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Food Chemistry 99 (2006) 30–37
Food Chemistry
www.elsevier.com/locate/foodchem

Characterization of new starches separated from different Chinese yam (*Dioscorea opposita* Thunb.) cultivars

Wang Shujun [a], Liu Hongyan [c], Gao Wenyuan [a,*], Chen Haixia [a], Yu Jiugao [b], Xiao Peigen [d]

[a] *The College of Pharmaceutical Science and Biotechnology, Tianjin University, Tianjin 300072, China*
[b] *The College of Science, Tianjin University, Tianjin 300072, China*
[c] *Henan Agricultural Academy of Sciences, Henan province, 350002, China*
[d] *Institute of Medicinal Plant, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100094, China*

Received 2 May 2005; received in revised form 5 July 2005; accepted 5 July 2005

Abstract

The starches separated from four different *Dioscorea opposita* Thunb. cultivars were investigated for morphological, thermal, crystal, and physicochemical properties, such as amylose content, swelling power, solubility and water-binding capacity properties. Amylose content of *D. opposita*starches from different cultivars ranged from 20.74% to 25.94%. The shape of starch granules separated from different *D. opposita* Thunb. cultivars varied from round to oval or elliptic. The mean particle diameter of starches ranged from 23.39 to 26.87 μm. The transition temperatures (T_o, T_p and T_c) and enthalpy of gelatinization (ΔH_{gel}) were determined using differential scanning calorimetry (DSC). T_o, T_p and T_c varied from 73.6 to 74.8, 78.8 to 81.0, and 83.3 to 87.2 °C, respectively. *D. opposita* cv. Jinchengerhao starch showed the highest ΔH_{gel} values (12.48 J/g) while *D. opposita* cv. Baiyu starch showed the lowest values (8.413 J/g). The crystal type of starches separated from different *D. opposita* cultivars was a typical C_B-type pattern. The degrees of crystallinity of the four *D. opposita* cultivars starches were about 50.52%, 32.99%, 33.57% and 36.16%, respectively.

Keywords: *Dioscorea opposita* Thunb.; Starch; Morphology; Thermal; Crystallinity; Amylose content

1. Introduction

Dioscoreae (Chinese name Shanyao), the rhizome of various species of genus *Dioscorea opposita* Thunb. (Dioscoreaceae), have been used as an important invigorants in traditional Chinese medicine (TCM) for many years (Zuo & Tang, 2003). They can invigorate the spleen and stomach, promote production of the body fluids and benefit the lung and invigorate the kidney. The *D. opposita* Thunb. is produced in Hebei, Shanxi, and Shandong, with the best from Xinxiang county of Henan province, and harvested in winter and processed in a procedure of washing, peeling, steaming with sulphur, drying, softening and slicing. The crude or stir-baked yam with bran can be used. The quality and utilization differ due to the different regions and cultivars. As reported in the literature, there are many chemical components contained in the *D. opposita* Thunb., such as mannan, allantoin, dopamine, batatasine, phytic acid, abscisin II, amino-acids, glucoprotein, choline, cholesterol, ergosterol, campesterol, saponins, starch, non-starch polysaccharide and others (Nie, Zhou, Dong, & Zhang, 1993; Zhao, Li, & Chen, 2003). The Agricultural Academy of Sciences of Shandong reported that the rhizoma of *D. opposita* Thunb. contains, on average, 43.7% starch, 14.5% protein, 3.48% crude fibre, 1.14% sugar, 2.26% potassium, 0.2% phosphorus, 0.2% calcium, 0.14% magnesium,

* Corresponding author. Tel.: +86 022 87 40 1895.
E-mail address: edwinwa@hotmail.com (G. Wenyuan).

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doi:10.1016/j.foodchem.2005.07.008

5.51% ash, 53.6 mg/kg of iron, 29.2 mg/kg of zinc, 10.6 mg/kg of copper and 5.38 mg/kg of manganese (Zhou, Wu, Zhang, & Yan, 2004).

Total carbohydrates of *D. opposita* Thunb. vary from 20% to 60%. Starch is the most abundant carbohydrate in the rhizoma of *D. opposita* Thunb. (Ni & Song, 2002). However, there are few investigations on the properties of starch contained in *D. opposita* Thunb. Starch, an important reserve carbohydrate in higher plants, in the form of birefringent, semi-crystalline granules deserves detailed research to understand better its biochemical and functional characteristics as well as variations. Extensive research has been conducted on the structure and functional properties of commercial starches obtained from seeds (corn, waxy corn, high amylose corn, wheat and various rice types) and from tubers and roots, particularly potato, sweet potato and cassava, due to their ready availability and their extensive utilization in food and non-food applications (Kaur, Singh, Sandhu, & Guraya, 2004; Singh, Singh, Kaur, Sodhi, & Gill, 2003). Starches from different sources vary, particularly in their quantitative and qualitative make-up, as well as in some of their physicochemical properties. In addition, researches on the characterization of starches from Indian potato, rice, corn, field pea, chickpea, yam bean, black bean and pinto bean cultivars have also been reported (Aggarwal, Singh, Kamboj, & Brar, 2004; Hoover & Ratnayake, 2002; Kaur, Singh, & Sodhi, 2002; Ratnayake, Hoover, Shahidi, Perera, & Jame, 2001; Singh & Singh, 2001; Singh, Sandhu, & Kaur, 2004; Sodhi & Singh, 2003). Therefore, the starches separated from different *D. opposita* Thunb. cultivars should also have different properties. In order to widen the application of *D. opposita* Thunb. and provide a new starch for the food and drug industry, the physicochemical, thermal, morphological and crystalline characteristics of starches separated from different *D. opposita* Thunb. cultivars were completely evaluated.

2. Materials and methods

2.1. Materials

Four different *D. opposita* Thunb. cultivars (cv.), namely *D. opposita* cv. Baiyu, *D. opposita* cv. Jichengerhao, *D. opposita* cv. Jiaxiangxichangmao and *D. opposita* cv. jinchengyihao were provided by Henan Agricultural Academy of Sciences and were identified by Researcher Liu hongyan, Henan Academy of Agricultural Science, Henan province, China.

2.2. Starch isolation

The four different dried *D. opposita* Thunb. cultivars were washed, cut into small pieces and ground with a plant micro-muller which were sieved with a 100 mesh sifter. After sieving, the *D. opposita* Thunb. powders were immediately steeped in water containing 0.1% $HgCl_2$ to prevent microbial growth. After depositing, the supernatant was removed by suction and the settled starch layer was resuspended in distilled water. After seven or eight cycles of depositing and resuspending repeatedly, the slurry containing starch was centrifuged in wide-mouthed cups at 3000 rpm for 10 min. The supernatant was discarded and the upper non-white layer was scraped off. The white layer was resuspended in distilled water and recentrifuged 3–5 times. The starch was then collected and dried at room temperature automatically.

2.3. Physicochemical properties of starch

2.3.1. Amylose content

Amylose content of the isolated starch was determined in triplicate by using the method of Williams, Kuzina, and Hiynka (1970).

2.3.2. Swelling power (%) and solubility (%)

Swelling power and solubility were determined in triplicate according to the method of Leach, McCowen, and Schoch (1959).

2.3.3. Water-binding capacity

Water-binding capacities (WBCs) of the starches from different *D. opposita* Thunb. cultivars were determined, using the method described by Yamazaki (1953), as modified by Medcalf and Gilles (1965). A suspension of 5 g starch (dry weight) in 75 ml distilled water was agitated for 1 h and centrifuged (3000×*g*) for 10 min. The free water was removed from the wet starch. After draining for 10 min, the wet starch was weighed.

2.4. Morphological properties

Scanning electron micrographs were obtained with an environmental scanning electron microscope (ESEM, Philips XL-3). Starch samples were suspended in acetone to obtain a 1% suspension. One drop of the starch–acetone suspension was applied on an aluminium stub using double-sided adhesive tape and the starch was coated with gold powder to avoid charging under the electron beam after the acetone volatilized. An accelerating potential of 30 kV was used during micrography.

2.5. Particle size analysis

Particle size analysis of starches from different *D. opposita* Thunb. cultivars was done using a laser light scattering particle size analyzer (Mastersizer S, version 2.15, Malvern instruments Ltd., Malvern, UK). The focal length was 100 mm.

2.6. Differential scanning calorimetry

Thermal characteristics of isolated starches were studied by using a differential scanning calorimeter-DSC204, HP (NETZSCH, Germany) equipped with a thermal analysis station. Each of the *D. opposita* Thunb. cultivar starches (3.5 mg, dry weight) was loaded into a 40 μl, capacity aluminium pan (Mettler, ME-27331) and distilled water was added with the help of a Hamilton microsyringe to achieve a starch–water suspension containing 70% water. Samples were hermetically sealed and allowed to stand for 1 h at room temperature before heating in the DSC. The DSC analyzer was calibrated using indium and an empty aluminium pan was used as reference. Sample pans were heated at a rate of 10 °C/min from 20 to 120 °C. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH_{gel}) were calculated automatically. The gelatinization temperature range (R) was computed as (T_c–T_o) as described by Vasanthan and Bhatty (1996). Enthalpies were calculated on a starch dry basis. The peak height index (PHI) was calculated by the ratio $\Delta H/(T_p - T_o)$, as described by Krueger, Knutson, Inglett, and Walker (1987).

2.7. X-ray diffractometry

X-ray powder diffraction measurements were done using a Panalytical X'Pert Pro diffractometer (PANalytical, Holland). Each sample of *D. opposita* Thunb. cultivar starches was packed tightly in a rectangular glass cell (15×10 mm, thickness 0.15 cm). The samples were exposed to the X-ray beam from the X-ray generator running at 40 kV and 40 mA. The scanning regions of the diffraction angle, 2θ, were 4–40°, which covered most of the significant diffraction peaks of the starch crystallites. The other operation conditions were as follows: $\lambda = 1.78901$, step size, 0.0330°, scan step time, 30.8451 s, divergence slit size, 0.2177°. The d-spacings were computed according to Bragg's equations ($n\lambda = 2d\sin\theta$; where d is the inter crystalline spacing, $n = 1$ and $\lambda = 1.78901$ Å). Duplicate measurements were made at ambient temperature.

2.8. Determination of the degree of crystallinity

The degree of crystallinity of samples was quantitatively estimated, following the method of Nara and Komiy (1983). A smooth curve, which connected peak baselines, was computer-plotted on the diffractograms (Fig. 1). The area above the smooth curve was taken as the crystalline portion, and the lower area between smooth curve and the linear baseline which connected the two points of the intensity, 2θ, of 30° and 4° in the samples was taken as the amorphous section. The upper diffraction peak area and the total diffraction area over the diffraction angle 4–30°2θ were integrated using Smadchrom software (Morgan and Kennedy Research, Australia). The ratio of upper area to total diffraction was taken as the degree of crystallinity.

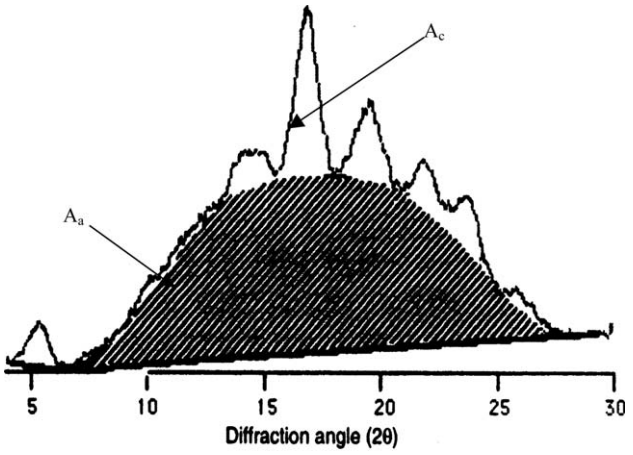

Fig. 1. Calculation of the relative degree of the crystallinity.

The equation of the degree of crystallinity is as follows:

$$X_c = A_c/(A_c + A_a),$$

where X_c refers to the degree of crystallinity; A_c refers to the crystallized area on the X-ray diffractogram; A_a refers to the amorphous area on the X-ray diffractogram.

2.9. Statistical analysis

The data reported in all the Tables are averages of triplicate observations. Statistical comparison of means was conducted using the Student's test in a general linear model (GLM) procedure on a SAS system (release 8.2, SAS Institute, Cary, NC).

3. Results and discussion

3.1. Physicochemical characteristics of D. opposita Thunb. cultivar starches

The amylose content, swelling power, solubility and water-binding capacity of starches separated from different *D. opposita* Thunb. cultivars are presented in Table 1. The amylose content of starches separated from different *D. opposita* Thunb. cultivars were in the range 20.74%–25.94%. *D. opposita* Thunb. cv. Baiyu starch had the lowest amylose content while *D. opposita* cv. Jichengerhao starch had the highest amylose content. The higher amylose content of the starches from *D. opposita* cv. Jichengerhao may be due to the presence of small-size granules. Sandhu, Singh, and Kaur (2004) reported that the small grain fraction had higher amylose content than its counterpart medium and large grain fractions in

Table 1
Amylose content, swelling power, solubility and water-binding capacity of starches separated from different *D. opposita* Thunb. cultivars

Samples	Amylose (%)	Swelling power (%)	Solubility (%)	WBC (%)
Dioscorea opposita cv. Baiyu	20.74c	10.61b	10.32b	112.03a
Dioscorea opposita cv. Jichengerhao	25.94a	10.92ab	11.70a	116.60a
Dioscorea opposita cv. Jiaxiangxichangmao	22.70b	11.78a	11.11a	99.38b
Dioscorea opposita cv. Jinchengyihao	22.53b	10.51b	10.21b	117.56a

[a] Means with the same letter are not significantly different ($P < 0.05$).

the three Pop corn. Swelling power of starches separated from different *D. opposita* Thunb. cultivars ranged from 10.51 to 11.78%, the highest for *D. opposita* cv. Jiaxiangxichangmao and lowest for *D. opposita* cv. Jinchengyihao. The solubility values were in the range 10.21–11.70%. The swelling power of starch has been reported to depend on water-binding capacity of starch molecules by hydrogen bonding (Lee & Osman, 1991). Hydrogen bonds stabilizing the structure of the double helices in crystallites are broken during gelatinization and are replaced by the hydrogen bonds with water and swelling is regulated by the crystalline properties of the starch (Tester & Karkalas, 1996). *D. opposita* cv. Jiaxiangxichangmao starch showed a higher solubility and swelling power than the other *D. opposita* Thunb. starches. Swelling power and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains. The extent of this interaction has been reported to be influenced by the amylose/amylopectin ratio, and by the characteristics of amylose and amylpectin in terms of molecular weight distribution, degree and length of branching, and conformation (Hoover, 2001). WBC of *D. opposita* Thunb. cultivars starches ranged from 99.38% to 117.56%, lowest for *D. opposita* cv. Jiaxiangxichangmao starch and highest for *D. opposita* cv. Jinchengyihao starch (Table 1). Low WBC of starches may be attributed to the involvement of a larger proportion of the hydroxyl groups in forming hydrogen and covalent bonds between starch chains than with water (Hoover & Sosulski, 1986).

3.2. Scanning electron microscopy

The starches separated from the four *D. opposita* Thunb. cultivars differed significantly in granule size and shape when viewed by scanning electron microscopy (SEM). Scanning electron micrographs of the starch granules from different *D. opposite* Thunb. cultivars are illustrated in Fig. 2.

The SEMs of starches separated from different *D. opposita* Thunb. cultivars showed the presence of starch granules, from small to large and round or oval to irregular or cuboidal, with diameter ranges between 5–20 and 20–50 μm, respectively, for small and large granules. The surface of the granules appeared to be smooth with no evidence of any fissures. *D. opposita* cv. Jichengerhao starch showed the presence of many small-sized, oval or elliptic-shaped granules. *D. opposita* cv. Jiaxiangxichangmao starch and *D. opposita* cv. Jinchengyihao starch showed the presence of a fairly large number of large-sized, oval-shaped or cake-shaped granules. However, *D. opposita* cv. Baiyu starch had more large-sized granules in a much larger number.

The variation in size and shape of starch granules may be due to biological origin (Svegmark & Hermansson, 1993). The morphology of starch granules depends on the biochemistry of the chloroplast or amyloplast, as well as physiology of the plant (Badenhuizen, 1969). Physicochemical properties, such as percent light transmittance, amylose content, swelling power and water-binding capacity, were significantly correlated with the average granule size of the starches separated from different plants.

3.3. Particle size distribution of D. opposita Thunb. cultivar starches

Fig. 3 shows the granule size distribution of four different *D. opposita* cultivar starches. The figure clearly indicates that particle diameter of the majority of the starch ranged from 7 to 60 μm with some granules having diameters in the range 1–6 μm. According to the result statistics, the average particle size of these starches ranged from 23.39 to 26.87 μm, the largest for *D. opposita* cv. Baiyu starch and the lowest for *D. opposita* cv. Jichengerhao starch, which was consistent with the results of SEM.

3.4. Thermal properties of D. opposita Thunb. cultivar starches

The results of DSC analysis of starches separated from different *D. opposita* Thunb. cultivars are summarized in Table 2. The transition temperatures (T_o, T_p, and T_c), range (T_c–T_o), enthalpies of gelatinization (ΔH_{gel}) and peak height indices (PHI) of starches from different *D. opposita* cultivars differed significantly. *D. opposita* cv. Jichengerhao starch showed the highest ΔH_{gel}value of 12.4 J/g which may be due to the lowest degree of crystallinity of its granules while *D. opposita* cv. Baiyu starch showed the lowest ΔH_{gel} value of 8.413 J/g which may be due to the highest degree of crystallinity of its granules. Tester and Morrison

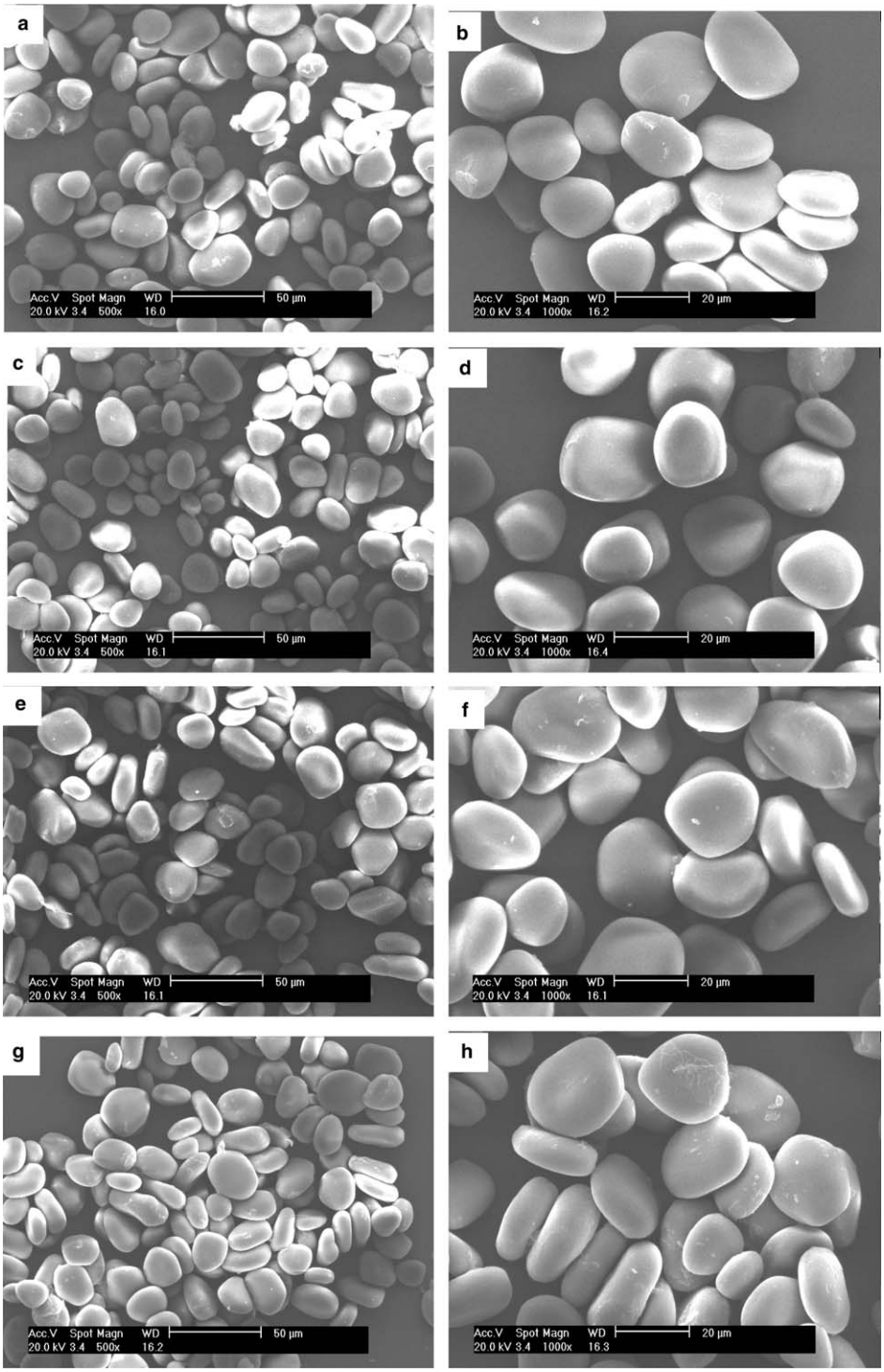

Fig. 2. SEM of four *D. opposita* Thunb. cultivar starches: (a), (b) *Dioscorea opposita* cv. Baiyu; (c),(d) *Dioscorea opposita* cv. Jichengerhao; (e),(f) *Dioscorea opposita* cv. Jiaxiangxichangmao; (g),(h) *Dioscorea opposita* cv. Jinchengyihao.

(1990) postulated that ΔH_{gel} reflects the overall measure of crystallinity (quality and quantity of crystallites) of amylopecin and is an indicator of the loss of molecular order within the granules. According to the X-ray diffraction data of starches from different *D. opposita* Thunb. cultivars (Table 3), it could be concluded that the ΔH_{gel} value increases with decrease of the degree of crystallinity of starches from different *D. opposita* Thunb. cultivars. In addition, granule shape, percentage of large and small granules and presence of phosphate esters have been reported to affect the gelatinization enthalpy values of starches (Stevens & Elton, 1971; Yuan,

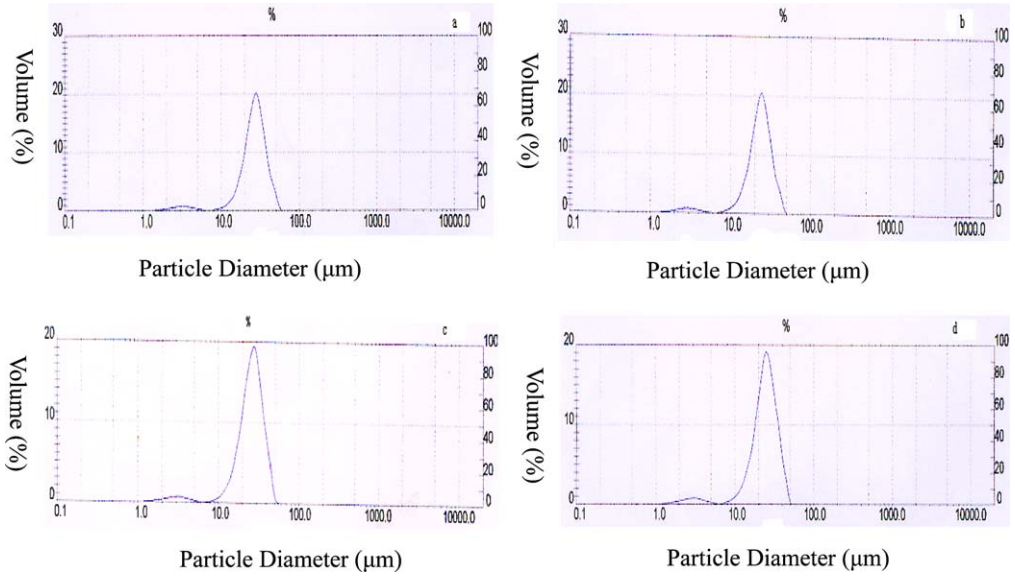

Fig. 3. Particle size analysis of the starches separated from different *D. opposita Thunb. cultivars*: (a) *Dioscorea opposita* cv. Baiyu; (b) *Dioscorea opposita* cv. Jichengerhao; (c) *Dioscorea opposita* cv. Jiaxiangxichangmao; (d) *Dioscorea opposita* cv. Jinchengyihao.

Table 2
Thermal properties of starches separated from different *D. opposita* Thunb. cultivars

Samples	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g)	PHI	R
Dioscorea opposite cv. Baiyu	74.0b	78.8c	83.3c	8.413d	1.75c	9.3d
Dioscorea opposite cv. Jichengerhao	73.6c	79.4b	85.b	12.4a	2.14a	11.7b
Dioscorea opposite cv. Jiaxiangxichangmao	74.8a	81.0a	85.7b	11.78b	1.90b	10.9c
Dioscorea opposite cv. Jinchengyihao	74.2b	80.7ab	87.2a	11.16c	1.72c	13.0a

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; R, gelatinization range (T_c–T_o); ΔH_{gel}, enthalpy of gelatinization (dwb, based on starch weight); PHI, peak height index $\Delta H_{gel}/(T_p–T_o)$.
[a] Means with the same letter are not significantly different ($P < 0.05$).

Table 3
X-ray diffraction data of starches from different*D. opposita* Thunb. cultivars

Samples	Diffraction peaks at 2θ values (° angle)				Degree of crystallinity (%)
	6	18	20	27	
Dioscorea opposita cv. Baiyu	6.64 15.43 (Å)	17.74 5.80 (Å)	20.01 5.15 (Å)	27.18 3.81 (Å)	50.52a
Dioscorea opposita cv. Jichengerhao	6.78 15.13 (Å)	17.69 5.82 (Å)	20.10 5.13 (Å)	27.14 3.81 (Å)	32.99c
Dioscorea opposita cv. Jiaxiangxichangmao	6.65 15.43 (Å)	17.87 5.76 (Å)	20.19 5.10 (Å)	27.09 3.82 (Å)	33.57c
Dioscorea opposita cv. Jinchengyihao	6.60 15.53 (Å)	17.74 5.80 (Å)	20.14 5.11 (Å)	27.23 3.80 (Å)	36.16b

[a] Means with the same letter are not significantly different ($P < 0.05$).

Thompson, & Boyer, 1993). *D. opposita* cv. Jiaxiangxichangmao starch showed the highest T_o (74.8 °C), followed by *D. opposita* cv. Jinchengyihao starch (74.2 °C), while it was lowest for *D. opposita* cv. Jichengerhao starch (73.6 °C). T_p and T_c of starches from different cultivars ranged from 78.8–81.0 to 83.3–87.2 °C, respectively. The transition temperatures observed for *D. opposita* Thunb. starches were higher than those earlier observed for corn, rice, potato and wheat starches (Singh et al., 2003). The differences in gelatinization temperature may be attributed to the differences in amylose content, size, form and distribution of starch granules, and to the internal arrangement of starch fractions within the granule. Noda et al. (1998) have postulated that

T_o, T_p, T_c are influenced by the molecular architecture of the crystalline region, which corresponds to the distribution of short chain amylopectin (DP, 6-11), and not by the proportions of crystalline regions, which corresponds to the amylose/amylopectin ratio. *D. opposita* cv. Jinchengyihao starch had the highest R value, while*D. opposita* cv. Baiyu starch had the minimum value. The differences in R value among the starches from different cultivars may be due to the presence of crystalline regions of different strength in the granules (Banks & Greenwood, 1975). PHI is the ratio of ΔH_{gel} for gelatinization to the gelatinization temperatures range and is a measure of uniformity in gelatinization. PHI of *D. opposita* cv. Jichengerhao starch was highest and that of *D. opposita* cv. Jinchengyihao starch was lowest.

3.5. Crystalline properties of D. opposita Thunb. cultivar starches

The X-ray diffractograms of the starches from four different *D. opposita* Thunb. cultivars are presented in Fig. 4. The corresponding X-ray diffraction parameters and crystallinity levels calculated from the ratio of diffraction peak area and total diffraction area are given in Table 3. The scattering angle, at which the diffraction intensities could be observed was 2θ, and the d spacing was used to discriminate the planes of different sites.

In the diffraction spectra of the four starches separated from different *D. opposita* Thunb. cultivars, there were four strong diffraction peaks at 6.5°, 17.8°, 20.1° and 27.4° 2θ . Of all the diffraction peaks, the peaks at around the 2θ value of 6.5° were characteristic of B pattern. At 27.4° 2θ, only one peak appeared which was indicative of the A pattern. Thus the four starches separated from the four different *D. opposita* Thunb. cultivars were classified as C-type, which is a mixture of A-type and B-type.

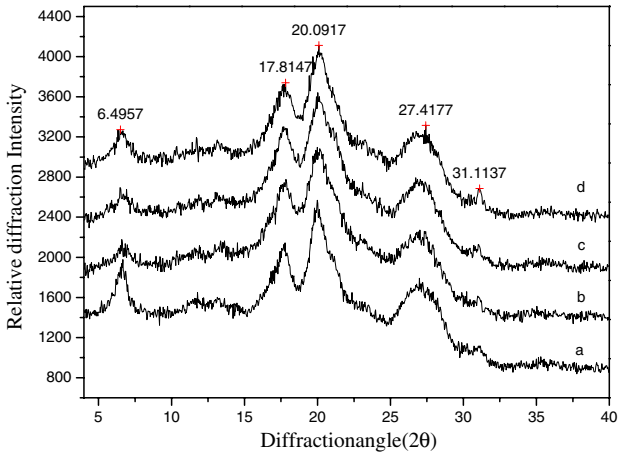

Fig. 4. X-ray diffraction spectra of the four *D. opposita* Thunb. cultivar starches: (a) *Dioscorea opposita* cv. Baiyu; (b) *Dioscorea opposita* cv. Jichengerhao; (c) *Dioscorea opposita* cv. Jiaxiangxichangmao; (d) *Dioscorea opposita* cv. jinchengyihao.

The degrees of crystallinity of four starches from different *D. opposita* Thunb. cultivars calculated from the above Figure are shown in Table 3. For this evaluation, we utilized the starches which had almost identical moisture contents, in order to minimize the effect of different moisture contents on crystallinity. Generally speaking, the higher the amylose content, the lower is the degree of crystallinity of starch. *D. opposita* cv. Baiyu starch had the lowest amylose content and the highest degree of crystallinity while *D. opposita* cv. Jiaxiangxichangmao starch showed the highest amylose content and lowest degree of crystallinity.

4. Conclusion

Starches separated from various *D. opposita* Thunb. cultivars showed significant differences in physicochemical, morphological, thermal and crystalline properties. Amylose content of *D. opposita* starches was observed to be from 20.74% to 25.94%. The four *D. opposita* cultivar starches showed different swelling powers, solubility and water-binding capacities. The shape of the four starch granules varied from round or oval to elliptical, similar to those of tuber starch granules. The average particle diameter of the four starches from different *D. opposita* Thunb. cultivars was in the range 23.39–26.87 μm. These starches showed different ΔH_{gel} values and transition temperature which were higher than the normal starches separated from corn, wheat and potato. The crystal type of the *D. opposita* Thunb. cultivar starches was a C_B-type pattern. The degrees of crystallinity of these starches varied from 32.99% to 50.52%, respectively.

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