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Morphological, Structural and Functional Properties of Starches from Different Legume Resources

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ABSTRACT

Background: Legume is well known for its high nutrition and health care values. Considering that starch is the main carbohydrate in legume, its properties directly affect the development and utilization of legume resources.

Methods: Starches were extracted from the seeds and root tubers of five legumes. The morphological, structural and functional properties of the starches were investigated and compared using scanning electron microscopy, X-ray diffraction and attenuated total reflectance-Fourier transform infrared spectroscopy.

Result: The granules of kudzu, broad bean and pigeon pea starches were kidney-shaped with a large size, while groundnut and white pea starches were small granules with a mixture of irregularly polyhedral and spherical shape. The five legume starches had different apparent amylose contents and exhibited remarkably different crystalline properties. Among the samples, the relative crystallinity of kudzu starch was the highest, while pigeon pea starch had the highest short-range ordered degree. The swelling power and water solubility of the five legume starches were also varied. Furthermore, white pea starch is more susceptible to acid and enzymatic hydrolysis than the four other starches. The results are important for the processing and utilization of legume starches and can provide reference for the development of legume-based functional food.

Key words: Legume, Physicochemical properties, Starch.

INTRODUCTION

Legume crops are widely cultivated minor grain crops next to rice, wheat and maize. Legume crops have many species, including soybean, broad bean, pea, mung bean, red bean, cowpea and lentil. As a basic food in many countries, legume is an adequate complement to increase the protein quality of cereals because of its high protein content. Therefore, legume plays an important role in improving dietary structure and enhancing human health (Jeong *et al.,* 2019). With the improvement of living standards and the deepening understanding of health care, the increase of legume intake has attracted extensive attention, especially for populations with high prevalence of diabetes, obesity and cardiovascular diseases (Kamboj and Nanda, 2018; Wu *et al.,* 2019). Current research on legume mainly focuses on variety breeding, functional components analysis and biological health properties (Mejri *et al.,* 2018; Kamalasundari *et al.,* 2019; Shi *et al.,* 2020).

In higher plants, starch provides nutrition and energy for humans and is widely used in food processing and industries. Starch derived from different plant sources exhibits various physicochemical properties, thus determining their utilization (Guo *et al.,* 2018). The structural and functional properties of starch from commercially important cereal crops, such as rice, wheat and corn, have been extensively studied, resulting in their extensive utilization in industrial applications or food products. The properties of starch in legume determine the usage of legumes. However, legume starches have not been extensively studied and have not been widely used in the food industry (Stevenson *et al.,* 2007). The physicochemical properties of legume starch have not been systematically compared. So in the present study, starches were isolated from the seeds and root tubers of five legumes and their morphological, structural and functional properties were determined to investigate the difference of legume starches. The results will be helpful for the processing and utilization of legume starches and serve as guide for further development of legume resources and legume-based functional foods.

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MATERIALS AND METHODS

Plant materials

The experiment was conducted in Yangzhou University in 2019. The legumes selected in this study were broad bean (*Vicia faba* Linn.), pigeon pea [*Cajanus cajan* (Linn.) Millsp.], white pea (*Pisum sativum* Linn.), kudzu [*Pueraria lobata* (Willd.) Ohwi] and groundnut (*Apios fortunei* Maxim.). All the materials were provided by the Institute of Agricultural Science of the Lixiahe District in Jiangsu Province. The seeds of broad bean, pigeon pea and white pea and the root tubers of kudzu and groundnut were used to isolate the starch (Fig 1).

Starch isolation

The seeds and root tubers chopped into small pieces were soaked in 0.5% (*w/v*) sodium metabisulfite solution. The samples were homogenized in water by using an agitator and the homogenate was extruded through eight layers of gauze. The extract was filtered through 100-mesh sieves and then centrifuged at 3,000 ×*g* for 5 min. The supernatant was discarded and the top dirty layer was scraped off. The remaining precipitate was resuspended with 0.2% NaOH solution and centrifuged (3,500 ×*g*, 10 min) for several times until the supernatant was clear. The purified starch was further washed with anhydrous ethanol for 3-4 times. The purified starch was dried at 40°C, ground into powder and collected after filtering through a 100-mesh sieve.

Apparent amylose content (AAC) determination

The AAC was determined using the iodine colorimetric method. The absorbance at 620 nm was recorded using a spectrophotometer and the percentage of amylose was calculated based on the standard curve of amylose standard samples with different concentrations (amylose from potato and amylopectin from waxy maize).

Morphology observation and granule size distribution analysis

The morphology of starch was observed and photographed under a normal light microscope (BX53, Olympus, Tokyo, Japan) and field-emission scanning electron microscope (S4800, Hitachi, Tokyo, Japan) as described by Chen *et al.* (2019). Granule size distribution analysis was performed based on the micrographs by using Image-Pro Plus software.

X-ray diffraction (XRD) analysis

Approximately 200 mg of starch was placed in the groove of quartz sample table and flattened with a glass slide. The sample was detected using an X-ray diffractometer (D8 Advance, Bruker, Karlsruhe, Germany). The relative crystallinity was determined using Jade software. Each sample was quantitatively analyzed in triplicate.

Attenuated total reflectance-Fourier transform infrared (ATR–FTIR) analysis

Approximately 30 mg of dried starch was mixed with 25 μL of ultrapure water in a centrifuge tube and the suspension was scanned using a Fourier transform infrared spectrometer (7000, Varian, Palo Alto, USA) equipped with the attenuated total reflection mode. Ultrapure water was scanned as the background spectrum and the spectrum of the starch sample from 1,200 cm^{-1} to 800 cm^{-1} was recorded followed by deconvolution. The intensity of bands at 1,045, 1,022 and 9,95 cm^{-1} were measured and the ratio of 1,045/ 1,022 cm^{-1} and 1,022/995 cm^{-1} was calculated.

Swelling power and water solubility determination

A certain amount of starch (m_0) mixed with water was placed in a pre-weighed centrifuge tube (m_1) and heated in a water bath from 55°C to 95°C at 10°C intervals for 1 h. The sample was then cooled to 20°C and centrifuged at 5,000 ×*g* for 10 min. The supernatant was decanted and the weight of the residues with the centrifuge tube (m_2) was obtained. The residues were finally dried to constant weight (m_3) at 70°C. The water solubility and swelling power were calculated according to the following equations:

swelling power (g/g) =

$$(m_2 - m_1)/(m_3 - m_1) \text{ and water solubility (\%)} = [m_0 - (m_3 - m_1)/m_0] \times 100\%.$$

Hydrolysis analysis

Starch was hydrolyzed by porcine pancreatic α-amylase (PPA), *Aspergillus niger* amyloglucosidase (AAG) and hydrochloric acid (HCl) as previously described (Chen *et al.,* 2020). The starch slurry was centrifuged at 5,000 ×*g* for 5 min after each hydrolysis stage and the supernatant was used to determine glucose content by using a glucose assay kit (K-GLUC, Megazyme, Bray, Ireland) according to the manufacturer's instruction.

Statistical analysis

All data were analyzed by mean values and standard deviations. Difference analysis was conducted using Fisher's least significant difference method with the SPSS 19.0 software.

RESULTS AND DISCUSSION

Morphology, granule size distribution and apparent amylase content (AAC) of starch

Kudzu, broad bean and pigeon pea starches had similar granule morphologies and exhibited large kidney-shaped or elliptical granules (Fig 2A-C). The morphologies of groundnut and white pea starches were small granules with a mixture of irregularly polyhedral and spherical shape, but the proportions of differently shaped granules varied between these two starches (Fig 2D, E). Most white pea starch granules were truncated polyhedral with uneven surface and some oval granules were observed. The similar poroid concave in the surface of maize starch granules was also reported by Sandhu *et al.* (2004) and these micropores may be related to the source of starches.

The results of the granule size analysis of the five legume starches presented as unimodal distributions (Fig 2F-J). The kudzu, broad bean and pigeon pea starches had similar granule size range and were significantly larger than groundnut and white pea starches. The pigeon pea starch had the largest granule size with the highest minimum and average particle diameter. Groundnut and white pea starches had smaller granule size with lower minimum,

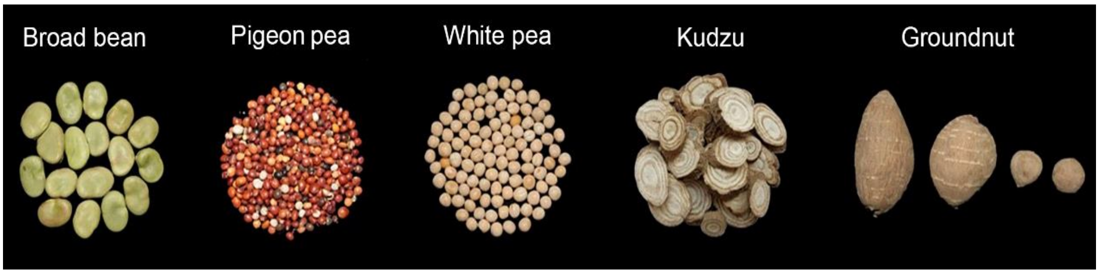

Fig 1: Morphology of seeds of broad bean, pigeon pea and white pea and root tubers of kudzu and groundnut.

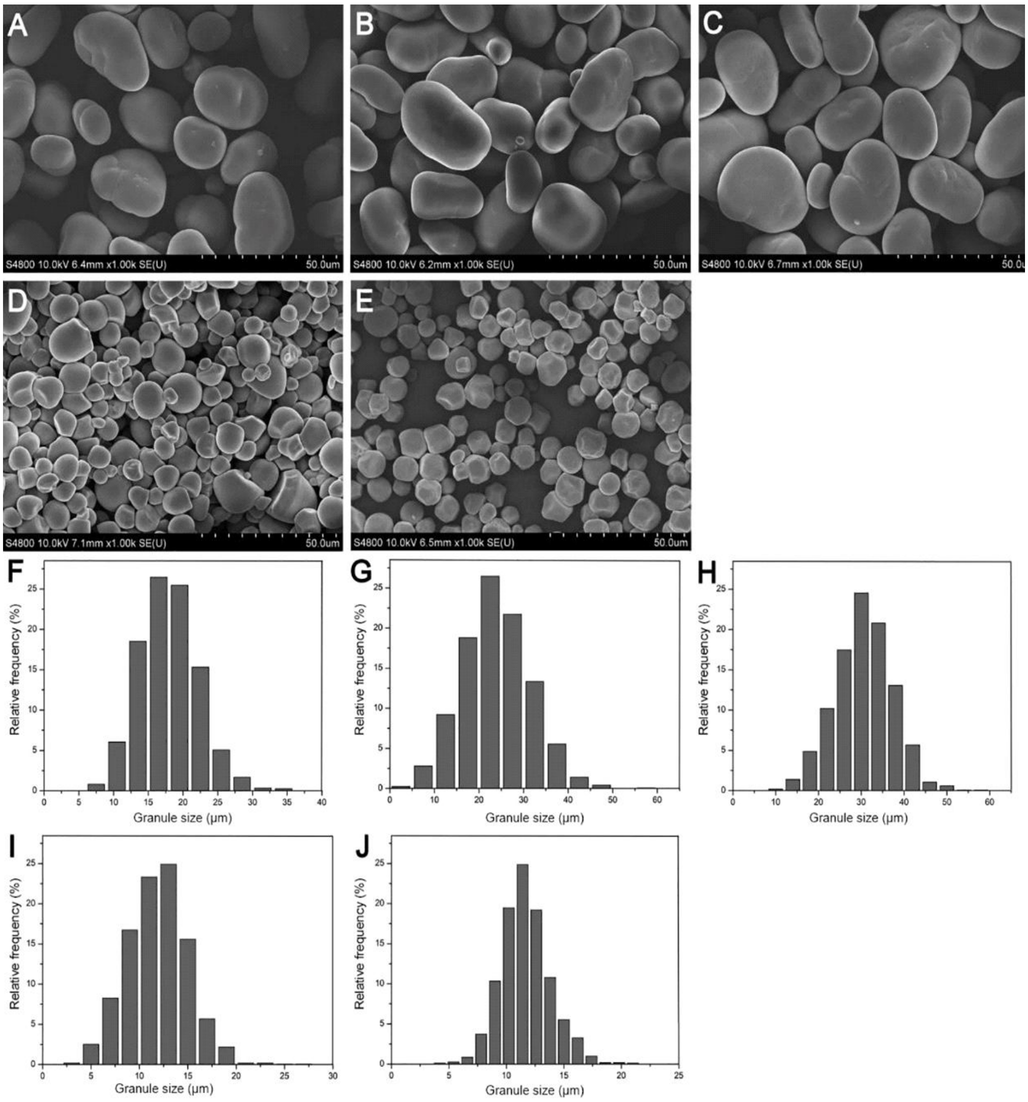

Fig 2: Morphology and granule size distribution of the five legume starches. (A-E) scanning electron microscope photograph of starch granules from kudzu, broad bean, pigeon pea, groundnut and white pea; (F-J) size distribution of starch granules from kudzu, broad bean, pigeon pea, groundnut and white pea.

maximum and average particle diameter. In addition, broad bean starch had the highest AAC, followed by groundnut and kudzu starches, whereas the AAC of pigeon pea and white pea starches was the lowest (Table 1). The differences in starch morphology, granules size and amylose content may be attributed to the different botanical origin, amyloplast biosynthetic mechanisms and plant physiological process (Sandhu *et al.,* 2004).

Crystal structure of starch

Based on the XRD spectra, the five legume starches exhibited strong diffraction peak at 2θ of approximately 17°

Table 1: Particle size and apparent amylose content of the five legume starches.

	Minimum particle size (μm)	Maximum particle size (μm)	Average particle size (μm)	Apparent amylose content (%)
Kudzu	6.80±0.16b	35.38±0.52b	17.94±0.96c	21.01±3.56c
Broad bean	2.07±0.67c	57.61±3.56a	23.82±0.52b	32.26±7.32a
Pigeon pea	11.01±0.19a	56.08±1.49a	30.51±0.87a	20.74±5.18d
Groundnut	3.35±0.15c	27.49±1.26c	11.87±0.50d	23.08±2.49b
White pea	4.03±0.24c	21.33±0.31bc	11.67±0.49d	20.66±2.15d

Data are shown as mean ± standard deviation, n = 3. Different lowercase in the same column indicate significant difference (p<0.05).

and weak diffraction peaks at approximately 5.6°, 15°, 20° and 23° (Fig 3A). The diffraction peak at 2θ of approximately 5.6° indicates B-type crystal and that at 2θ of 23° indicates A-type crystal. Therefore, the five legume starches had the characteristics of C-type crystal, which contained the characteristics of both A- and B-type crystal. Although the XRD patterns of five legume starches were similar, two shoulder peaks were observed in kudzu starch, indicating that it was a C_B-type starch, a mix of A- and B-type crystals with a high proportion of B-type crystalline structure. This result supported the findings of Hung and Morita (2007). Although no significant difference was observed in the XRD spectra, the relative crystallinities of five legume starches ranged from 15.01% to 22.00% (Fig 3B). Among the starch samples, kudzu and pigeon pea starches had the highest relative crystallinity, followed by broad bean and groundnut, whereas the relative crystallinity of white pea starch was the lowest.

The ATR-FTIR spectra of starches with various conformations show different infrared rays (IR) absorbance intensity of bands at 1,045, 1,022 and 995 cm^{-1}. The absorbance ratio of 1,045/1,022 cm^{-1} can be used to quantify the ordered degree of starch and the ratio of 1,022/995 cm^{-1} can reflect the proportion of amorphous to ordered carbohydrate structure in starch (Zhu *et al.,* 2017). The ATR-FTIR spectra of five legume starches exhibited similar resonance peaks (Fig 3C). However, the IR ratio of 1,045/1,022 and 1,022/995 cm^{-1} ranged from 0.63 to 0.74 and 1.04 to 1.42, respectively (Fig 3D). Among the five legume starches, the IR ratio of 1,045/1,022 cm^{-1} was the highest in pigeon pea starch and the lowest in kudzu starch, indicating that pigeon pea starch had the highest short-range ordered

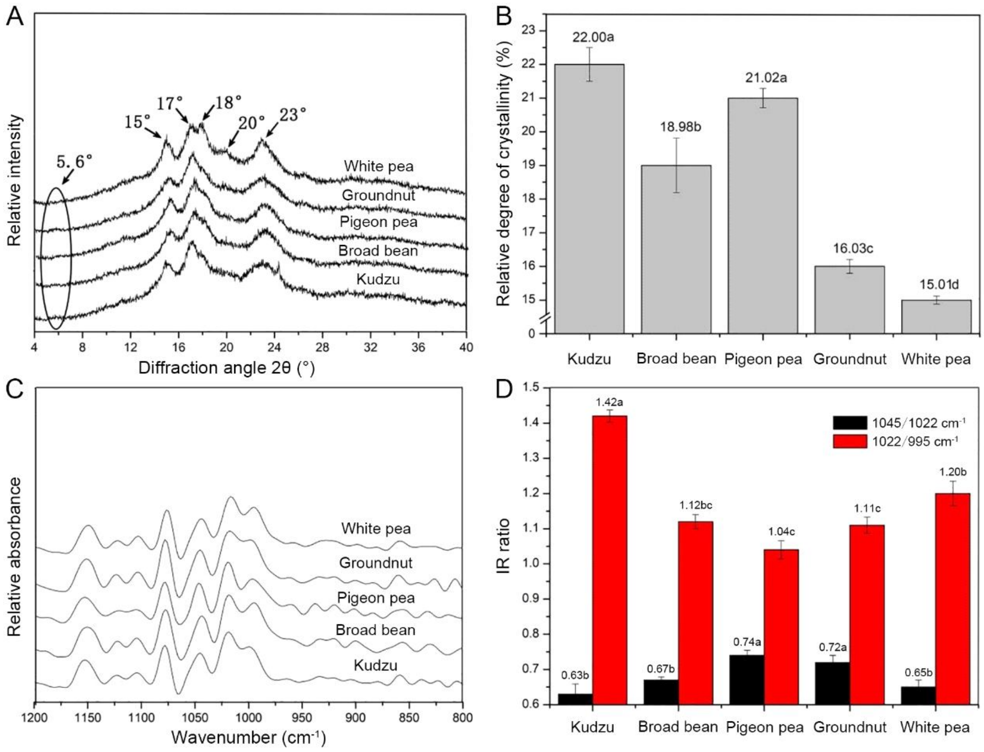

Fig 3: Crystal structure analysis of the five legume starches. (A) XRD spectra; (B) relative degree of crystallinity; (C) ATR-FTIR spectra; (D) IR ratio. The arrows in (A) represent peaks at 2θ of approximately 5.6°, 15°, 17°, 20° and 23°. Numbers with different lowercase above histogram indicate significant difference at a p<0.05 level.

degree, whereas kudzu starch had the lowest short-range ordered degree. The IR ratio of 1,022/995 cm^{-1} in kudzu starch was the highest, showing its highest proportion of amorphous structure.

Swelling power and water solubility of starch

The solubility and swelling power of five legume starches increased with the increase of temperature. The swelling power of pigeon pea starch rapidly rose at 75°C but for other legumes it was at 65°C (Fig 4A). A sharp increase in water solubility was observed in the four legume starches (kudzu, broad bean, pigeon pea and white pea, except groundnut) from 65°C to 85°C (Fig 4B). The similar tendency of swelling power and water solubility in legume starches was also been reported (Yadav *et al.,* 2011). At 95°C, the swelling power and water solubility of five legume starches varied. White pea starch had the greatest swelling power, while kudzu starch had the highest water solubility. The swelling power of starch indicates the water absorption capacity and the water solubility reflects the dissolution degree of starch during gelatinization (Falade and Okafor, 2013; Guo *et al.,* 2018). Swelling power and water solubility of starch are affected by several factors, including starch granule size, amylose content, protein and lipid content, amylopectin fine structure and crystalline structure (Huang *et al.,* 2015). The difference in swelling power and water solubility among these five legume starch samples may be attributed to their significantly different morphology and structural properties.

Hydrolytic properties of starch

The dynamic hydrolysis of the five legume starches was studied. The five legume starches exhibited rapid hydrolysis at the early stage, followed by a gradual reduction of hydrolysis rate and a gentle trend of hydrolysis. However, their susceptibilities to acid and enzymatic hydrolysis significantly differed. All five legume starches exhibited rapid hydrolysis rates on PPA hydrolysis from 0 h to 12 h. White pea starch had the highest hydrolysis degree and pigeon pea starch had a markedly less hydrolysis degree (Fig 5A). On AAG hydrolysis, kudzu starch showed relatively rapid rate at the initial phase from 0 h to 12 h, whereas the rapid hydrolysis stage of the four other legume starches occurred from 0 h to 24 h. The hydrolysis degree of white pea starch was higher than that of the four other legume starches from 24 h to 48 h (Fig 5B). Hence, white pea starch was easily hydrolyzed by enzymes and might be conducive to human absorption. On HCl hydrolysis, the hydrolysis degree of five legume starches gradually increased with increasing hydrolysis time. The difference in hydrolysis degrees of the five legume starches indicated that white pea and pigeon pea starches presented the lowest and highest resistant ability to the acid, respectively (Fig 5C). Starch hydrolysis is complex and can be influenced by numerous factors, such as granule shape, size, surface area, integrity, porosity, structural heterogeneity, crystallinity and component (Blazek and Gilbert, 2010). In this study, the five legume starches had significant difference in terms of amylose content and

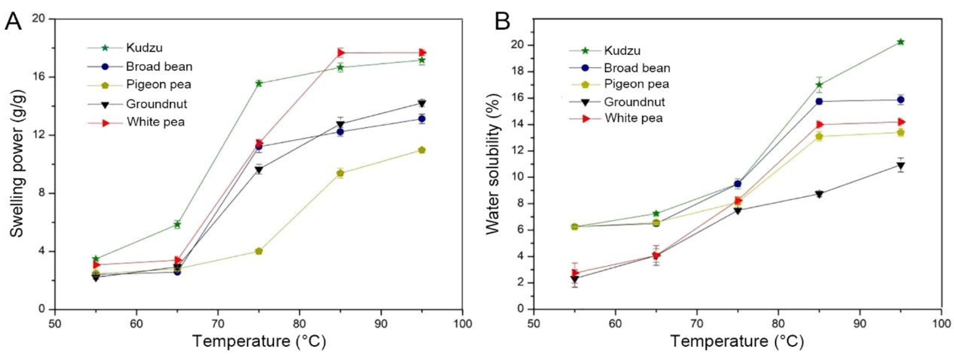

Fig 4: Swelling power and water solubility of the five legume starches. (A) Swelling powers; (B) water solubility.

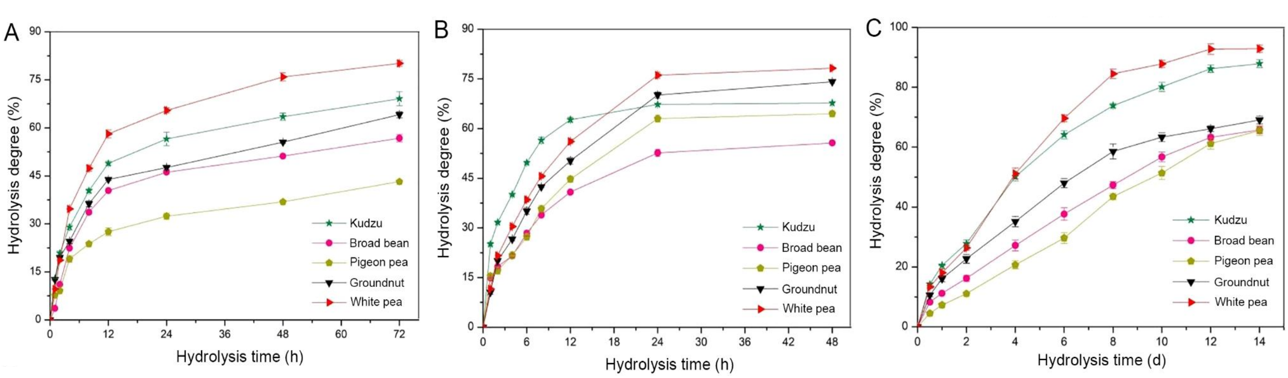

Fig 5: Hydrolysis analysis of the five legume starches. (A) PPA hydrolysis; (B) AAG hydrolysis; (C) HCl hydrolysis.

morphological and structural properties, resulting in different hydrolysis properties.

CONCLUSION

The physicochemical properties of starch isolated from five legumes were compared. The granules of kudzu, broad bean and pigeon pea starches were kidney-shaped with a large size, while groundnut and white pea starches were small granules with a mixture of irregularly polyhedral and spherical shape. The AAC of broad bean starch was the highest, followed by groundnut and kudzu starches, whereas pigeon pea and white pea starches had the lowest AAC. The five legume starches exhibited the characteristics of C-type crystal, but significant differences were detected in terms of relative crystallinity and short-range ordered structure. Kudzu starch had the highest relative crystallinity, pigeon pea starch had highest short-range ordered degree and kudzu starch had high swelling power and water solubility. Moreover, white pea starch is more susceptible to acid and enzymatic hydrolysis than the four other starches. The results may provide references for the processing and utilization of legume starches in food and nonfood industries.

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