

*Full Length Research Paper*

# Resistant starch content, molecular structure and physicochemical properties of starches in Virginia-grown corn, potato and mungbean

Yixiang Xu<sup>1\*</sup>, Cory Grizzard<sup>1</sup>, Edward N. Sismour<sup>1</sup>, Harbans L. Bhardwaj<sup>1</sup> and Zhenxing Li<sup>2</sup>

<sup>1</sup>Agricultural Research Station, Virginia State University, Petersburg, VA 23806, USA.

<sup>2</sup>Food Safety Lab, Ocean University of China, Qingdao, Shangdong Province, P. R. 266003 China.

Accepted 28 January, 2013

Starches were isolated from Virginia-grown corn, potato, and mungbean, and their resistant starch content, molecular structure and physicochemical properties were investigated for potential applications. All starches, extracted with combination of chemical and physically method exhibited high purity with low protein, fat and ash, and high carbohydrate. Potato starches had the highest resistant starch content, while mungbean starches showed the highest amylose content. Amylose content as well as the starch granule size and structure were responsible for resistance to digestibility. Compared to their mungbean and corn counterparts, potato starches had the highest amylopectin molecular weights and largest granular size. A typical A-and B-type crystalline structure was assigned to corn and potato starches, respectively, while mungbean starches had a C<sub>A</sub>-type crystalline pattern. Both potato and mungbean starch granules were smooth, oval and irregular ellipsoids, while corn starches had polyhedral granules. The gelatinization transition temperatures ( $T_o$ ,  $T_p$ , and  $T_c$ ) of the starches were significantly different, with the order of corn > mungbean > potato. Water absorption capacity of corn starches was lower than that of potato and mungbean starches. The results would assist food scientists in determining the potential end-uses of starches.

**Key words:** Resistant starch, molecular structure, physicochemical properties, starch, Virginia.

## INTRODUCTION

Starch is the most abundant storage reserve carbohydrate, and is mainly found in the seed, root, tubers, and fruits of plants. It is the most important carbohydrate in the human diet, and accounts for 60 to 70% of total dietary energy intake (National Nutrition Monitoring Bureau, 1991). Historically, starch has been disparaged as an underlying cause of weight gain and obesity, because it was considered to be fully digestible and absorbable in the small intestine. However, recent human studies have indicated that a starch fraction, termed 'resistant starch', is indigestible in the small intestine and enters the large intestine where it is fermented by colonic bacteria to short-chain fatty acids (Sajilata et al., 2006), which has the potential to prevent

colon cancer, diabetes, atherosclerosis, and obesity-related complications (Sharma et al., 2008; Topping and Clifton, 2001).

Corn and potato are starch-rich traditional crops grown in Virginia with starch accounting for up to 70% of total solids in kernels and tubers (Shapours and Salassi, 2006; Stark and Love, 2003). The productions of these two crops in 2010 were approximately 21 million bushels and 952,000 cwt, and ranked 2nd and 7th in production value, respectively (USDA-NASS, 2011). Beside the traditional crops, non-traditional food legume crops in Virginia are being researched extensively as alternative crops for Virginia farmers to promote income diversification due to changes in tobacco production (Bhardwaj et al., 1996, 1999). Tobacco had been the historical mainstay of many local farm-based economies in Virginia until production fell significantly from the early 1990's to 2009 (USDA-NASS, 2011). Parallel initiatives should be taken during

\*Corresponding author. E-mail: [yixu@vsu.edu](mailto:yixu@vsu.edu)

adoption or establishment of new crops to develop or enhance their utilization to increase cash value. Mungbean, chickpea and pigeon pea are non-traditional legume crops in Virginia that show great potential for further development. Generally, protein and carbohydrate are the two main constituents in legume seed. Legume seeds contain about 60% carbohydrate with starch as the major carbohydrate component. In addition, compared to other sources such as cereals, legume starches have reduced digestibility since they are characterized by high resistant starch contents (Hoover and Zhou, 2003).

Composition and properties of starch vary among crop cultivars due to genetics, environmental factors and agronomic practices (Hoover and Ratnayake, 2002). Currently, there is no information regarding the compositions and properties of starches from Virginia-grown corn, potato and mungbean. Therefore, the objectives of this study were to evaluate the compositions and properties of starches extracted from selected Virginia-grown corn, potato and mungbean varieties, with the intent to provide the baseline information for: (1) plant breeders to develop or select cultivars with their starches having certain functional properties, and (2) food scientists to determine potential end-uses of these starches.

## MATERIALS AND METHODS

Two corn varieties (Southern States 731 and Pioneer 35F37) were obtained from a local farmer. Two mungbean cultivars (Berken and TexSprout) were cultivated at Randolph Farm of Virginia State University. The corn and mungbean were harvested in the fall of 2011. The kernels and seeds were cleaned and dried prior to starch extraction. Two potato cultivars (Atlantic and Superior), were harvested from Virginia Polytechnic Institute and State University's Eastern Shore Agricultural Research and Extension Center in the summer of 2012. All chemicals used are reagent-grade.

### Starch isolation

Starches were extracted from the corn kernels, mungbean seeds, and potato tubers following the method described by Vasanthan (2001) with some modifications. Briefly, corn kernels and mungbean seeds were softened by steeping in water containing 0.2% (w/v)  $\text{SO}_2$  at 50°C for 24 h with a steep water-to-seed ratio of 2:1 (w/w). Seed coats were removed manually from the mungbean seeds. The samples were macerated in water with ice in a blender until smooth slurry formed. Starch slurries were filtering through nylon mesh. Potato tubers were peeled and macerated in water with ice as described above. No steeping was necessary for potato tubers. Starch was recovered by washing, filtering, and centrifuging. The recovered starch was oven dried at 40°C prior to analysis.

### Proximate composition

Analyses of crude protein, crude lipid, and ash were performed using the methods as described by Association of Official Analytical Chemists (AOAC, 2000). Crude protein content was measured using the combustion method (AOAC, 2000) with a Vario MAX CN (Elementar Americas, Inc., Mt. Laurel, NJ, USA). Organic N in the

samples was converted into nitric oxide by combustion, which was reduced further to molecular nitrogen. Crude protein content was calculated by multiplying nitrogen concentration by a conversion factor of 6.25. Oil was extracted from dried and ground samples using hexane in a Soxhlet apparatus for 8 hrs (AOAC method 948.22, 2000). Hexane was separated from the extracted oil by evaporation at ambient temperature in a fume hood. The cups were cooled and the crude lipid content weighed. Ash was determined using dried samples following AOAC method 950.49 (2000). The samples were weighed into separate porcelain crucibles and placed in a preheated muffle furnace (600°C) for 2 h. The crucibles were transferred into desiccators, cooled and weighed. Carbohydrate content was determined by subtracting the total percentage of other components from 100.

### Determination of resistant starch

Resistant starch contents of all samples were determined using Megazyme<sup>®</sup> Resistant Starch Assay Kits (Megazyme International Ireland Ltd. Wicklow, Ireland) based on AOAC Method 2002.02 (AOAC International, 2002). In brief, a sample (100 mg) was mixed with 4 ml  $\alpha$ -amylase containing amyloglucosidase followed by incubating in a shaking water bath for 16 h to hydrolyze digestible starch. The resistant portion was washed using ethanol (95%) and centrifuged at 3600 rpm for 20 min. The solid was twice washed with 50% ethanol followed by centrifuging at 3600 rpm for 20 min. The supernatant from each wash was collected to measure hydrolysable starch. Potassium hydroxide solution (2 M) was added to solubilize the resistant starch, and the pH of the solution was adjusted to 4.75 using 8 ml of 1.2 M sodium acetate buffer (pH 3.8). After incubation with amyloglucosidase (0.1 ml, 3300 U/ml) at 50°C for 30 min, the samples were centrifuged at 3600 rpm for 10 min. Three milliliters of glucose-oxidase-peroxidase-aminoantipyrine was added to aliquots (0.1 ml) of the supernatant, and the mixture was incubated at 50°C for 20 min. Sodium acetate buffer (100 ml, 100 mM, and pH 4.5) was added to the hydrolyzed samples collected from the ethanol washings, and aliquots (0.1 ml) were incubated with 10  $\mu$ l of dilute amyloglucosidase (3300 U/ml) solution at 50°C for 20 min. Glucose-oxidase-peroxidase-aminoantipyrine (3 ml) was then added, and tubes were incubated at 50°C for a further 20 min. Absorbance for both resistant and hydrolysable starch was measured using an evolution 60 s spectrophotometer (Thermo Scie) at 510 nm for glucose concentration).

### Amylose content

Amylose content of the starches was determined following the method of Hoover and Ratnayake (2001) with some modifications. A starch sample was first de-fatted using hexane in a Soxhlet apparatus for 6 h, followed by oven drying for 12 h. Lipid-free starch (20 mg) was added into a round-bottom tube containing 8 ml 90% DMSO and mixed vigorously for 5 min using a vortex mixer. The starch dispersion was heated in a water bath at 85°C for 15 min, followed by cooling to the room temperature. The sample subsequently was diluted to 25 ml with water in a volumetric flask. An aliquot of diluted sample (1.0 ml) was transferred to a 50 ml volumetric flask, and 5 ml iodine solution was added. The volume was adjusted to 50 ml and the absorbance was measured at 600 nm. Amylose concentration was determined from a standard curve developed using amylose and amylopectin blends.

### Molecular weight distribution

Molecular weight ( $M_w$ ) distribution of the starches was determined using high-performance size-exclusion chromatography (HPSEC)

as described by Ratnayake and Jackson (2007). For each assay, sample of 50 mg (dry basis) was dispersed in 10 ml of 90% (v/v) DMSO/water solution and maintained at room temperature for 5 days on a multi-tube rotator (Model: 4632Q, Thermo Scientific, Essex, UK) with a fixed shaker speed of 30 rpm. Dispersed samples were filtered through a 1.2 µm Magna nylon supported membrane (GE Osmonics, Minnetonka, MN), and 100 µL of filtrate was injected into the HPSEC system (equipped with Shimadzu LC-20AD pump, Shimadzu CTO-20A column oven, Shodex RI-101 detector). Size exclusion columns (Shodex OHPack SB-807G, SB-807 HQ, SB-806 M HQ, SB-804 HQ and SB-802.5 HQ) used in the system were connected in series and maintained at 50°C. Degassed distilled water was used as the mobile phase at 1 ml/min flow rate. Pullulan standards (Showa Denko K.K., Tokyo, Japan) P-5, P-10, P-20, P-50, P-100, P-200, P-400, and P-800 representing molecular weights of  $0.53 \times 10^4$ ,  $1.2 \times 10^4$ ,  $2.08 \times 10^4$ ,  $4.67 \times 10^4$ ,  $9.54 \times 10^4$ ,  $19.4 \times 10^4$ ,  $33.8 \times 10^4$ , and  $75.8 \times 10^4$ , respectively, were used to create the standard curve. The molecular weights of samples were calculated using the following equation ( $R^2 = 0.987$ ):

$$\text{Molecular weight} = 10^{-0.2905RT + 14.759}$$

where RT = retention time (min)

### Crystalline structure

Crystalline structure of the starches was analyzed as described by Xu et al. (2004) using a Panalytical X'pert Pro MPD X-Ray Diffractometer (XRD, Panalytical B.V., Almelo, the Netherlands). Samples were scanned with a CuKα target at 40 kV and 30 mA from  $2\theta = 2$  to  $40^\circ$  with a scanning speed of  $2^\circ \text{ min}^{-1}$ . The degree of crystallinity of samples was quantitatively estimated by following the method of Nara and Komiy (1983).

### Morphology structure

Morphological structure of the starch granules was determined using a SU-70 scanning electron microscope (Hitachi, Japan) (Li et al., 2011). The samples were mounted on an aluminum specimen holder using double-sided tape and were coated with gold palladium to a thickness of 30 nm. The coated samples were examined at an accelerating potential of 2 to 5 kV.

### Thermal properties

Thermal properties of the starches were measured using a differential scanning calorimeter (DSC-2000) (TA Instruments, New Castle, DE) as described by Singh et al. (2004). Starch was weighed into high pressure DSC pans, and distilled water was added to make suspensions with 70% moisture content. Pans were hermetically sealed and equilibrated for 1 h at ambient temperature before heating in the DSC. The heating range and rate were 30 to  $100^\circ\text{C}$  and  $10^\circ\text{C}/\text{min}$ , respectively. Indium and zinc were used for calibration and an empty pan was used for reference. Onset temperature ( $T_o$ ), peak temperature ( $T_p$ ), conclusion temperature ( $T_c$ ), gelatinization temperature range ( $\Delta T$ ), and enthalpy ( $\Delta H$ ) for gelatinization were determined.

### Water absorption capacity

Water absorption capacity (WAC) was determined following AACC method 56-20 (1983) with some modifications. Samples (about 2 g) were dispersed in 30 ml of distilled water in pre-weighed centrifuge tubes. Starch was agitated using stir bars in the centrifuge tubes at

room temperature for 1 h and were then centrifuged at  $5000 \times g$  40 min. Free water was removed, and the wet starch was drained for 5 min and weighed. WAC was calculated as:

$$\text{WAC} = \frac{\text{weight of wet starch} - \text{weight of dry starch}}{\text{weight of dry starch (d.b.)}} \times 100\%$$

### Statistical analyses

Three replications were used to obtain average values and standard deviations for all tests. All results were analyzed with SAS version 9.2 statistical software (SAS Institute Inc., Cary, NC). ANOVA with Duncan's Multiple Range test was used to evaluate the differences in composition and properties. Probability ( $P$ )  $\leq 0.05$  indicates significance.

## RESULTS AND DISCUSSION

### Proximate composition and starch content

Proximate compositions, specifically crude protein, crude lipid, ash, and total carbohydrate, of starches isolated from Virginia-grown corn, potato, and mungbean cultivars are presented in Table 1. All starches were characterized by low protein, fat and ash, and high carbohydrate. This indicated that starches extracted from each crop had high purity and were suitable for further evaluation. Potato starch had significantly ( $p < 0.05$ ) higher resistant starch content (49.4% for Atlantic and 46.1% for Superior) compared to starches extracted from mungbean (31.5% for Berken and 35.0% for TexSprout) and corn (28.3% for Pioneer and 20.0% for Southern State). These results are in agreement with previous reports that tuber and legume starches contain higher amount of resistant starch than cereal starches (Chen et al., 2010; Liu et al., 2006). There are four types of resistant starch (RS), termed  $RS_1$ ,  $RS_2$ ,  $RS_3$ , and  $RS_4$  based on differences in composition, structure, and response to processing methods. The type of resistant starch in the native starch granules is  $RS_2$ , due to intact and ungelatinized granular structure. The differences in resistant starch contents between different varieties for both potato and mungbean were not significant ( $p > 0.05$ ) while a significant difference was observed between the two corn varieties.

Virginia-grown corn, potato and mungbean starches had amylose contents of 19.9 to 26.9%, 23.0 to 26.7%, and 33.6 to 37.9%, respectively, which were close to those grown at other locations as reported in other studies, e.g. 16.9 to 21.3% for corn starch (Sandhu and Singh, 2007), 15.0 to 23.1% for potato starch (Kaur et al., 2007), and 29.9 to 33.6% for mungbean starch (Kaur et al., 2011). Mungbean starches had significantly ( $p < 0.05$ ) higher amylose content than corn and potato starches. The differences in amylose content between different cultivars for all crops were significant ( $p < 0.05$ ). Within the same crop, there was positive correlation between amylose content and resistant starch, for example corn starches ( $r = 0.97$ ,  $p < 0.001$ ), potato starches ( $r = 0.91$ ,

**Table 1.** Proximate composition, resistant starch composition, and amylose content ( $\text{g} \cdot 100 \text{g}^{-1}$  dry weight) of starches isolated from Virginia-grown corn, potato, and mungbean cultivars

Cultivar	Crude protein (%)	Crude oil (%)	Ash (%)	Total carbohydrate (%)	Resistant starch (%)	Amylose content (%)
<b>Corn</b>						
Southern States 731	0.37±0.01 <sup>a</sup>	0.11±0.03 <sup>a</sup>	0.03±0.00 <sup>bc</sup>	99.5±0.03 <sup>a</sup>	20.0±0.58 <sup>d</sup>	19.9±0.66 <sup>e</sup>
Pioneer 35F37	0.39±0.02 <sup>a</sup>	0.12±0.03 <sup>a</sup>	0.01±0.00 <sup>c</sup>	99.5±0.03 <sup>a</sup>	28.3±1.21 <sup>c</sup>	26.9±0.44 <sup>c</sup>
<b>Potato</b>						
Atlantic	0.25±0.03 <sup>b</sup>	0.03±0.01 <sup>b</sup>	0.18±0.01 <sup>a</sup>	99.5±0.03 <sup>a</sup>	49.4±1.14 <sup>a</sup>	26.7±1.19 <sup>c</sup>
Superior	0.24±0.03 <sup>b</sup>	0.04±0.01 <sup>b</sup>	0.26±0.01 <sup>a</sup>	99.5±0.04 <sup>a</sup>	46.1±1.79 <sup>a</sup>	23.0±1.47 <sup>d</sup>
<b>Mungbean</b>						
Berken	0.28±0.01 <sup>b</sup>	0.09±0.04 <sup>a</sup>	0.05±0.01 <sup>b</sup>	99.6±0.04 <sup>a</sup>	31.5±0.57 <sup>bc</sup>	33.6±1.50 <sup>b</sup>
TexSprout	0.28±0.02 <sup>b</sup>	0.12±0.05 <sup>a</sup>	0.04±0.01 <sup>b</sup>	99.6±0.03 <sup>a</sup>	35.0±0.79 <sup>b</sup>	37.9±2.44 <sup>a</sup>

Data are expressed as mean ± standard deviation (n=3), Means followed by the same letter within a column indicate no significant ( $p>0.05$ ) difference among cultivars.

**Table 2.** Structural and morphological properties of starches isolated from Virginia-grown corn, potato and mungbean cultivars.

Cultivar	Molecular weight (Da)		Granular size ( $\mu\text{m}$ )	Percentage of crystallinity (%)
	Amylopectin ( $\times 10^8$ )	Amylose ( $\times 10^5$ )		
<b>Corn</b>				
Southern States 731	3.06±0.34 <sup>c</sup>	2.03±0.65 <sup>b</sup>	16.8±1.65 <sup>d</sup>	31.0±0.36 <sup>d</sup>
Pioneer 35F37	2.56±0.20 <sup>c</sup>	1.79±0.34 <sup>b</sup>	15.6±0.89 <sup>d</sup>	29.3±0.30 <sup>e</sup>
<b>Potato</b>				
Atlantic	57.3±13.7 <sup>a</sup>	8.50±0.78 <sup>a</sup>	24.0±1.37 <sup>b</sup>	38.2±0.34 <sup>a</sup>
Superior	37.5±8.22 <sup>b</sup>	7.45±0.29 <sup>a</sup>	28.2±4.83 <sup>a</sup>	35.7±0.46 <sup>b</sup>
<b>Mungbean</b>				
Berken	3.58±0.03 <sup>c</sup>	6.19±0.16 <sup>a</sup>	19.3±1.36 <sup>c</sup>	33.9±0.16 <sup>c</sup>
TexSprout	3.39±0.05 <sup>c</sup>	7.01±0.51 <sup>a</sup>	22.7±1.18 <sup>bc</sup>	33.4±0.30 <sup>c</sup>

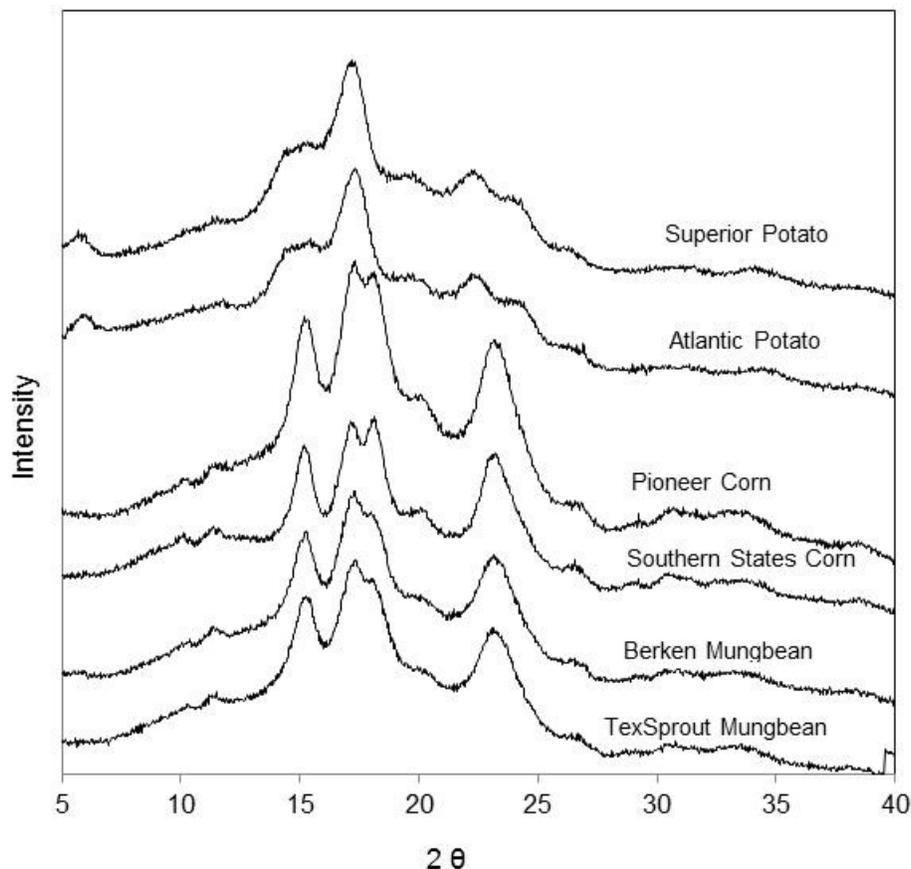
Data are expressed as mean ± standard deviation (n=3), Means followed by the same letter within a column indicate no significant ( $p>0.05$ ) difference among cultivars.

$p<0.01$ ) and mungbean starches ( $r = 0.886$ ,  $p < 0.05$ ). This is consistent with the results obtained by previous studies (Polesi et al., 2011; Themeier et al., 2005). However, compared to their corn and mungbean counterparts, potato starches had intermediate amylose content but the highest resistant starch levels. Large size of granules might be responsible for the high resistance of raw potato starch (Leszczyński, 2004). Granular size of potatoes starches was found to be significantly ( $p<0.05$ ) larger than those of corn and mungbean starches (Table 2). It is well known that starch hydrolysis requires enzyme adsorption on the surface of starch granules (Leloup et al., 1992). The large potato starch granular size results in smaller surface area, therefore, lowering degree of enzyme

adsorption on the surface.

### Structural and morphological properties

Molecular weight (Mw) distributions of Virginia-grown corn, potato, and mungbean starches are summarized in Table 2. Two peaks, identified as amylopectin and amylose, were present in the all samples. Potato starches had significantly ( $p<0.05$ ) higher amylopectin Mw (10 to 22 times) than corn and mungbean starches. The difference in amylopectin Mw between corn and mungbean starches was not significant. The starches from Virginia-grown mungbean had similar amylopectin



**Figure 1.** X-ray diffraction patterns of starches extracted from Virginia-grown corn, potato, and mungbean cultivars.

$M_w$  ( $3.8 \times 10^8$  Da) as reported for starches isolated from mungbean grown at other locations. Compared to corn and potato starches isolated from crops grown at other locations, Virginia-grown corn starches had lower amylopectin  $M_w$  while a significantly higher  $M_w$  was observed for starches from Virginia-grown potatoes (Yoo and Jane, 2002). Amylopectin  $M_w$  was found to be positively correlated to relative crystallinity of the starch ( $r = 0.861$ ,  $p < 0.05$ ). This is consistent with the result obtained in our previous study of chickpea starch (Xu et al., 2013). No difference was found for amylose  $M_w$  among potato and mungbean starches, while amylose  $M_w$  of corn starches was significantly lower.

X-ray diffraction was used to study the crystalline structure of the starch granules. The diffraction patterns of corn, potato and mungbean starches are shown in Figure 1. Corn starches showed a typical A-type crystalline structure with the diffraction peaks at  $2\theta$  around 15 and 22.8°, an unresolved doublet at 17.0 and 18.2°, and a small peak at 19.5°, whereas a B-type pattern was observed for potato starches with a strong reflection peak at  $2\theta$  of 17° and several medium peaks at  $2\theta$  about 5.4, 14.4 and 21.7° (Jiranuntakul et al., 2011). Although mungbean starches exhibited a C-type crystalline structure

(a mixture of A- and B-type structure), the A-type crystalline predominated. A unique peak at  $2\theta$  of about 5°, which is typically found in the B-type crystal, was not detected. Furthermore, although split peaks at  $2\theta$  about 17.0 and 18° were observed in our mungbean starches, they were not obvious as supposed to be for a typical A-type crystal. This implied that mungbean starches had a  $C_A$ -type crystalline pattern. These results are in agreement with previous reports regarding the crystalline structure of mungbean starches (Hoover et al., 1997; Kim et al., 2007). The percent crystallinity of the starches was significantly different (Table 3). Crystal size, amount of crystalline regions, orientation of the double helices within the crystalline domains, and extent of interaction between double helices might be responsible for the differences in degree of crystallinity between starches (Hoover and Ratnayake, 2002), all of which could be influenced by the location of origin of the crop and environmental growth conditions (Huang et al., 2007).

The morphological structures of starch granules from Virginia-grown corn, potato and mungbean examined by SEM show significant variations in shape and size (Figure 2 and Table 3). Corn starches granules exhibited polyhedral appearance whereas potato and mungbean

**Table 3.** Thermal and water absorption properties of starches extracted from Virginia-grown corn, potato, and mungbean cultivars.

Cultivar	Thermal properties					Water absorption (%)
	T <sub>o</sub> (°C)	T <sub>p</sub> (°C)	T <sub>c</sub> (°C)	ΔT (T <sub>c</sub> -T <sub>o</sub> , °C)	ΔH (J/g)	
<b>Corn</b>						
Southern States 731	69.6±0.36 <sup>ab</sup>	74.3±0.33 <sup>a</sup>	80.4 ±0.57 <sup>a</sup>	10.8 ±0.57 <sup>ab</sup>	2.03±0.09 <sup>c</sup>	77.9±0.37 <sup>b</sup>
Pioneer 35F37	70.1±0.64 <sup>a</sup>	74.1±0.16 <sup>a</sup>	81.5±0.47 <sup>a</sup>	11.4 ±0.11 <sup>a</sup>	2.27±0.06 <sup>b</sup>	76.1±2.80 <sup>b</sup>
<b>Potato</b>						
Atlantic	66.3±0.69 <sup>d</sup>	69.0 ±0.54 <sup>c</sup>	75.6±0.52 <sup>c</sup>	9.28 ±0.12 <sup>ab</sup>	2.75±0.54 <sup>a</sup>	86.1±1.62 <sup>a</sup>
Superior	65.4±0.15 <sup>d</sup>	68.2±0.18 <sup>c</sup>	74.5±0.15 <sup>c</sup>	8.97 ±0.35 <sup>ab</sup>	2.57±0.28 <sup>ab</sup>	84.8±1.96 <sup>a</sup>
<b>Mungbean</b>						
Berken	67.8±0.16 <sup>c</sup>	72.2±0.47 <sup>b</sup>	77.1±0.57 <sup>b</sup>	9.37±0.40 <sup>ab</sup>	1.46±0.08 <sup>d</sup>	83.6±2.59 <sup>a</sup>
Texas Sprout	69.1±0.30 <sup>bc</sup>	72.7±0.39 <sup>b</sup>	77.3±0.24 <sup>b</sup>	8.25±0.06 <sup>b</sup>	1.14±0.11 <sup>e</sup>	85.8±1.98 <sup>a</sup>

Data are expressed as mean ± standard deviation (n=3), Means followed by the same letter within a column indicate no significant (p>0.05) difference among cultivars.

granules had smooth, oval or irregular ellipsoidal appearance. Corn starch granules were the smallest with mean size of 15.6 μm for Pioneer and 16.8 μm for Southern States. On the other hand, potato starches had the largest granules with mean granular sizes of 24.0 μm for Atlantic and 28.2 for Superior, followed by mungbean starches with mean granular sizes of 19.3 μm for Berken and 22.7 μm for TexSprout. The granular size of starches from Virginia-grown corn, potato and mungbean *fell* within a *range* similar to those already reported (Kim et al., 2007; Swinkels, 1985).

### Thermal and water absorption properties

The gelatinization transition temperatures [T<sub>o</sub> (onset), T<sub>p</sub>(peak), and T<sub>c</sub> (conclusion)], and enthalpy of gelatinization (ΔH) for starches from corn, potato and mungbean are presented in Table 3. DSC thermographs of the starches are shown in Figure 3. Significant differences were observed in T<sub>o</sub>, T<sub>p</sub>, and T<sub>c</sub> among the starches with the order of corn> mungbean> potato. However, the differences between cultivars of each crop for T<sub>o</sub>, T<sub>p</sub> and T<sub>c</sub> were not significant. T<sub>o</sub>, T<sub>p</sub> and T<sub>c</sub> of corn starches were in the ranges of 69.6-70.1, 74.1-74.3 and 80.4-81.5°C, respectively. Mungbean starches had an T<sub>o</sub> of 67.8-69.1°C, T<sub>p</sub> of 72.2-72.7°C, and T<sub>c</sub> of 77.1-77.3°C, while the ranges of T<sub>o</sub>, T<sub>p</sub> and T<sub>c</sub> of potato starches were 65.4-66.3, 68.2-69.0°C and 74.5-75.6°C, respectively. These values were in agreement with the previous reports for the starches from corn, mungbean, and potato (Kaur et al., 2007, 2011; Li and Yeh, 2001; Sandhu and Singh, 2007).

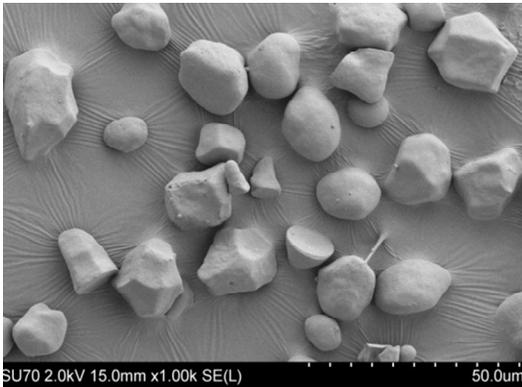
A variety of factors, including form and distribution of starch granules, internal arrangement of starch fractions within the granule, and amylose content and size, could contribute to the differences in gelatinization temperature

(Sandhu and Lim, 2008). The differences in the gelatinization temperature range (ΔT) among different starches were not significant. Enthalpy of gelatinization (ΔH) for the starches ranged from 1.14 to 2.75 J/g, which was much lower than those values reported by others for corn, potato, and mungbean starches (Kaur et al., 2007, 2011; Li and Yeh, 2001; Sandhu and Singh, 2007). Potato starches had the highest enthalpy followed by corn and mungbean. The lower enthalpy of gelatinization is suggested to be mainly due to the disruption of the double helices A high ΔH suggests that more energy must be applied to unravel and melt strongly associated double helices (formed by the outer branches of adjacent amylopectin chains) within the native granule during gelatinization (Hoover et al., 1997). Our results suggest a lower degree of association between double helices in starches from these Virginia-grown crops compared to similar crops from other locations.

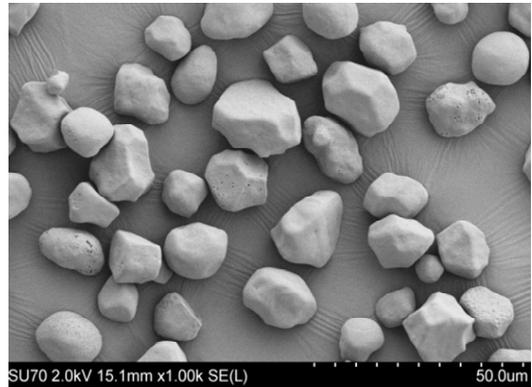
Water absorption capacity (WAC) ranged from 76.1% for Pioneer corn starch to 86.1% for Atlantic potato starch (Table 3). The difference in WAC of the starches among the different cultivars may be attributed to the starch structure and extent of interactions between starch chains and water. A-type crystalline structure is more dense and binds less water than B- and C type structures, which results in lower WAC of corn starches compared to potato and mungbean starches (Wang et al., 1998).

### Conclusions

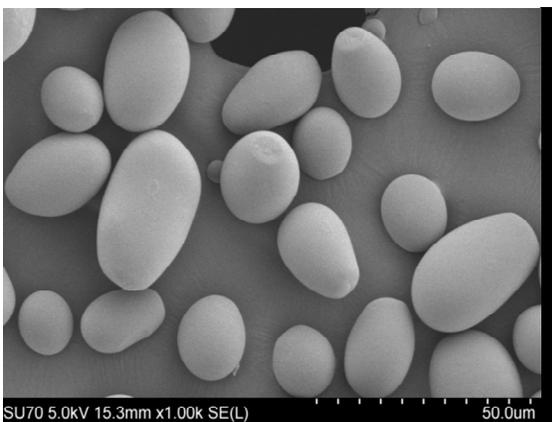
All extracted starches had high purity and were characterized by low protein, fat and ash, and high carbohydrate. Compared to corn and mungbean starches, potato starches had the highest resistant starch content but intermediate amylose content. The resistance of starch



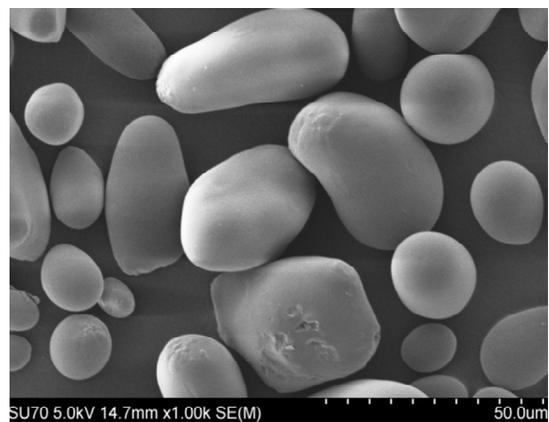
Pioneer 35F37 Corn



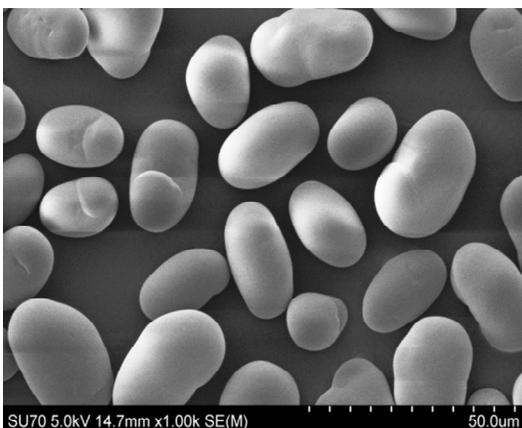
Southern States 731 Corn



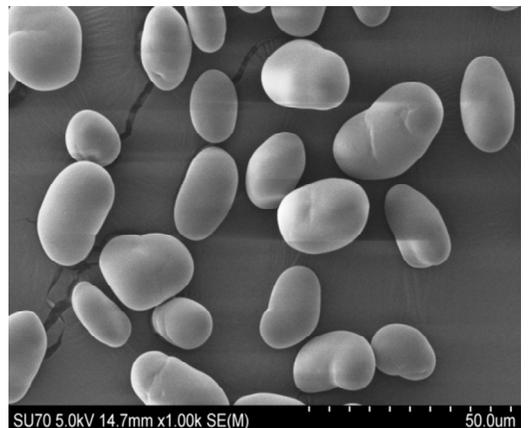
Atlantic Potato



Superior Potato



TexSprout Mungbean

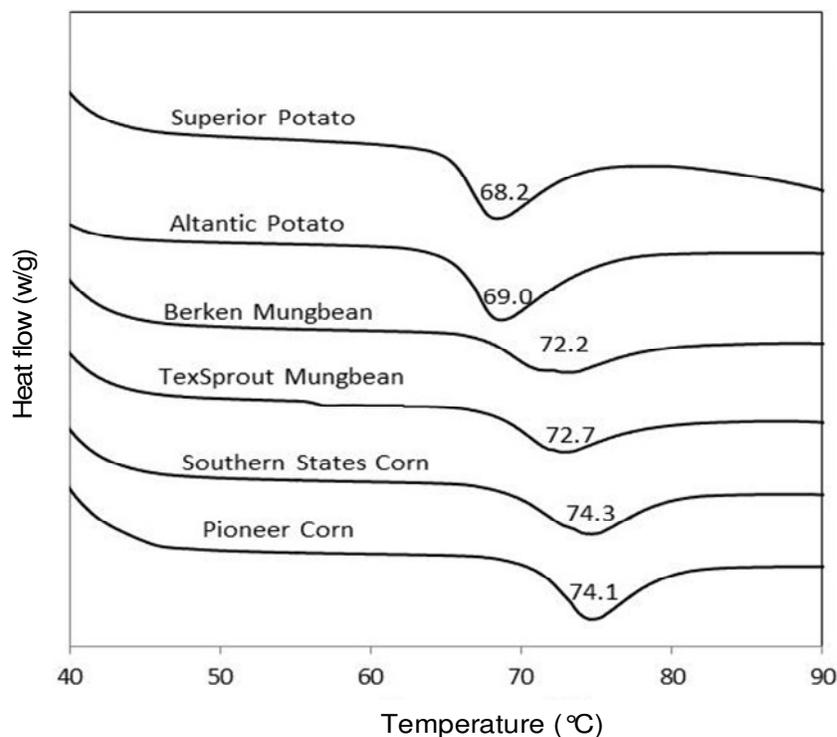


Berken Mungbean

**Figure 2.** Scanning electron micrographs of starches extracted from Virginia-grown corn, potato, and mungbean cultivars.

to enzymatic hydrolysis was dependent on amylose content and the size and structure of starch granules. Higher amylopectin molecular weight and large particle size also contributed to high resistance of potato starches.

Crystallinity, granular morphology, and thermal properties of the starches from Virginia-grown corn, potato and mungbean were in agreement with those already reported for same crop. The results not only provide



**Figure 3.** DSC thermographs of starches extracted from Virginia-grown corn, potato, and mungbean cultivars.

baseline information for plant breeders in developing new cultivars with improved properties, but also assist food scientists in determining the potential end-uses of the starches.

## ACKNOWLEDGMENTS

The authors thank Dr. Anwar Hamama, Mrs. Zaid Abraha-Eyob, and Mrs. Naomi Pearson of the VSU Agricultural Research Station, Wajira Ratnayake of Food Processing Center at the University of Nebraska-Lincoln, and Drs. Everett E Carpenter and Dimitri Pestov of the Virginia Commonwealth University Nano materials Core Characterization Facility for technical support. We thank Drs. Glenn Chappell and Josh Freeman for providing corn kernels, potato tubers, and mungbean seeds, respectively. Funding was provided through the USDA Evans-Allen program and the research was conducted at Virginia State University Agricultural Research Station (Journal Series number 301).

## REFERENCES

- AACC International (1983). Approved Methods of Analysis, 8th Ed. Method 56-20, Hydration capacity of pregelatinized cereal products. AACCI, St. Paul, MN.
- AOAC International (2000). Approved Methods of Analysis, 17th Ed. Method 948.22, Lipid and oil; Method 950.49, Ash; Method 990.03, Crude protein; Association of Official Analytic Chemists International: Gaithersburg, MD, USA.
- AOAC International (2002). Method 2002.02, Resistant starch in starch and plant materials. J. AOAC Int. 85:1103.
- Bhardwaj HL, Hankins A, Mebrahtu T, Mullins J, Rangappa M, Abaye O, Welbaum GE (1996). Alternative crops research in Virginia. Prog. in New Crops, J. Janick, ed. ASHS Press: Alexandria, VA. pp. 87-96.
- Bhardwaj HL, Rangappa M, Hamama AA (1999). Chickpea, faba bean, lupin, mungbean, and pigeonpea: potential new crops for the mid-Atlantic Region of the United States. Pers. on New Crops and New Uses, J. Janick, ed. ASHS Press: Alexandria, VA. pp. 202-205.
- Chen L, Liu R, Qin C, Meng Y, Zhang J, Wang Y, Xu G (2010). Sources and intake of resistant starch in the Chinese diet. Asia Pac. J. Clin. Nutr. 19(2):274-282.
- Hoover R, Li YX, Hynes G, Senanayake N (1997) Physicochemical characterization of mungbean starch. Food Hydrocolloid. 11(4):401-408.
- Hoover R, Ratnayake WS (2001). Determination of total amylose content of starch. Curr. Prot. Food Anal. Chem. E 2.3.
- Hoover R, Ratnayake WS (2002). Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada. Food Chem. 78:489-498.
- Hoover R, Zhou Y (2003). *In vitro* and *in vivo* hydrolysis of legume starches by  $\alpha$ -amylase and resistant starch formation in legumes - A review. Carbohydr. Polym. 54:401-417.
- Huang J, Schols HA, van Soest JJG, Jin Z, Sulmann E, Voragen A G J (2007). Physicochemical properties and amylopectin chain profiles of cowpea, chickpea and yellow pea starches. Food Chem. 101:1338-1345.
- Jiranuntakul W, Puttanlek C, Rungsardthong V, Pancha-arnon S, Uttapap D (2011). Microstructural and physicochemical properties of heat-moisture treated waxy and normal starches. J. Food. Eng. 104:246-258.
- Kaur A, Singh N, Ezekiel R, Guraya HS (2007). Physicochemical, thermal and pasting properties of starches separated from different potato cultivars grown at different locations. Food Chem. 101:643-

- 651.
- Kaur M, Sandhu KS, Singh N, Lim ST (2011). Amylose content, molecular structure, physicochemical properties and *in vitro* digestibility of starches from different mungbean (*Vigna radiate* L.) cultivars. *Starch/Stärke*. 63:709-716.
- Kim SH, Lee BH, Baik MY, Joo MH, Yoo SH (2007). Chemical structure and physical properties of mungbean starches isolated from 5 domestic cultivars. *J. Food Sci.* 72(9): C471-477.
- Leloup VM, Colonna P, Ring SG (1992). Physico-chemical aspects of resistant starch. *J. Cereal Sci.* 16: 253-266.
- Leszczyński W (2004). Resistant starch - classification, structure, production. *Pol. J. Food Nutr. Sci.* 13: 37-50.
- Li JY, Yeh AI (2001). Relationship between thermal, rheological characteristics and swelling power for various starches. *J. Food Eng.* 50:141-148.
- Li S, Gao Q, Ward R (2011). Physicochemical properties and *in vitro* digestibility of resistant starch from mungbean (*Phaseolus radiatus*) starch. *Starch/Stärke* 63:171-178.
- Liu Q, Donner E, Yin Y, Huang, RL, Fan MZ (2006). The physicochemical properties and *in vitro* digestibility of selected cereals, tubers and legumes grown in China. *Food Chem.* 99(3):470-477.
- Nara S, Komiy T (1983). Studied on the relationship between water saturated state and crystallinity by the diffraction method for moistened potato starch. *Starch* 35:407-410.
- National Nutrition Monitoring Bureau (1991). Trends in nutrition. *Nutr. News* 12(3):1-4.
- Polesi LF, Sarmento SBS, Anjos, CBP (2011). Composition and characterization of pea and chickpea starches. *Braz. J. Food Tech.* 14(1):74-81.
- Ratnayake WS, Jackson DS (2007). A new insight into the gelatinization process of native starches. *Carbohydr. Polym.* 67:511-529.
- Sajilata MG, Singhal RS, Kulkarni PR (2006). Resistant starch-a review. *Compr. Rev. Food Sci. Food Safety* 59(1):1-17.
- Sandhu KS, Singh N (2007). Some properties of corn starches II: physicochemical, gelatinization, retrogradation, pasting and gel textural properties. *Food Chem.* 101:1499-1507.
- Sandhu KS, Lim ST (2008). Digestibility of legume starches as influenced by their physical and structural properties. *Carbohydr. Polym.* 71:245-252.
- Sharma A, Yadav BS, Ritika (2008). Resistant starch: physiological roles and food applications. *Food Rev. Int.* 24:193-234.
- Shapours H, Salassi M (2006). The economics feasibility of ethanol production from sugar in the United States. A report through a cooperative agreement between OEPNU, OCE, USDA and LSU. Available online: (November 27, 2012). <<http://www.usda.gov/oce/reports/energy/EthanolSugarFeasibilityReport3.pdf>>
- Singh N, Sandhu KS, Kaur M (2004). Characterization of starches separated from Indian chickpea (*Cicer arietinum* L.) cultivars. *J. Food Eng.* 63:441-449.
- Stark JC, Love SL (2003). Tuber quality. *Potato Production Systems*. JC Stark, SL Love, eds. Univ. Idaho Extension: Moscow. pp. 329-343.
- Swinkels JJM (1985). Composition and properties of commercial native starches. *Starch/Stärke* 37:S.1-5.
- Themeier H, Hollmann J, Neese U, Lindhauer MG (2005). Structural and morphological factors influencing the quantification of resistant starch II in starches of different botanical origin. *Carbohydr. Polym.* 61:72-79.
- Topping DL, Clifton PM (2001). Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol. Rev.* 81(3):1031-1064.
- USDA-NASS (2011). Quick Stats database - Virginia. United States Department of Agriculture, National Agricultural Statistics Service, <http://www.nass.usda.gov> (November 27, 2012).
- Vasanthan T (2001). Overview of laboratory isolation of starch from plant materials. *Curr. Prot. Food Anal. Chem.* E2.1.1-E2.1.6.
- Wang TL, Bogracheva TY, Hedley CL (1998). Starch: as simple as A, B, C? *J. Exp. Bot.* 49:481-502.
- Xu, YX, Miladinov V, Hanna MA (2004). Synthesis and characterization of starch acetates with high degree of substitution. *Cereal Chem.* 81(6):735-740.
- Xu Y, Sismour EN, Narina SS, Dean D, Bhardwaj HL, Li Z (2013). Composition and properties of starches from Virginia-grown kabuli chickpea (*Cicer arietinum* L.) cultivars. *Int. J. Food Sci. Tech.* doi:10.1111/j.1365-2621.2012.03217.x. 48(3):539-547.
- Yoo SH, Jane JL (2002). Molecular weight and gyration radii of amylopectin determined by high-performance size exclusion chromatography equipped with multi-angle laser light scattering refractive index detectors. *Carbohydr. Polym.* 49:307-314.