

Morphological, Thermal and Physicochemical Properties of Starches from Squash (*Cucurbita maxima*) and Pumpkin (*Cucurbita moschata*)

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Abstract

The properties of starches isolated from two squash cultivars (*Cucurbita maxima*) were studied and compared with those of two pumpkin cultivars (*Cucurbita moschata*) and one normal potato cultivar. The amylose content of pumpkin and squash starches ranged from 16.18% to 21.29%. The pumpkin and squash starches appeared as a mixture of spherical, polyhedral and dome shaped granules. The pumpkin and squash starch pastes had pasting temperature of 63.3°C-70.9°C. The enthalpy of gelatinization of starches from pumpkin and squash range from 9.73 J/g-13.58 J/g. The pumpkin and squash starches exhibited the B-type X-ray diffraction pattern. The swelling power and solubility of squash starch increased with the rise of temperature, which were lower than potato starch. The hardness, adhesiveness and chewiness of squash starch were lower than potato starch, while the cohesiveness and recoverability of squash starch was higher than potato starch.

Keywords: *Cucurbita maxima*; *Cucurbita moschata*; Starch; Morphological; Thermal; Physicochemical

maxima and compared the physicochemical characteristics of the starches with those of potato starch.

Introduction

Cucurbita species (*Cucurbita moschata*, *Cucurbita maxima*, *Cucurbita pepo*, *Cucurbita mixta* and *Cucurbita ficifolia*) are important sources of minerals, vitamins and nutritional carbohydrates, which are grown and consumed worldwide [1]. *Cucurbita* species are popular in numerous culinary uses, such as vegetable or ingredient in pies and soups [2]. *Cucurbita* species are also suitable for various systems of traditional medicine such as antibacterial, anticancer, antidiabetic, antihypertensive, antiinflammation, antiparasitic, antalgic, hypocholesterolemic, immunomodulatory and intestinal [3].

Starch, which is the major reserve polysaccharide of plants, have received extensive attention in relation to structural and physicochemical properties [4,5]. Starch consists of amylose which possesses unbranched α -1,4-linkages and amylopectin which possesses the branched α -1,6-linkages. Starch is used in the food, pharmaceutical, textile and chemical industries [4,6]. The functional behaviour of starches depends on the morphological, thermal, rheological and physicochemical properties [7]. The morphological, thermal, rheological and physicochemical properties such as granule shape and size, amylose to amylopectin ratio, gelatinization temperature and swelling behavior vary with genotype, environmental conditions and cultivatory practices [4,8]. Starches have been widely used in food and industrial applications as adhesive, thickener, colloidal stabiliser, gelling agent, bulking agent and water retention agent. Industrial interest in new value-added products has resulted in many studies being carried out on the physicochemical properties of squash starch [9,10]. However, no study has been yet to be conducted to compare physicochemical properties of *Cucurbita moschata* and *Cucurbita maxima*. In the present work, we studied morphological and thermal properties of starches from *Cucurbita moschata* and *Cucurbita*

Materials and Methods

Plant materials

Two pumpkin cultivars (XIAOMOPAN and MIBEN) and two squash cultivars (ZHONGLI NO.3 and 729) fruits were obtained from the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China (2011 harvest). The potatoes of a modern-day cultivar (Beijing, China, 2011 harvest) were used. All the chemicals and solvents used in the study were of analytical grade.

Potato starch was isolated according to the method described earlier [9,11]. The starches from different cultivars of pumpkin and squash were isolated as previously reported [9].

Amylose content

Starch sample of 50 mg was dissolved in 10 ml 90% dimethyl sulphoxide (DMSO). The mixed sample heated for 10 min at 100°C with continuous stirring and then cooled to ambient temperature. The diluted solution (1.0 ml) was mixed with 40 ml distilled water and 2 ml solution of iodine (I) and potassium iodide (KI) and then adjusted to a final volume of 50 ml. Blank sample was also prepared and absorbance was taken at 600 nm.

Pasting properties

The pasting properties of the samples were analyzed using Rapid Visco-Analyser (RVA-4, Newport Scientific, Australia). Starch sample of 2.5 g was added to 25 ml deionised water in aluminum RVA sample canister. A programmed heating and cooling cycle under constant shear (160 rpm) was used, where the samples were held at 50°C for 1

min, heated to 95°C at a rate of 12°C/min, held at 95°C for 2.5 min, then cooled at a rate of 12°C/min to 50°C and held at 50°C for 2 min. The parameters of the RVA measurement included peak viscosity, trough viscosity, final viscosity, breakdown viscosity (peak viscosity minus trough viscosity), setback viscosity (final viscosity minus trough viscosity) and pasting temperature. All samples were analyzed in triplicate.

Thermal properties

Thermal analysis experiments were performed on differential scanning calorimetry (DSC-Q200, TA-Instruments, USA) equipped with thermal analysis software. Starch sample (2 mg, dry weight basis) was precisely weighed in an aluminum pan, mixed with 40 µL distilled water. An empty sealed aluminium crucible was used as reference. All the samples were equilibrated for at least 12 h at room temperature and then heated in the calorimeter from 20°C-100°C at a rate of 10°C/min. The onset temperature (To), peak temperature (Tp), conclusion temperature (Tc), and gelatinization enthalpy (ΔH) were recorded. All the experiments were performed in triplicate.

Starch granule morphology

Starch granule morphology was obtained by scanning electron microscope (S-3700 N, Hitachi, Japan) at different magnifications. Starch samples were sprinkled on to double-sided adhesive tape attached to a circular aluminum stub, and then coated with 20 nm gold under vacuum. The samples were viewed and photographed with a scanning electron microscope at an acceleration potential of 15 kV and magnification of 2000.

Crystalline structure

The crystalline structure of starch samples were obtained from X-ray diffractometer (D/Max-2200, Rigaku Denki Company, Tokyo, Japan). Starch samples were equilibrated at ambient temperature for 24 h. The samples were scanned at target voltage 40 kV and target current 100 mA with 4°-35° of the scanning range and 2.0°/min of the scanning rate. The crystallinity of starch was calculated according to the method described earlier [12].

Particle size distribution

The starch particle size distribution was determined with a laser diffraction particle size analyser (Zetasizer, model Nano ZS90, Malvern Instruments Limited, UK). Starch samples of 50 mg were dispersed in 25 ml distilled water and mixed in an ultrasound sonicator for 10 min

at room temperature prior to measurement. The starch granules volumes were calculated as previously reported [13].

Swelling power and solubility

Starch suspension (2%, w/v) was heated at 50, 60, 70, and 80°C for 30 min, separately. The suspension was then centrifuged at 4000 rpm for 20 min. Swelling power and solubility were measured according to the method previously reported [14].

Freeze-thaw stability

Starch suspension (5%, w/w) was heated at 95 °C under constant agitation for 1 h. The paste was frozen in the refrigerator for 24 h before thawing at room temperature. It was centrifuged at 4000 rpm for 20 min. Five cycles of freeze thaw were performed by freezing at -16°C for 24 h and thawing at 25°C for 6 h. The freeze thaw stability of the samples was determined in terms of syneresis according to the method described [15].

Light transmittance

Starch suspension (2%, dry basis) was heated at 100°C for 30 min with constant stirring. The suspension was cooled to room temperature. Samples were stored at 4°C after cooling to room temperature. The light transmittance (%) was determined at 0, 24, 48, 72, 90 and 120 h by measuring the absorbance at 640 nm against a blank water sample with a UV spectrophotometer (Varian, Inc. Corporate, USA).

Textural properties

Starch samples were poured into aluminum canisters and stored at 4°C to cause gelation. Textural properties were measured using the TA/XT2 texture analyzer (TAHDI, Stable Microsystems, Surrey, England). The conditions used for the experiments were as follows: pretest 10.0 mm/s, test 2.0 mm/s, posttest 10.0 mm/s, and trigger force 0.1 N.

Statistical analysis

All experiments were conducted in triplicate and analyzed using analysis of variance (ANOVA). The treatments were considered significantly different at the 5% significance level. The statistical analysis of the data was performed using SPSS v17.0 software.

Results and Discussion

Samples	Amylose content (%)	Peak viscosity (cP)	Trough viscosity (cP)	Final viscosity (cP)	Breakdown (cP)	Setback (cP)	Pasting temperature (°C)
XIAOMOPAN	18.65ab	3521c	2179bc	2557bc	1242bc	378bc	66.3b
MIBEN	21.29a	3959b	2811a	3282a	1148b	471a	64.8bc
ZHONGLI NO.3	19.07ab	3088d	2011c	2368c	1077c	357c	70.9a
729	16.18b	4404a	2659b	3060b	1745a	401b	63.3c

Table 1: The amylose contents and pasting properties of pumpkin and squash starches.

Amylose content is influenced by the factors such as botanical sources, climatic conditions, soil types and harvest time [16]. Amylose

content affects the physicochemical properties of the starches, including pasting, thermal, retrogradation and swelling properties

[17]. The amylose contents of the starches are shown in Table 1. The amylose contents were significantly different between the starches of pumpkin and squash cultivars. Amylose content was observed in the range of 16.18–21.29%. The amylose content of MIBEN was highest (21.29%) of all samples, while the amylose content of 729 was lowest (16.18%). The amylose contents observed in the study was in agreement with that reported by Stevenson et al. [10].

The pasting properties of starch are affected by amylose content, amylopectin chain length distribution, granule morphology and crystallinity structure [18]. The peak viscosity, trough viscosity, final viscosity, breakdown, setback and pasting temperatures of pumpkin and squash starches are shown in Table 1. The cultivars of pumpkin and squash starches displayed similar pasting patterns. Peak viscosity and breakdown followed the order: 729>MIBEN>XIAOMOPAN>ZHONGLI NO.3. Trough viscosity indicates decrease in starch paste viscosity due to shear and high temperature, while final viscosity represents increase in starch paste viscosity on cooling. Trough viscosity, final viscosity and setback followed the order: MIBEN>729>XIAOMOPAN>ZHONGLI NO.3. The peak viscosity is attained at the most swollen state of starch granules. The granules rupture and the viscosity would fall when starch paste heats beyond this point [19]. Breakdown viscosity represents the stability of the paste while setback viscosity reflects the degree of retrogradation [20]. The high peak viscosity and breakdown of 729 might be associated with by low amylose content. The final viscosity and setback have closed relationship with re-ordering and polymerization of leached amylose and long linear amylopectin [21]. The high final viscosity and setback of MIBEN might be explained by high amylose content. Pasting temperature indicates resistance toward swelling. The pasting temperatures of pumpkin and squash starches ranged from 63.3°C-70.9°C, which was consistent with the present study [10].

Samples	To (°C)	Tp (°C)	Tc (°C)	Tc-To (°C)	ΔH (J/g)
XIAOMOPAN	62.01a	65.73ab	77.6a	15.59a	13.58a
MIBEN	56.06c	60.17c	67.57bc	11.51b	12.32ab
ZHONGLI NO. 3	58.97b	66.11a	72.5b	13.53ab	11.61b
729	57.32bc	62.29b	66.47c	9.11c	9.73c

Table 2: Thermal properties of pumpkin and squash starches.

Gelatinization is an order–disorder phase transition of starch granules during heating in the presence of water. The gelatinisation transition temperatures (To, Tp and Tc), gelatinization temperature range (Tc–To) and gelatinisation enthalpy (ΔH) from pumpkin and squash starches are exhibited in the Table 2. To, Tp, Tc, Tc–To and ΔH from pumpkin and squash starches ranged from 56.06°C-62.01°C, 60.17°C-66.11°C, 66.47°C-77.6°C, 9.11°C-15.59°C, and 9.73 J/g-13.58 J/g, which was consistent with those reported for other squashes [9]. The gelatinisation enthalpy corresponds to genetic varieties, provenance, climatic conditions and disappearance of double helical structure [22,23]. XIAOMOPAN starch showed higher gelatinization enthalpy than that of the other cultivars, which indicates that more energy was needed to break the intermolecular bonds in XIAOMOPAN starch granules for gelatinization. The gelatinization transition temperature indicates the stability of starch crystallinities which are influenced by molecular architectures of crystalline regions [21,24]. The Tc of XIAOMOPAN starch was much higher than that of

the other cultivars, which could be explained by higher melting enthalpy. The gelatinization temperature range have closed relationship with internal arrangement of amylose and amylopectin, and the distribution of amylopectin short chains (degree of polymerization) [10,25]. Difference in Tc–To reflected the extent of heterogeneity of crystallites within the granules of pumpkin and squash starches. XIAOMOPAN had the widest gelatinisation temperature range (15.59°C), while 729 had the narrowest gelatinisation temperature range (9.11°C). The higher gelatinization temperature range of XIAOMOPAN starch compared to other starches also suggested higher degree of association between amylose components. The gelatinisation enthalpy of pumpkin and squash starches followed the order: XIAOMOPAN>MIBEN>ZHONGLI NO.3>729. Difference in enthalpy values between pumpkin and squash starches was due to the crystalline structures [26].

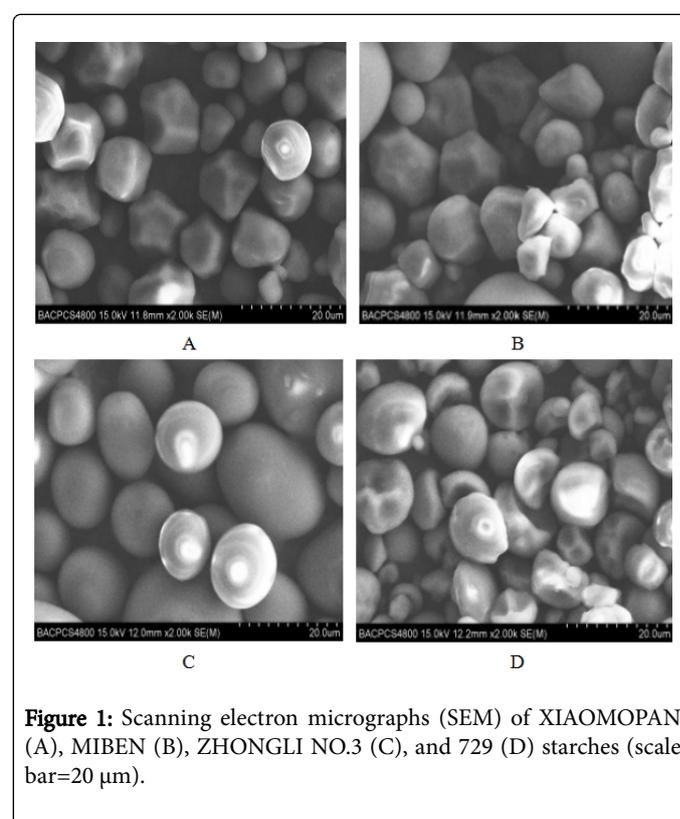


Figure 1: Scanning electron micrographs (SEM) of XIAOMOPAN (A), MIBEN (B), ZHONGLI NO.3 (C), and 729 (D) starches (scale bar=20 μm).

The morphology of starch granules from different botanical sources vary with the genotype and cultural practices. Scanning electron micrographs of the starch granules from pumpkin and squash are illustrated in Figure 1. Starch granules in pumpkin and squash cells were polyhedral, spherical and oval varied with size. Morphological characteristics of starches depends on the biochemistry of the chloroplast or amyloplast, as well as the physiology of the plant. Physico-chemical properties, such as amylose content, light transmittance, swelling power, solubility, and water-binding capacity were significantly correlated with the starch granule size [11,27]. The smaller starch granules were polyhedral and spherical, while the larger starch granules appeared oval. The variation in the size and shape of starch granules is attributed to the plant sources [28]. The average granule size ranges from 1-10 μm for small and 15-40 μm for large starch granules. Many of the polyhedral and spherical starch granules

showed indentations on their surfaces in contrast to oval starch granules, which was in agreement with previous observations [9].

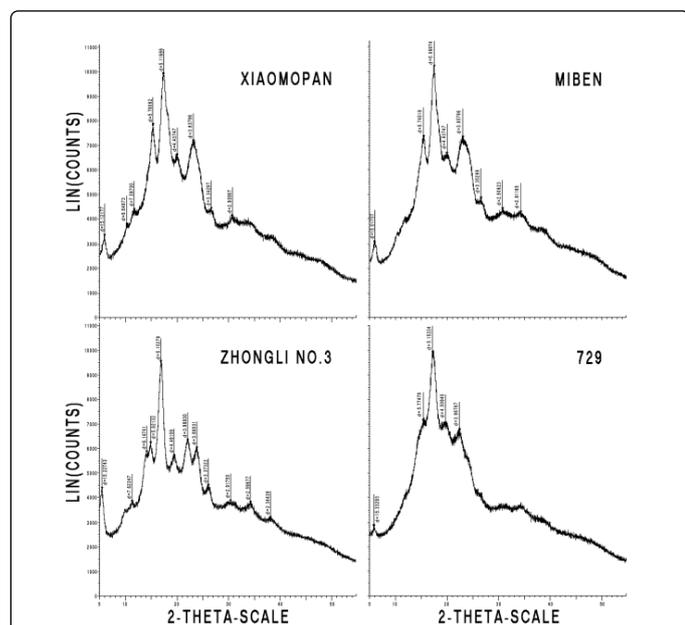


Figure 2: X-ray diffraction patterns of pumpkin and squash starches.

Starches of different botanical sources exhibit different X-ray diffraction patterns namely A-type, B-type and C-type [21]. The cereal and tuber starches exhibit the A-type and B-type X-ray diffraction pattern respectively, while the legume starches show the C-type X-ray pattern, mixture of the A and the B type starch [29]. X-ray diffraction diffractometry has been used to reveal the crystalline structure characteristics of the starch granules [30]. X-ray diffraction patterns of pumpkin and squash starches are shown in Figure 2. Starches isolated from pumpkin and squash all exhibited B-type X-ray diffraction patterns. The pumpkin and squash starches showed peaks at diffraction angles 2θ of 15° , 18° , 20° and 23° . The starch crystallinity varies with amylopectin component, crystal size and amount of crystalline region [31]. The starch crystallinity of XIAOMOPAN, MIBEN, ZHONGLI NO.3 and 729, calculated based on X-ray diffraction peak intensity, was 44.9%, 41.3%, 48.2% and 39.6%, which was in agreement with the previous study [10]. The relative crystallinity ranged between 39.6% and 44.9% for pumpkin and squash starches, which was greater than other native starches, such as 32.4%-38.9% reported for wheat, triticale and barley starches [32].

The granule size distribution of starches vary with different plant sources and development stages [33]. The granule size of different winter squashes cultivars varied between 1.5 and 13 μm , while the size of Kamo Kamo (*Cucurbita pepo*) starch granules ranged from 3 to 23 μm [9,10]. The granule size distributions of potato, corn, rice and wheat starches exhibited ranges of 1 μm -85 μm , 1 μm -25 μm , 3 μm -5 μm and 1 μm -35 μm , respectively [9,34,35]. The granule size distribution of pumpkin and squash starches are shown in Figure 3. The granule size of pumpkin and squash starches ranged from 3 μm -40 μm .

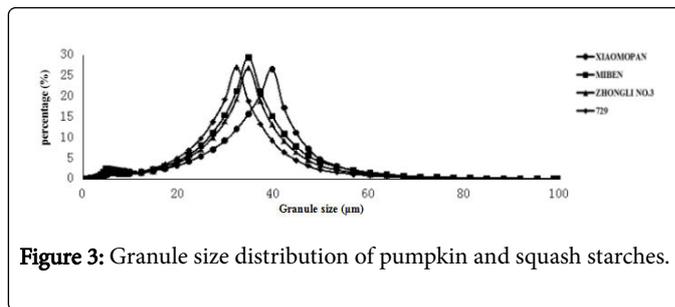


Figure 3: Granule size distribution of pumpkin and squash starches.

Starch granules with different size present different properties. Small size starches could be used as fat substitutes, stabilisers, face (dusting) powders and starch-filled biodegradable films [36]. Small starch granules, such as *Okenia hypogaea* (fox peanut), could be suitable for food and cosmetic industries due to their high adsorbent capacity [37]. The pumpkin and squash starches granules were small, which could have applications in food industry for noodle making and encapsulating flavours as well as cosmetic, medicine and paint industries.

Starch	Temperature ($^\circ\text{C}$)			
	50	60	70	80
Swelling power (g/g)				
Squash	1.37 \pm 0.04	2.01 \pm 0.07	4.67 \pm 0.13	6.22 \pm 0.16
Potato	1.59 \pm 0.05	3.13 \pm 0.11	5.31 \pm 0.14	7.04 \pm 0.18
Solubility (g/100 g)				
Squash	22.65 \pm 0.19	38.37 \pm 0.15	52.16 \pm 0.23	69.27 \pm 0.29
Potato	31.23 \pm 0.21	46.18 \pm 0.22	63.38 \pm 0.17	78.65 \pm 0.35
Values expressed are mean \pm standard deviation.				

Table 3: Swelling power and solubility of squash and potato starch pastes.

The starch polymer molecules become solvated while heating in excess amount of water. At the same time, both crystalline and amorphous structures are disrupted, which causes the increase in granule swelling and starch solubility [30,38]. The swelling power and solubility of starches from different botanical sources vary with the genotype and cultural practices. Potato starch has much higher swelling power than corn, rice and wheat starches [8]. The swelling power and solubility of squash and potato starches were investigated over a temperature range from 50 to 80 $^\circ\text{C}$. The results of these experiments are summarized in the Table 3. In case of squash and potato starches, the swelling power was found to be 1.37 g/g-6.22 g/g and 1.59 g/g-7.04 g/g, whereas the solubility ranged from 22.65 g-69.27 g/100 g and 31.23 g-78.65 g/100 g, respectively. The increase in the temperature weakened the hydrogen bonding interactions of granules starch and improved the swelling power and solubility of squash and potato starches. Swelling power corresponds to the content, phosphate groups and chain branch length of amylopectin as well as amylose/amylopectin molecular weight and distribution [19]. Solubility is contributed by hydrophilicity, amylose content as well as granule structure and organization [39]. The swelling power and solubility is also attributed to phosphorus content and compounds, such as phospholipids and phosphate monoesters [40]. The squash starch had

lower swelling power and solubility than that of potato starch from 50°C-80°C, which was in accordance with the research earlier [9]. The strong internal organization within the starch granules may have resulted in the low swelling power of squash starch. The low large granule percentage and mean granule volume may be responsible for the low solubility of squash starch.

Starch	Time (h)					
	0	24	48	72	90	120
Syneresis (%)						
Squash	11.62 ± 0.03	17.25 ± 0.09	28.16 ± 0.08	31.58 ± 0.17	38.37 ± 0.15	46.52 ± 0.21
Potato	15.44 ± 0.06	21.37 ± 0.11	33.49 ± 0.14	41.49 ± 0.24	52.76 ± 0.26	60.31 ± 0.19
Light transmittance (%)						
Squash	55.65 ± 0.33	48.31 ± 0.27	42.26 ± 0.29	36.17 ± 0.15	24.73 ± 0.11	22.58 ± 0.09
Potato	60.13 ± 0.29	52.62 ± 0.31	49.49 ± 0.22	41.36 ± 0.19	30.65 ± 0.13	27.31 ± 0.12
Values expressed are mean ± standard deviation.						

Table 4: Syneresis and light transmittance of squash and potato starch pastes.

Syneresis is used to evaluate the freeze thaw ability of starches to withstand the undesirable physical changes during freezing and thawing [41]. Thus, the amount of water released due to syneresis is a useful indicator of the tendency of starch to retrograde. The freeze thaw stability of squash and potato starches is presented in Table 4. The syneresis increased with the number of freeze thaw cycles and varied with the botanical sources (squash and potato), which was in accordance with the previous study [42]. The increase of syneresis is caused by molecular interaction associations between leached amylose and amylopectin chains, which results in the expulsion of water from the gel structure [21,43]. Compared with squash starch, potato starch rapidly lost the stability after the first freeze thaw cycle where the syneresis level was larger than 10%. Subsequent freeze thaw cycles increased the syneresis degrees of squash and potato starches, which achieved 28.51% and 48.63% after the 5th cycle respectively. Squash starches would be considered as the “clean-label” ingredients for frozen food application as a result of high freeze thaw stability.

Light transmittance is incident light fraction at specified wavelength passing through starch sample, which can indicate the paste clarity and retrogradation process of starch [21]. Light transmittance is an important starch quality attribute in formulation of jellies and fruit pastes [44]. The light transmittance of squash and potato starches is shown in Table 4. Light transmittance of squash and potato starches decreased with the increase of storage time, which may be due to the initial broken bonds re-association of starch structure [45]. Similar time-dependent reduction in transmittance has been reported earlier for rice starch pastes [46]. Light transmittance of potato starch was higher than squash starch after equivalent storage time, which might be attributed to larger dissolved molecules proportion.

Sample s	Hardness	Adhesiveness	Cohesiveness	Chewiness	Recoverability
Squash	43.11 ± 0.98	8.67 ± 0.11	0.89 ± 0.03	12.42 ± 0.09	0.78 ± 0.02
Potato	70.21 ± 1.34	13.51 ± 0.17	0.73 ± 0.02	17.45 ± 0.12	0.41 ± 0.01
Values expressed are mean ± standard deviation.					

Table 5: Textural properties of squash and potato starch pastes.

Textural properties, including hardness, adhesiveness, springiness, brittleness, chewiness, elasticity, recoverability, and conglutination degree, were conducted to evaluate the potential applicability of starches as food additive [47]. The textural properties of squash and potato starches using Texture profile analysis (TPA) tests were shown in Table 5. The textural properties of starches are affected by the ratio, distribution, molecular weight and chain length of amylose and amylopectin [48]. The textural properties of pastes of the starches from the two different sources varied to a great extent, which may be attributed to different starch structure. Compared with potato starch, squash starch showed considerably higher cohesiveness and recoverability. The hardness, adhesiveness and chewiness of squash starch were observed to be lower than potato starch.

Conclusions

In summary, we have reported the morphological, thermal and physicochemical properties of starches from squash preparation and physicochemical characterization of acetylated lotus rhizome starches as well as a preliminary investigation their applications in the food industry. Squash starch exhibited similar morphological and thermal properties to those of pumpkin starch. The squash starch presented considerable differences from potato starch in physicochemical properties (swelling power, solubility, light transmittance, freeze-thaw stability and textural properties), which could endow the food product with good appearance and quality. As mentioned above, squash starch could find suitable applications in the food industry such as soups, noodles, puddings and fruit jelly.

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Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest.

Compliance with ethics requirements: This article does not contain any studies with human or animal subjects.

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