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Studies on the physicochemical, morphological, thermal and crystalline properties of starches separated from different *Dioscorea opposita* cultivars

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Abstract

The physicochemical, morphological, thermal and crystal properties of the starches separated from four different *Dioscorea opposita* Thunb. cultivars (Taigu, Ribenbai, Wenxi and Zhongbowen) were studied. Amylose contents of *D. opposita* Thunb. starches from different cultivars ranged from 21.17% to 25.00%. The shape of starch granules separated from different *D. opposita* Thunb. cultivars varied from round or oval to elliptic or caky. The surface of the starch granules appeared to be smooth without any fissures. The average particle diameter of starches from different *D. opposita* Thunb. cultivars ranged from 25.90 to 28.06 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, T_c and ΔH_{gel} of *D. opposita* Thunb. starches ranged from 73.1 to 73.9, 77.6 to 80.4, 82.1 to 85.9 °C and 6.548 to 12.13 J/g, respectively. The crystal type of starches separated from different *D. opposita* Thunb. cultivars was a typical C-type pattern. The relative degree of crystallinity of the four *D. opposita* Thunb. cultivars starches were about 38.79%, 39.88%, 41.67% and 49.03%, respectively.

Keywords: *Dioscorea opposita* Thunb.; Starch; Physicochemical; Morphological; Thermal; Crystallinity

1. Introduction

Dioscoreae (Chinese name Shanyao), the rhizome of various species of genus *Dioscorea opposita* Thunb. (Dioscoreaceae), has been used as an important ingredient for invigorating the spleen and stomach, promoting production of the body fluids and benefiting the lung and invigorating the kidney. It has also been used as one of the important foods in the people's everyday life (Zuo & Tang, 2003). There are many *D. opposita* Thunb. cultivars in China, especially in Henan province. As reported in the literature, there are many chemical components contained in *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,

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14.5% protein, 3.48% crude fibre, 1.14% sugar, 2.26% potassium, 0.2% phosphorus, 0.2% calcium, 0.14% magnesium, 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).

In recent years, the bioactive polysaccharides from *D. opposita* Thunb. have been paid more and more attention by researchers. Zhao et al. (2003) reported the structure and antitumor activity of RDPS-Ipolysaccharide from Chinese yam. However, there have been few investigations on the properties of starch contained in *D. opposita* Thunb.. Studies of properties on *D. opposita Thunb*. starch are very important due to their ready availability and their potential extensive utilization in food and non-food applications. Starches from different sources vary, particularly in their quantitative and qualitative make-up, as well as in some of their physicochemical properties (Kaur, Singh, Sandhu, & Guraya, 2004; Singh, Singh, Kaur, Sodhi, & Gill, 2003). Therefore, the starches separated from different *D. opposita* Thunb. cultivars should also have different properties. In order to make full use of the resource of D*ioscorea opposita* Thunb. and widen its industrial application, the physicochemical, thermal, morphological and crystalline characteristics of starches separated from different *D. opposita* Thunb. cultivars were studied.

2. Materials and methods

2.1. Materials

Four different *D. opposita* Thunb. cultivars (cv.), i.e. *Dioscorea opposita* cv. Taigu (*D*. TG); *Dioscorea opposita* cv. Ribenbai (*D*. RBB); *Dioscorea opposita* cv. Wenxi (*D*. WX) and *Dioscorea opposita* cv. Zhongbowen (*D*. ZBW) 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. opposite* Thunb. cultivars were washed, cut into small pieces and ground with a plant micro-muller, and sieved with a 100 mesh sifter. After sieving, the *D opposite* 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 depositing and resuspending cycles, 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 (WBC)

WBC of starches from different *D. opposita* Thunb. cultivars was 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, which was drained for 10 min, and 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. cultivars starches (3.5 mg, dry weight) was loaded into a 40 rmul 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. opposite* 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 equation ($n\lambda = 2d\sin\theta$; where $d =$ inter crystalline spacing, $n = 1$ and $\lambda =$ 1.78901 Å). Duplicate measurements were made at ambient temperature. The degree of crystallinity of samples was quantitatively estimated, following the method of Nara and Komiy (1983). 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.

2.8. 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

Amylose content of starches separated from different *D. opposita* Thunb. cultivars ranged between 21.17% and 25.00% (Table 1). *D*. WX starch had the lowest whereas *D*. RBB starch had the highest amylose content. The amylose content observed for *D. opposita* Thunb. starches was similar to the amylose content observed for corn or potato starches (Singh et al., 2003). Moisture content of starches from different *D. opposita* Thunb. cultivars ranged from 9.76% to 12.67%, the lowest for *D*. TG and highest for *D*. WX starch. The moisture content was the inherent physicochemical properties of starch granule which was influenced by the crystalline structure of the starch granule (Cheetham & Tao, 1998). In addition, the moisture contents of starches were related to the environment temperature, humidity and other effects. The ability of the starches to swell in excess water and solubility also differed significantly. Swelling power of *D. opposita* Thunb. cultivars starches varied from 10.97% to 12.47%, whereas solubility values were in the range 10.6–11.3%. Starch swelling occurs concomitantly with loss of birefringence and precedes solubilisation. Swelling power was observed to be the highest for *D*. WX and lowest for *D*. TG starch. Highest solubility was observed for *D*. ZBW, whereas *D*. RBB starch had the lowest value. Swelling power has been reported to be influenced by strongly bonded micellar networks (Gujska, Reinhard, & Khan, 1994) and amylopectin molecular structure (Tester, Morrison, & Schuiman, 1993). A low swelling power of starches may be attributed to the presence of a large number of crystallites formed by the association between long amylopectin chains. Crystallite formation increases granular stability, thereby reducing the extent of granular swelling. WBC of *D. opposita* Thunb. cultivar starches ranged from 99.83% to 107.57%. The lowest was for *D*. RBB and highest for *D*. TG 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).

Table 1
Amylose contents, moisture contents, swelling powers, solubilities, water-binding capacities and mean diameters of starches separated from different *D. opposita* Thunb. cultivars

Samples	Amylose (%)	Moisture content (%)	Swelling power (%)	Solubility (%)	WBC (–)	Mean diameter (μm)
Dioscorea opposite cv. Taigu	23.13ab	9.76c	10.97b	10.96ab	107.57a	28.06
Dioscorea opposite cv. Ribenbai	25.00a	10.11c	11.50ab	10.6b	99.83a	26.37
Dioscorea opposite cv. Wenxi	21.17c	11.36b	12.47a	10.82b	102.78a	26.62
Dioscorea opposite cv. Zhongbowen	22.87b	12.67a	11.34ab	11.3a	103.71a	25.90

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

3.2. Scanning electron microscopy (SEM)

The scanning electron micrographs of starches separated from different *D. opposita* Thunb. cultivars showed the presence of large oval, caky or polygonal to small spherical-shape granules (Fig. 1). The SEM revealed the granule surface of starches to be smooth with no evidence of any fissures.

Four representative curves of granule diameter of *D. opposita* Thunb. cultivars starches are shown in Fig. 2. The diameter of the majority of starch granules ranged from 8 to 75, 8 to 50, 7 to 68 and 7 to 50 μm with some granules having diameters in the range 1.0–8 μm for *D.* TG, *D.* RBB, *D.* WX and *D.* ZBW, respectively. *D.* TG starch had the largest granules with a mean diameter of 28.06 μm, whereas *D.* ZBW starch

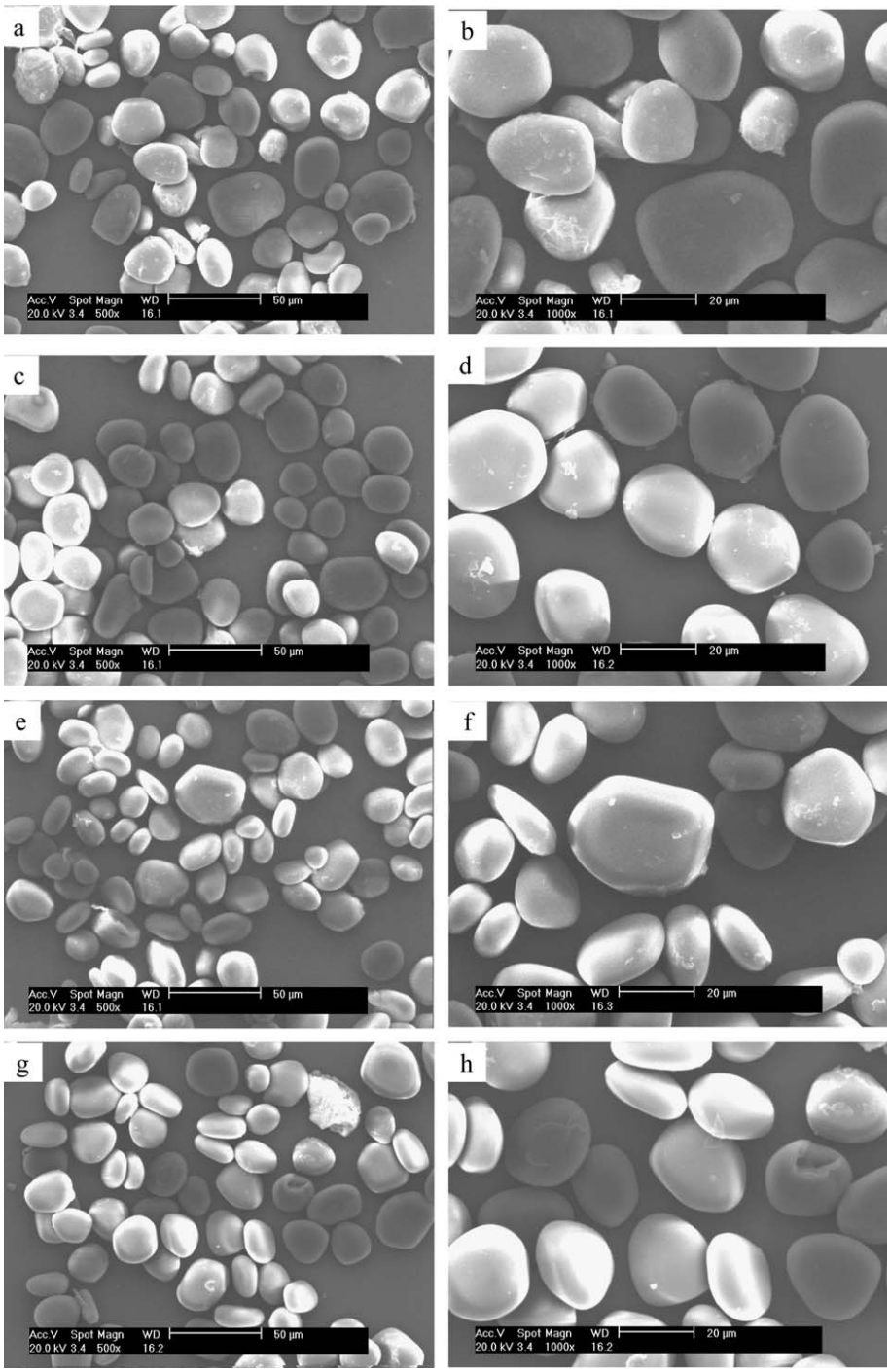

Fig. 1. SEM of the four *D. opposita* Thunb. cultivar starches: (a) *D.* TG 500×; (b) *D.* TG 1000×; (c) *D.* RBB 500×; (d) *D.* RBB 1000×; (e) *D.* WX 500×; (f) *D.* WX 1000×; (g) *D.* ZBW 500×; (h) *D.* ZBW 1000×.

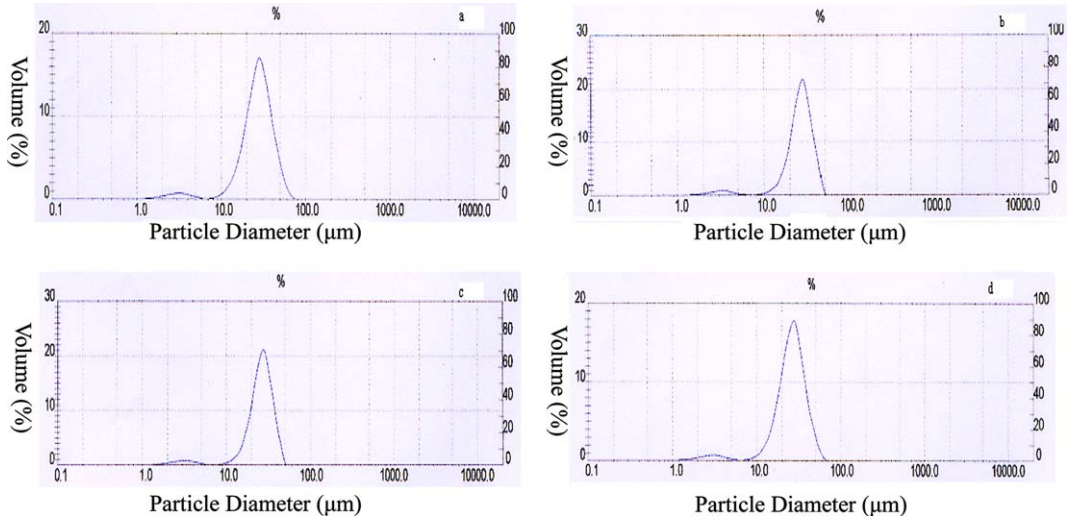

Fig. 2. Particle size analysis of the starches separated from different *D. opposita* Thunb. cultivars: (a) *D.* TG; (b) *D.* RBB; (c) *D.* WX; (d) *D.* ZBW.

had the smallest granules, with mean diameters of 25.90 μm (Table 1).

The variation in size and shape of starch granules may be due to their 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. Thermal properties of *D. opposita* Thunb. cultivar starches

DSC parameters of starches separated from different *D. opposita* Thunb. cultivars are summarized in Table 2. The transition temperatures (T_o, T_p, and T_c), gelatinization range ($T_c - T_o$), enthalpies of gelatinization (ΔH_{gel}) and peak height indices (PHI) of starches from different *D. opposita* Thunb. cultivars differed significantly. ΔH_{gel} values of *D. opposita* Thunb. starches ranged from 6.548 to 12.13 J/g. The lowest and highest ΔH_{gel} values among different *D. opposita* Thunb. cultivars were in the starches isolated from *D.* TG and *D.* ZBW, respectively. ΔH_{gel} reflected the loss of double helical structure rather than crystalline order (Cooke & Gidley, 1992). The gelatinization enthalpy values of starches have been reported to be affected by many factors, such as granule shape, percentage of large and small granules and presence of phosphate esters (Stevens & Elton, 1971; Yuan, Thompson, & Boyer, 1993). Small differences ($P < 0.05$) were observed in T_o among starches separated from different *D. opposita* Thunb. cultivars. The T_o values of *D.* TG, *D.* RBB, *D.* WX and *D.* ZBW starches were 73.1, 73.9, 73.9, and 73.5 °C, respectively. However, significant differences were observed in T_p and T_c among these four starches. T_p and T_c for *D. opposita* Thunb. cultivars starches ranged from 77.6 to 80.4 °C and 82.1 to 85.9 °C, respectively. The transition temperatures observed for different *D. opposita* Thunb. cultivars 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 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 amylopectin chain (DP, 6–11), and not by the proportions of

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. Taigu	73.1a	78.1b	84.1b	6.548d	1.31bc	11.0b
Dioscorea opposite cv. Ribenbai	73.9a	80.4a	84.6b	9.307b	1.43b	10.7b
Dioscorea opposite cv. Wenxi	73.9a	79.8ab	85.9a	7.377c	1.25c	12.0a
Dioscorea opposite cv. Zhongbowen	73.5a	77.6c	82.1c	12.13a	2.96a	8.6c

Means with the same letter are not significantly different ($P < 0.05$).
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, $\Delta H_{gel}/(T_p - T_o)$.

crystalline regions, which corresponds to the amylose/amylopectin ratio. *D*. WX starch showed the maximum *R* values PHI were narrow for the same whereas *D*. ZBW starch had the minimum *R* values and the maximum PHI. The differences in *R* value among the starches from different *Fritillaria* may be due to the presence of crystalline regions of different strength in the granules (Banks & Greenwood, 1975).

3.4. Crystalline properties of *D. opposita* Thunb. cultivar starches

Fig. 3 shows the X-ray diffractograms of starches separated from four different *D. opposita* Thunb. cultivars. The corresponding X-ray diffraction parameters and relative degrees of crystallinity calculated from the ratio of diffraction peak area and total diffraction area are listed 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.

All the four *D. opposita* Thunb. starches showed a characteristic C-type pattern, with strong reflection at 2θ about 6.5°, 17.8°, 20.1° and 27.4°. Of all the diffraction peaks, the peaks at around 2θ values of 6.5° with a *d* spacing of 15.3 Å were characteristic of the B pattern, while, at 27.4° 2θ, only one peak appeared which was indicative of the A pattern. Thus, the starches separated from the four different *D. opposita* Thunb. cultivars were classified as C-type.

The relative crystallinities of the starches followed the order: *D*. ZBW (49.03%) > *D*. WX (41.67%) > *D*. RBB (39.88%) > *D*. TG (38.79%). Generally, differences in relative crystallinity between starches could be attributed to the following: (1) crystal size, (2) amount of crystalline regions (influenced by amylopectin content and amylopectin chain length), (3) orientation of the double

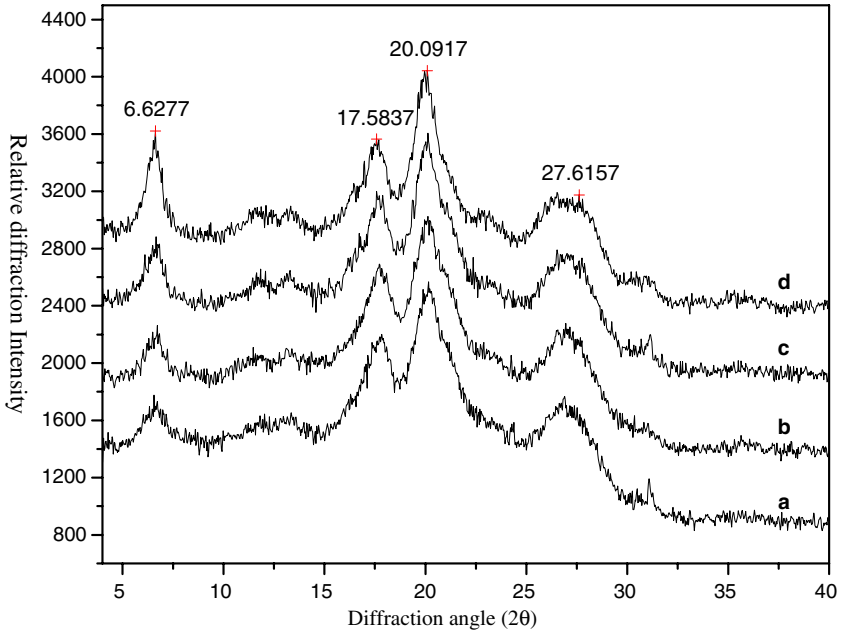

Fig. 3. X-ray diffraction spectra of the four *D. opposita* cultivar starches: (a) *D*.TG; (b) *D*. RBB; (c) *D*. WX; (d) *D*. ZBW.

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

Samples	Diffraction peaks at 2θ values (° angle)				Relative degree of crystallinity (%)
	6°	18°	20°	27°	
Dioscorea opposite cv. Taigu	6.65 (15.43 Å)	18.01 (5.72 Å)	20.23 (5.09 Å)	27.14 (3.81 Å)	38.79c
Dioscorea opposite cv. Ribenbai	6.74 (15.23 Å)	17.78 (5.78 Å)	20.14 (5.11 Å)	27.14 (3.81 Å)	39.88bc
Dioscorea opposite cv. Wenxi	6.69 (15.33 Å)	17.78 (5.79 Å)	20.14 (5.11 Å)	27.14 (3.81 Å)	41.67 b
Dioscorea opposite cv. Zhongbowen	6.69 (15.33 Å)	17.56 (5.86 Å)	20.06 (5.14 Å)	27.00 (3.83 Å)	49.03a

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

helices within the crystalline domains and (4) extent of interaction between double helices (Hoover & Ratnayake, 2002). The differences in relative crystallinity (Table 3) of starches cannot be attributed to amylose content (Since *D*. WX starch with a lower amylose content exhibited the lower relative crystallinity). Therefore, the differences in relative crystallinity could be due to all four influencing factors.

4. Conclusion

Starches separated from various *D. opposita* Thunb. cultivars showed significant differences in physicochemical, morphological, thermal and crystal properties. Amylose content of *D. opposita* Thunb. starches was 21.17–25.00%, similar to that of corn and potato starches. The four *D. opposita* cultivars starches showed different moisture contents, swelling powers, solubilities and water-binding capacities. The shape of the four starches varied from round or oval to elliptical-shaped granules, similar to those of tuber starch granules. Transition temperatures of the *D. opposita* Thunb. starches were higher than those of corn, rice, wheat and potato. The crystal type of the *D. opposita* Thunb. cultivar starches was a C-type pattern. The relative degree of crystallinity of these starches varied from 38.79% to 49.03%.

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