Morphological Structure, Physicochemical Properties Analysis and Application in Processing Vermicelli of the Kidney Bean Starch Grown in Qianjiang, China

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Abstract: The structure, morphology, physicochemical properties and potential use in vermicelli of kidney bean starch, grown in Qianjiang, China, were carried out. Results pointed out that the scanning electron micrographs revealed the presence of kidney or ovoid- to globose-shaped granules and with a diameter ranging from 5 to 50 μm. And the kidney bean starch exhibited an A-type X-ray diffraction pattern. The freeze-thaw stability was poor that the rate of water release was only 41.90%. But the thermal stability and cold stability were good and it showed a C- type Brabender viscosity curve. Besides, the kidney bean vermicelli was prepared by the optimized process. The viscosity and swelling of kidney bean vermicelli were less than mung bean vermicelli, which lead to the acceptable unable to equal to mung bean vermicelli. But considering the price and big cooking loss, weak tensile strength and weak shear strength of other starch vermicelli, the kidney bean starch vermicelli still has important application value.
Key words: Kidney bean starch; Morphology structure; Physicochemical properties; Properties of kidney bean vermicelli

1. Introduction

Legumes were widely grown and played an important role in the human diet of developing countries owing to their high protein and carbohydrate contents. Kidney bean (*Phaseolus vulgaris L.*), as one species of food legume, was widely planted in the west of China, which the planting area was second only to India and Brazil [1]. It was an underutilized beans resources in high carbohydrates that was produced at an altitude of 1400m above Chongqing Qianjiang, China [2]. It was also regarded as an important food resource in the west of China.

Starch and its products are important sources of carbohydrates and act as a primary source of calories [3]. Starch from different sources could affect food in deep processing on the quality of viscosity, hardness and chewiness [4,5]. And in the processing, the stirring, mixing, conveying and energy loss of the raw materials are also closely related to the rheological properties of the starch paste, such as the granule size had been reported to influence the baking properties and the pasting behaviour of starch [6]. In the legume starch, amylose is the majority and its content have obvious influence on starch solubility, gelatinization characteristics and thermal properties. Traditionally, mung bean starch is regarded as the best for all kinds of starch vermicelli because of its' high amylose content and suitable amylopectin length [7]. And the mung bean vermicelli has the advantages of good taste, little cooking loss, low breaking rate and high tensile strength [8]. Previous studies have shown that Qianjiang kidney bean also had a higher content of amylose. Therefore, the structure, physical and chemical properties of kidney bean starch and the quality characteristics of kidney bean vermicelli were studied to explore the potential use of kidney bean starch as a raw material for vermicelli processing.

2. Materials and methods

2.1 Materials

Kidney bean (*Phaseolus vulgaris L.*) landrace, cultivated in the Santangga plantation, with altitude above 1400 m, Qianjiang district, Chonqging city, China, located at latitude of 29°04′29″–29°52′10″N and longitude of 108°28′04″–108°56′56″E. All chemicals used were of analytical grade except the potassium iodide which was of spectroscopically pure.

2.2 Starch isolation

Starch granules were isolated from kidney bean according to the method of Wagner [9] with some modifications. The kidney beans (50g) were soaked in 0.45 g/100mL sodium sulfite solution (150mL) and settled overnight. Using a micro-mixer grinding slurry and then filtered through a 53 -μm-mesh sieve to separate the fibre. The filtered slurry was then centrifuged at 3000 rpm for 30 min at 4°C. The aqueous phase obtained on centrifugation was collected for the recovery of proteins, whereas the sediment obtained was scraped off from the surface and the lower white portion was recovered as starch. The starch was dried at 40℃ in a hot air oven (DHG-9140A, Shanghai Qixin Scientific Instrument Co., Ltd, China) for 48h and through a 100mesh sieve.

2.3 Preparation of kidney bean vermicelli

Starch and water were mixed at 1:10 and gelatinized for 80s in boiling water at 100℃ for getting the starch paste. Add warm distilled water (50℃) in the starch until the water content up to 40% and add 4% starch paste. Preserve heat at 50℃ for 25min to get the dough. Then transfer the dough to injector (2mm aperture) of 50mL in time. Squeeze the dough into the boiling water with keeping about 20mm in height from orifice to the surface of water. After about 8-15s, fish out the vermicelli when it floating on the surface of water and then cooling for 15min at the cold water. Arrange the cooled vermicelli into bundles and put them on the shelf. Then freeze at 4℃ for 6h and followed by -2℃ for 6h. Finally, dry them at 40℃ for 6h in the hot air oven until the water content down to about 10%.

2.4 Starch granule morphology and optical performance

Starch granules were examined using the S-4800 field emission scanning electron microscope (S-4800, Hitachi Co., Ltd, Japan). The granules were mounted on an aluminium stub using double-sided adhesive tape. The stubs were coated with gold and the starch was viewed at an accelerating voltage of 3 kV. The optical performance was estimated using the OLYMPUS microscope (BX43, Olympus Co., Ltd, Japan). Starch granule (0.3g) was mixed with 10 mL distilled water and then stirred for 30min. And the sample was removed to the slide and viewed at the mode of ordinary light and polarized light respectively.

2.5 X-ray diffraction (XRD)

X-ray diffraction (XRD) patterns of the starch samples were obtained using an X-ray powder diffractometer (D8 ADVANCE, Brook Co., Ltd, German) at 40 KV and 100 mA. The intensity was measured from 4 to 40° as a function of 2θ and at a step size of 0.02°. The crystalline and amorphous areas were calculated according to the method of Kong[10]. Crystallinity was calculated as follows:

$$\text{Degree of crystallinity (\%)}=A_c/\ (A_c + A_a) \tag{1}$$

where A_c is total area of ten crystalline peaks, and A_a is amorphous area on the diffractogram.

2.6 Swelling power and solubility index

Swelling power and water solubility index were determined according to the method of Yang [11] with some modifications. The starch (0.5 g, dry weight basis) was weighed directly into screw-cap centrifuge tubes, and 40 mL distilled water was added to each. The capped tubes were then placed on a vortex mixer for 10 s and incubated in 50, 60, 70, 80 and 90°C water baths for 30 min with additional mixing by vortex at 5 min intervals. The tubes were then cooled to room temperature in an ice water bath and centrifuged at 3800rpm for 20 min, after which the supernatant was removed with suction. The supernatant was dried to constant weight (W_1) in an air oven at 100°C. And the material adhering to the wall of the tube was considered sediment and weighed (W_2). The solubility index (SI) and swelling power (SP) were calculated as follows:

$$SI=W_1/0.5 \times 100\% \tag{2}$$

$$SP=W_2/\ (0.5-W_1) \times 100\% \tag{3}$$

2.7 Light transmittance and sedimentation characteristics

The determination method of the transparency of the kidney bean starch was following and improving the method of Ren [12]. An aqueous starch suspension (1% dwb) was prepared by heating at 100°C in a water bath (HH-6, Jintan Fuhua Instrument Co., Ltd, China) for 15 min with constant stirring. The suspension was cooled to 30°C. the sample were stored for 10 hours and the transmittance was determined every 2 hours by measuring absorbance at 620 nm against a water blank with a UV spectrophotometer (UV-2450, Shimadzu, Japan).

The sedimentation characteristics was examined by the method of Xu [13] with few modifications. Starch suspensions (1%, w／w dwb) were heated for 20 min in a boiling water bath and cooled to the room temperature. And the sample was put into 100 mL measuring cylinders and stood for 10 hours at 4°C. The supernatant volume was determined every 2 hours.

2.8 Pasting properties

Pasting properties of kidney bean starch samples were determined using a Rapid Visco-Analyzer (RVA) according to approved method. Kidney bean starch (2 g) was added to 100 ml deionized water and took out of 10 ml starch slurries in RVA canister. The starch slurries were held at 50°C for 1 min before heating from 50 to 95°C at a rate of 10°C /min and held at 95°C for 2.5 min. The slurry was then cooled at rate of 10°C /min to 50°C and held for 2 min [14]. Parameters recorded were pasting temperature, peak viscosity, trough viscosity, final viscosity (viscosity at 50°C), breakdown viscosity, setback value, and peak time.

2.9 Freeze-thaw stability

Freeze–thaw stability was determined by the method of Hoover [15] with few modifications. Aqueous starch slurry (3%, w/v dwb) was heated in a boiling water bath for 20 min and cooled to the room temperature. Then the gels were frozen at -20~-15℃ for 24 h. The gels (6% db) were subjected to cold storage at 4℃ for 16 h (to increase nucleation) and then frozen at -16℃. To measure freeze-thaw stability, the gels frozen at -16℃ for 24 h, were thawed at 25℃ for 6 h and then refrozen at -16℃. Five cycles of freeze-thaw were performed. The excluded water was determined by centrifuging the tubes at 3000rpm for 20 min after thawing.

2.10 Cooking quality and texture characteristics of vermicelli

The cooking loss and cooked weight were tested by the method of Yadav [16] with few modifications. Sample was weighed (W_0) and dried at 105℃ for 4 hours under normal pressure, and weighed the dry matter(W_1). Boiling the sample for 15min in 100ml distilled water with gently stirring. Then taking out the vermicelli, cooling them and absorbing the water on the surface and weighing (W_2). Finally, dried them for 4h at 105℃ and weighed (W_3). The swelling and cooking loss were calculated as follows:

$$\text{Swelling (\%)} = (W_2/W_3) \times 100\% \tag{4}$$

$$\text{Cooking loss (\%)} = (W_1 - W_3)/W_1 \times 100\% \tag{5}$$

The breaking rate (BR) was tested by the method of Li [17] with few modifications. 20 of kidney bean starch vermicelli of about 10 cm were boiled in 500 ml distilled water for 50 min, and count the total number (X), the breaking rate (BR) were calculated as follows:

$$BR = (X - 20)/20 \times 100\% \tag{6}$$

Texture characteristics analysis of kidney bean starch vermicelli was performed as described by Li [18] with minor modifications. 10 of vermicelli (10cm) were boiled in 200 distilled water for 10 min and cooled to room temperature and placed them in distilled water until ready to test. The hardness, deformation, peak strain, viscosity and elasticity were measured and recorded by the CT3 texture analyser (Brookfield Co., Ltd, America) with TA7 detector.

2.11 Statistical analysis

All the analysis was determined in triplicates and Statistical analysis was performed using Excel and Origin8.5 software and expressed as mean ± standard deviation.

3. Results and discussion

3.1 Morphological properties

The polarized light microscopic and light microscopic images of kidney beans starch are presented in Fig. 1. There is obvious polarized cross in the starch granules with the polarization cross point in the centre location shown a state of black cross. The appearance of polarized cross is a performance of highly ordered expression of starch granules. After the processing of starch, water molecules entered into the starch granules and the internal crystalline structure of starch granules was destroyed that caused the disappearance of the birefringence of starch granules [19]. Therefore, the change of the cross section of starch can reflect the change of its crystalline structure and judge the gelatinization degree of starch to a certain extent.

The typical scanning electron micrographs of isolated kidney bean starch granules are presented in Fig. 2. Many of the physicochemical properties of starch, such as swelling ability, water binding capacity, gelatinization and gelation ability are related to the average particle size of starch reported by Puncha-arnon [20]. And the larger the particle size, the stronger the water holding capacity with the worse the elasticity and chewiness of starch [21]. In Fig. 2, most of starch granules are showing an oval-shaped, part is the kidney-shaped that similar to the shape of kidney beans. The size of kidney bean starch granules measured by electron microscope scale are about 5-50 μm equal to the mung starch (4-74 μm), but lower than that of wheat, corn, cassava and potato [22]. The morphological properties show that the expansion and water holding capacity of the kidney bean starch are relatively weak, but the processed products have better elasticity and chewiness.

3.2 X-ray diffraction analysis

The X-ray diffraction pattern of kidney bean starch is shown in Fig. 3. According to the classification of X-ray diffraction spectroscopy, starch crystal structure can be divided into A, B and C-type. The crystal type of most legume starch belongs to type C, such as broad bean, lentils and chickpeas while the cow gram and part of mung bean belong to type A. In the Fig. 3, the kidney bean starch displayed "A" type diffraction pattern with peak intensities observed at 15.45, 17.19, 18.02 and 23.20° that are comparable with the findings of Hoover [23] on part of mung bean starch. It shows that the kidney bean starch has a tight structure, a high crystallinity and it’s hard to be influenced by external force and chemical reagent.

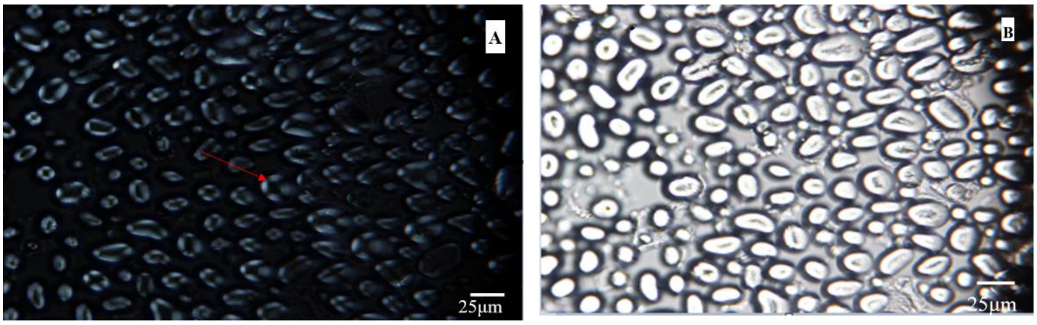

Fig. 1 Polarized light microscopic and light microscopic images of kidney beans starch

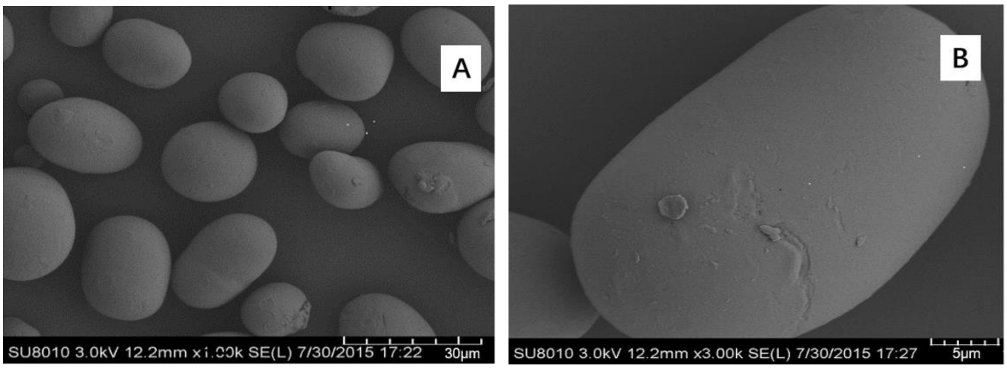

Fig. 2 Typical scanning electron micrographs of isolated kidney bean starch granules: (A image was at magnification level of 1000×. B image was at magnification level of 3000×.)

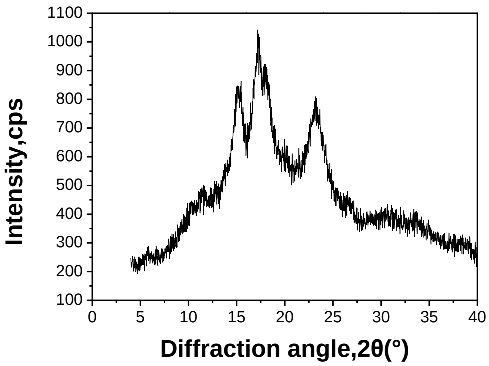

Fig. 3 X-ray diffraction pattern of kidney beans starch granule

3.3 Swelling power and solubility

Swelling power of the kidney bean starch ranged from 3.0 to 13.37 %, where solubility varied from 3.0 to 34.4 % within the temperature range of 55 - 95℃ as shown in Fig. 4. The trend showed by the curves related to swelling power and solubility of starches was found similar upon increase in the temperature. The solubility and swelling power of starch can reflect the swelling and paste in the process of cooking and it related to the size, morphology and composition of starch, the ratio of amylose and amylopectin, and the proportion of long chain or short chains in amylopectin [24]. Therefore, the experimental results show that the deep processing products of kidney bean starch may have the better cooking characteristics, especially in vermicelli products.

3.4 Light transmittance and sedimentation characteristics

Transmittance reflects the mutual solubility and swelling power of starch and water. Transparency of starch paste are closely related to the content of amylose starch and phosphorus also to starch granule structure. The higher the amylose content, the tighter the association of starch molecules, and the lower light transmittance. The initial transmittance of kidney bean starch was 10.3% and decreased gradually as the time went by. When placed for 60h, the transmittance decreased to 7.1% (in Fig. 5), which lower than that of lentil starch (54.85%), corn starch (21.25%), sweet potato starch (20.45%) and mung bean starch (15.85%) [25]. It can be concluded that the kidney bean starch is easy to gelatinization and aging. And the processing characteristics of kidney bean starch are similar to that of mung bean starch.

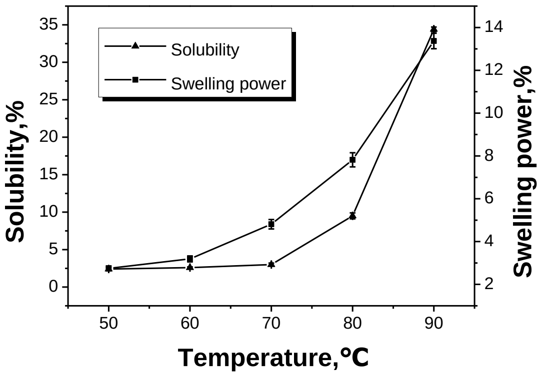

Fig. 4 Curves of solubility and swelling power in different temperatures of kidney bean starch

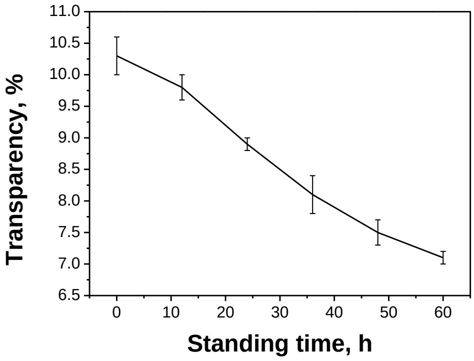

Fig. 5 Transparency of kidney beans starch

The volume ratio of the supernatant of kidney bean starch slowed down after the rapid increase (in fig. 6) which indicated that the retrogradation of kidney bean starch was fast and the starch was prone to aging. Starch paste had a high rate of sedimentation, which might be related to its high amylose content or moderate relative molecular weight. The ratio of amylose and amylopectin of kidney bean starch was 0.69 which was slightly higher than that of pea starch (0.48~0.54) and mung bean starch (0.5) but much higher than that of potato starch (0.33) and corn starch (0.33) [24,26-27]. The comparison showed that the retrogradation characteristics of kidney bean starch were similar to that of pea starch and mung bean starch. The preparation of vermicelli and other starch deep processing products was the use of starch retrogradation mechanism. Therefore, kidney bean starch is suitable for the vermicelli production.

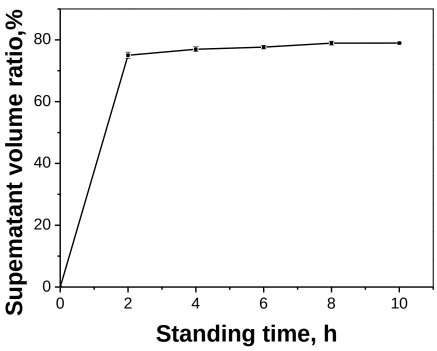

Fig. 6 Coagulation sink rate of kidney beans starch

3.5 Pasting properties

Pasting properties provide imminent information about the cooking behaviour of starches during heating and cooling cycles [28]. As the RVA viscograms are shown in Fig. 7, there was no obvious viscosity peak which showed a C- type Brabender viscosity curve. And the viscosity of the kidney starch was found to increase continuously in the temperature of 50 ℃ to 95 ℃, which indicated that it had good thermal stability and cold paste stability.

The pasting characteristic parameter of kidney bean starch paste are shown in Table 1. Pasting temperature is the minimum temperature required to cook the starch. The kidney bean starch showed a higher pasting temperature of 89.6℃ than lima bean (83℃) and mung bean (73.2℃) [29]. The high pasting temperature of kidney bean starch indicates the higher resistance of starch granules towards the swelling, which may be attributed to the higher amylose content and the tighter granular structure. It was reported that the higher the amylose content, the slower the swelling of starch particles because amylose can limit swelling properties by decreasing amylopectin concentration and/or forming helical complexes with lipid molecules, especially phospholipids [30]. The setback value was the difference between the two kinds of viscosity that one was the new molecular chain structure of starch that rearranged during aging and another was peak viscosity. In general, when the setback value is negative, the product has a high viscosity and when the setback value is positive and increasing, the product will become harder and rougher [31]. Thus, the deep products of kidney bean starch may be hard and rough owing to the high setback value. It was Reported by Hong [32] that the limited swelling, the high amylose content, the C-type Brabender viscosity curve of starch was three conditions for making the better vermicelli. Thus, the kidney bean starch is an excellent raw material for making vermicelli.

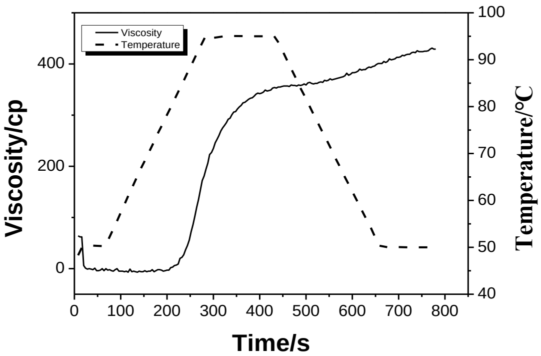

Fig. 7 RVA viscograms of isolated kidney beans starch

Table 1 Pasting characteristics parameters of kidney bean starch

PV (cp)	Trough (cp)	BDV (cp)	FV (cp)	SV (cp)	PT (min)	T_S (℃)
378±4	350±3	27±0.34	442±5	80±2	6.93±0.29	89.57±0.35

PV, peak viscosity; Trough, trough viscosity; BDV, break down viscosity; FV, final viscosity; SV, Setback value; PT, peak time; T_S, swelling temperature

3.6 Freeze-thaw characteristics

The freeze-thaw stability reflects the ability of starch paste to withstand severe physical changes such as freezing, melting, and so on [33]. It was determined by the estimation of the amount of water (% w/w) exuded from the kidney bean starch gel system stored at low temperatures [34]. The results showed that the average water release rate of kidney bean starch was 41.9%, which was similar to that of mung bean starch (41.26%), much higher than that of corn starch (0.37%) and sweet potato starch (4.41%). Therefore, kidney bean starch is not suitable for processing frozen foods which is similar to most bean starch.

3.7 Cooking properties and texture characteristics of kidney bean vermicelli

Mung bean vermicelli was often used as reference to compare the quality of vermicelli [35]. The results of the previous experiments showed that the characteristics of the kidney bean starch were similar to that of the mung bean [36] starch. Therefore, the vermicelli of kidney bean and mung bean were produced by the optimized process, and the cooking quality and texture of the two kinds vermicelli were compared and analysed. The results are showed in Tab. 2. The breaking rate and boiling loss rate of the two kinds samples were lower compared with other vermicelli products, which was consistent with the related quality of vermicelli reported by Sandhu [5]. The swelling degree of kidney bean vermicelli was much lower than mung bean vermicelli, which indicated that kidney bean vermicelli was more resistant to cooking and not easy to paste soup, but its smoothness was less than that of mung bean vermicelli. Texture analysis showed that the hardness, elasticity and shear deformation of kidney bean vermicelli were higher than that of mung bean vermicelli, which also indicated that the kidney bean vermicelli was resistant to cooking and chewing [18,37]. The adhesive force of kidney bean vermicelli was less than that of mung bean vermicelli, indicating that it was difficult to bond [38], which might lead to poor taste and affect the acceptability. Kidney bean vermicelli has the higher hardness, elasticity and the less cooking loss, which indicated that kidney bean starch can be used for vermicelli production. But the viscosity and swelling of kidney bean vermicelli are less than mung bean vermicelli, the acceptable can't equal to mung bean vermicelli.

Table. 2 Comparative analyses on cooking quality and mechanical properties of vermicelli of kidney bean and mung bean. [a]

Sample	Breaking rate (%)	Swelling degree (%)	Loss of boiling water（%）	Hardness (g)	Elasticity	Shear deformation	Adhesive force (g)
Kidney bean	0.67±0.58a	425.87±0.67b	1.94±0.20b	24.00±2.00a	0.34±0.02a	0.38±0.03a	6.67±1.15b
Mung bean	0.33±0.58a	518.09±3.82a	2.02±0.02a	17.33±1.15b	0.23±0.02b	0.27±0.01b	11.33±1.89a

[a] Mean followed by same letter in a column do not differ significantly ($P \geq 0.05$), n = 3 (±SD).

4. Conclusions

The starch particle presented the kidney or ovoid- to globose-shaped granules and with a diameter ranging from 5 to 50 μm. And the kidney bean starch exhibited an A-type X-ray diffraction pattern. It also had good thermal stability and showed a C-type Brabender viscosity curve. And the solubility and swelling degree of it were small. The freeze-thaw stability of kidney bean starch was poor, and the rate of water evolution is 41.90% which indicated that it was not suitable for the production of quick-frozen food. The studies on morphological structure and physicochemical properties of kidney bean starch showed that Qianjiang kidney bean starch were suitable for the production of vermicelli.

Qianjiang kidney bean vermicelli has a higher hardness, elasticity and a less cooking loss, which indicated that kidney bean starch can be used for vermicelli production. The viscosity and swelling of kidney bean vermicelli are less than mung bean vermicelli, so the acceptable of kidney bean vermicelli unable to equal to mung bean vermicelli. But considering the high price of mung bean starch, and the big cooking loss, weak tensile strength and weak shear strength of the vermicelli made of other starch material, the application of kidney bean starch in vermicelli processing still has important application value. Further study will focus on improving the quality of kidney bean vermicelli.

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