.8**a** ± 0.1	24.0**a** ± 0.1
Distal end	7.2**c** ± 4.1	61.7	30.5**c** ± 0.3	20.6**a** ± 0.1	48.0 ± 0.2	13.9 ± 0.0	17.5**a** ± 0.2	23.7**a** ± 0.0

[a] Different letters following the mean values within the same columns indicate statistically different mean values ($p < 0.05$).
[b] Number-percentage of the granules with a granule diameter > 5 μm.
[c] Ave.CL: average branch-chain length.
[d] Group 2 granules: the group of starch granules of *Apios* 2127 mother tuber that gelatinized at higher temperatures.
[e] The starch granules isolated from the bud end, center part, and distal end (opposite to the bud end) of *Apios* 2127 mother tuber.

Table 5
Pasting properties of *Apios* tuber starches.[a]

Sample			Pasting Temp. (°C)	Peak (RVU)	Hold(RVU)	Final(RVU)	Breakdown(RVU)	Setback(RVU)
2013	898	Child	72.3**b** ± 0.1	119.8**a** ± 2.4	71.5 ± 1.4	124.0 ± 2.8	48.3**a** ± 1.0	52.5**a** ± 1.4
		Mother	75.4**a** ± 0.4	96.5**b** ± 0.4	64.5 ± 0.2	113.4 ± 1.6	31.9**b** ± 0.2	48.9**a** ± 1.5
	2127	Child	74.1**b** ± 0.3	111.5**a** ± 1.7	82.0 ± 1.8	144.0 ± 2.1	29.4**a** ± 0.1	61.9**a** ± 0.2
		Mother	75.8**a** ± 0.0	97.6**b** ± 1.8	72.1 ± 1.6	128.4 ± 1.5	25.5**b** ± 0.2	56.3**b** ± 0.1
	2155	Child	73.8**b** ± 0.3	99.8**a** ± 2.5	68.8 ± 1.1	123.8 ± 1.1	31.0**a** ± 1.4	55.0**a** ± 0.0
		Mother	76.0**a** ± 0.1	94.3**a** ± 1.9	73.7 ± 0.8	125.3 ± 1.1	20.6**b** ± 2.8	51.6**a** ± 1.9
2014	898	Child	71.4**b** ± 0.0	112.9**a** ± 0.9	74.3 ± 1.2	128.2 ± 1.2	38.5**a** ± 0.3	53.8**a** ± 0.0
		Mother	75.9**a** ± 0.0	86.5**b** ± 0.5	74.0 ± 0.1	124.0 ± 1.1	12.5**b** ± 0.5	50.0**b** ± 1.1
	2127	Child	72.5**a** ± 0.3	88.2**a** ± 0.2	72.0 ± 0.1	128.8 ± 0.5	16.2**a** ± 0.4	56.8**b** ± 0.4
		Mother	73.3**a** ± 0.3	84.5**b** ± 0.1	72.5 ± 0.0	134.4 ± 0.6	12.0**b** ± 0.1	61.9**a** ± 0.6
	2155	Child	70.9**b** ± 0.2	102.7**a** ± 2.8	81.2 ± 0.9	135.3 ± 1.6	21.5**a** ± 1.9	54.0**a** ± 2.5
		Mother	75.6**a** ± 0.0	94.0**b** ± 1.7	72.1 ± 1.4	117.5 ± 0.1	22.0**a** ± 0.3	45.5**b** ± 1.5

[a] Different letters following the mean values within the same columns and same breeding lines indicate statistically different mean values between the child and mother tuber starches ($p < 0.05$). RVU = Rapid visco-units.

in the second year, and also annealed for two years. Therefore, the Group 2 starch gelatinized at a higher temperature.

3.7. Starch pasting properties

The child tuber starches showed significantly lower ($p < 0.05$) pasting temperatures (72.3–74.1 °C for 2013 samples and 70.9–72.5 °C for 2014 samples) but higher peak viscosities (99.8–119.8 RVU for 2013 samples and 88.2–112.9 RVU for 2014 samples) than their counterpart mother tuber starches (75.4–76.0 °C for 2013 samples and 73.3–75.9 °C for 2014 samples, and 94.3–97.6 RVU for 2013 samples and 84.5–94.0 RVU for 2014 samples, respectively) (Table 5). The differences in pasting properties between the child and mother tuber starches could be attributed to the larger amylose and lipid contents of the mother tuber starches than that of the child tuber starches (Table 1). It is known that amylose-lipid complex intertwines with amylopectin and restricts granule swelling, contributing to a higher pasting temperature and lower viscosity of the starch (Jane et al., 1999; Srichuwong, Sunati, Mishima, Isono, & Hisamatsu, 2005b). In addition, the *Apios* mother tubers were grown in the field for consecutive two years and went through a longer annealing process. Annealing is known to enhance double-helical crystalline structures of starch, reduce the rate of starch hydration, and restrict granule swelling (Tester, 1997; Tester, Debon, & Sommerville, 2000), which contributes to the higher pasting temperatures and lower viscosities of the mother tuber starches.

4. Conclusions

In this study, physicochemical properties of the child and mother tuber starches of a new food crop, *Apios americana*, were analyzed, and the mechanism underlying the two-peak gelatinization of *Apios* mother tuber starch were investigated and illustrated. The mother tuber starches of *Apios* displayed larger granule sizes in general, greater crystallinity, larger gelatinization enthalpy-changes, longer branch-chain lengths of amylopectin, and lower pasting viscosity than their counterpart child tuber starches. The mother tubers were growing for two years, and the starch went through further growth and was subjected to a longer annealing process, resulting in the greater crystallinity, higher pasting temperature, and lower pasting viscosity of the mother tuber starch. The mother tuber starch of *Apios* 2127 showed distinct two gelatinization peaks, which was attributed to two groups of starch granules gelatinizing at different temperatures. The starch granules initiated and synthesized in the second year of planting located at the bud and distal end of the mother tuber gelatinized at a lower temperature, whereas the granules located in the central region of the mother tuber, which were likely carried over from previous year and went through further growth and a longer annealing process, gelatinized at a higher temperature. These results indicated that starch biosynthesis in the mother tuber during the second year of growth was more active at the bud and distal end than in the central region of the tuber.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2016.05.062.

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