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Morphological, structural, pasting, thermal and functional characteristics of starches from chestnuts produced in different cultivation regions in China

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Running title: Physicochemical properties of chestnut starches

ABSTRACT

This study was to compare the characteristics of 21 starches isolated from chestnuts produced in different regions in China, also to investigate their potential food applications. Starches were isolated from chestnuts under the *Castanea mollisima* Blume variety with sub-varieties of *Banli, Youli,* and *Maoli*. Several properties of the starch samples were examined, including the moisture content, amylose content, morphological characteristics, color values, light transmittance, syneresis, swelling power, water solubility index, pasting properties, FTIR characteristics, X-ray diffraction (XRD) patterns, and thermal properties. The results showed that starches isolated from *Youli* presented higher resistance to shear and swelling during heating, indicating more suitability for high-heat cooking. The FTIR spectra confirmed the polysaccharide nature of all the chestnut starches. The XRD patterns showed most chestnut starches were in C_b-type, while only five were in C_a-type. Overall, this comparative study would be relevant for the further exploration of the potential utilization of chestnut starch in both food and non-food industries.

Key words: chestnut starch; morphology; physicochemical properties; RVA; DSC

1. Introduction

As the most common carbohydrate in human diets, starch serves as a major energy source and can be found in large amounts in staple foods such as potato, corn, rice, and wheat. Most commercial starches are isolated from these crops through wet milling process. The isolated starches have much utilization as both nutritional food ingredients and functional additives in foods. For instance, based on their characteristics, starch can be used as stabilizers, gel formers, emulsifiers, and thickening agents. In addition, starches can be used to produce sweeteners (e.g., sugars, syrups, and sugar alcohols) and ethanol. Moreover, starch is widely used in non-food applications, e.g., in papermaking, pharmaceuticals, and even mining industries. Therefore, native starch is in high demand for meeting the global needs for the production of various goods, which raises the significance of exploring more botanical sources for starch.

While properties of mainstream starches (e.g., potato and corn starches) have been extensively studied, few have been focusing on chestnut starch, especially a comparative study of that from different regions. The present study was to examine the physicochemical properties of starches isolated from chestnuts that were harvested in different regions in China. As an important food crop cultivated in many parts of the world, chestnut is a group of fruits belongs to the genus *Castanea* spp. in the family of Fagaceae, with major sub-species include *Castanea mollissima* in China, *C. crenata* in Japan, *C. dentata* in North America, and *C. sativa* in Europe [1, 2, 3, 4]. It is widely distributed and consumed in Asia, America, and Europe, with an increasing world production during the last few decades. China serves as one of the top producers of chestnuts according to the Food and Agriculture Organization of the United Nations [4]. Under the variety of Chinese *C. mollissima*, chestnuts are commonly classified into three sub-groups based on their morphological characteristics, which are *Banli, Youli*, and *Maoli* in their common names. *Banli* has an overall large size and flat shape, with a darker mat and unpolished skin, and is the most common chestnut variety in China. *Youli*, on the other side, presents a relatively smaller size and round shape, and its skin appears deep brown with shiny and glossy surface. *Maoli* has the smallest size with a hemispherical shape, and its fruit is less plump compared to *Banli* [5]. Unlike other culinary nuts, chestnuts have very low fat and protein content. Their calories come chiefly from carbohydrates, with almost twice as much starch as in potato on a dry basis [6]. According to studies conducted by Liu, Wang, Chang, and Wang [7] and Moreira, Chenlo, and Arufe [8], the total starch content of chestnut fruits (varieties of *C. mollissimia*) range from 42.4% to 53.8%. Due to its relatively high starch content, it is expected that chestnut quality is highly associated with its starch properties. Further, the chestnut starch may serve as a novel starch source with great potential applications both food and non-food industries [9]. Therefore, the in-depth study of the properties of chestnut starch is of great significance.

The chestnut cultivars in China are mainly grown in the middle regions, north-eastern, eastern, and south-eastern areas of the country, distributed in five basic temperature zones based on their climate characteristics, which are warm-temperature zone, north subtropical zone, mid subtropical zone, south subtropical zone, and tropic zone. Chestnuts are widely cultivated except for a few areas like Qinghai, Ningxia, Xizang, and Xinjiang, while provinces of Hebei, Henan, Liaoning, Shandong, Shaanxi, and Hubei are some famous origin places of chestnuts. Favoring low hills, gentle slopes and river banks, chestnuts grow largely depend on the environmental factors such as temperature, water, soil, and sunlight. In addition, the properties of starches isolated from these chestnuts can also be affected by various environmental factors around where they are grown.

Although there are previous studies on the structural and functional properties of chestnut starches [7, 9, 10, 11, 12], there have been few comparative studies on the properties of starches isolated from Chinese chestnut varieties in different regions. Therefore, to meet high demand of new-source starches and extend the utilization of chestnut, this study aimed to examine the physical and chemical properties of starches isolated from chestnuts produced in 21 cultivation regions in China and to make comparisons among them, including the moisture contents, morphological characteristics, color values, physio-chemical properties, pasting properties, FTIR spectra, X-ray diffraction patterns, and thermal properties. Information generated from this study will be useful for guiding their potential applications and possible contributions to both food and non-food industry.

2. Materials and Methods

2.1. Materials

Chestnut fruits were collected from 21 different regions in China, which were distributed in the five major temperature zones. Based on the identification performed by Dr. Zhang Jingzheng (Hebei Normal University of Science and Technology), all of the 21 chestnuts were under the major variety of *C. mollisima* Blume and were classified into three sub-varieties with different common names, i.e., *Banli, Youli,* and *Maoli*. The cultivation region, variety, common name, size, temperature zone of the region, and the respective picture of each chestnut sample were presented in **Table 1**. Chemical reagents, including sodium sulfite (Na_2SO_3) and ethanol, were of analytical grade.

2.2. Isolation of starches from chestnut fruits

The chestnut starches were extracted according to the methods of Cruz et al. [9] and Zhang [11] with some modifications. Chestnuts were peeled with a chestnut peeler (550W-Medium, Kenong Technology Co., Ltd, Jiangsu, China) and ground with a grinder (2200W-Small, Xulang Technology Co., Ltd, Guangzhou, China) into fine grains, which were then steeped into 0.025 M sodium sulfite (Na_2SO_3). The ratio of chestnuts to sodium sulfite solution was 1 g: 10 mL. The mixture was homogenized with a homogenizer, and the suspension was placed still at room temperature for 1 hour. Afterwards, the supernatant was discarded, and the sediment was added with distilled water and filtered through a 60-mesh screen. The residue left on the screen was discarded, while the filtrate was collected with a beaker, and added with distilled water to set still. After the layer separation occurred, the upper turbid layer was removed, and the white residue left was added with distilled water to be filtered again. The washing and filtration process were repeated for 4 times with 80-mesh, 100-mesh, 120-mesh, and 200-mesh screens sequentially, and for the last time, distilled water was replaced by 95% aqueous ethanol. When the supernatant appeared almost transparent, the upper layer (ethanol-rich layer) was discarded and the white sediment was dried at room temperature (25°C) overnight. The resultant dry samples were collected into a sample bottle.

2.3. Moisture content analysis

The determination of moisture content of the chestnut starch samples was carried out according to AACC method 44-15.02 [13]. All the experiments were conducted in triplicate.

2.4. Particle size analysis

The particle size distribution of the isolated chestnut starches was analyzed using a laser diffraction particle size analyzer (LA-920, Horiba Ltd., Tokyo, Japan).

2.5. Scanning electron microscopy (SEM) analysis

The surface morphology of starches was examined using a scanning electron microscope (SEM) (JSM-6390A, JEOL, Tokyo, Japan) operated at an accelerating voltage of 15 kV.

2.6. Color values testing

Color values of the starch samples were measured using a colorimeter (D-25, Hunter Lab Associates Inc, Reston, VA, U.S.A). The instrument was standardized with Hunter lab color standards, and L^* (lightness), a^* (redness and greenness), and b^* (yellowness and blueness) values were measured. L^* stands for the lightness ranging from 0 (black) to 100 (white); a^* indicates red with “+” and green with “-”; while b^* indicates yellow with “+” and blue with “-”. The whiteness (W) was calculated as:

$$W = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$

2.7. Light transmittance testing

The light transmittance of chestnut starches was measured using the method of Wani, Sogi, Wani, Gill, and Shivhare [14] with some modifications. Starch sample (0.5 g) and distilled water (50 mL) were added into a 50 mL centrifuge tube, which was then heated at 90°C for 15 min in a water bath (SYG-6, Langyue Instrumental Production Inc., Changzhou, China), with stirring at each 5 min intervals. The suspension was cooled to room temperature. The absorbance (time 0) at 620 nm was measured against a water blank using a UV-visible spectrophotometer (TU-1901, Persee

General Instrument Co., Ltd., Beijing, China). Afterwards, the absorbance was measured after 24 h, 48 h, and 72 h.

2.8. Syneresis properties determination

The syneresis properties of chestnut starches were measured using the method described by Wani et al. [14] with some modifications. Starch sample (3 g) and distilled water (50 mL) were added into a 50 mL centrifuge tube. The suspension was heated at 90°C for 20 min in the water bath with stirring at each 5 min intervals. The suspension was cooled in the refrigerator at 2°C for 24 h, and was centrifuged at 3000 rpm for 15 min. The syneresis was calculated as the percentage of water released after centrifugation.

$$\text{Syneresis} = \text{Weight of water released} / \text{Weight of starch suspension} \times 100\ \%$$

2.9. Swelling power (SP) and water solubility index (WSI) determination

The SP and WSI of chestnut starches were measured using the method of Wani et al. [14] with some modifications. Starch sample (0.6 g) and distilled water (30 mL) were added into a 50 mL centrifuge tube and was heated at 80°C for 30 min in the water bath with stirring at each 5 min intervals. After centrifuged at 3000 rpm for 15 min, the starch suspension in the supernatant was poured onto a petri dish and dried in an air over at 105 °C for 10 h. Both the dried residues of supernatant and the sediment were weighed. The SP and WSI were calculated based on the following formulas:

$$\text{WSI (g/100g)} = \text{Weight of dried residue of supernatant} / \text{Weight of dry sample} \times 100\%$$

$$\text{SP (g/100g)} = \text{Weight of sediment} / (\text{Weight of dry sample} \times 100 - \text{WSI}) \times 100\%$$

2.10. Water retention capacity determination

The water retention capacity (WRC) of chestnut starches was determined based on the study conducted by Raghavendra, Rastogi, Raghavarao, and Tharanathan [15]. The WRC was defined as the amount of water bound to the hydrated sample after centrifugation. Starch sample (1 g) and distilled water (30 mL) were added into a 50 mL centrifugal tube and were allowed to hydrate for 18 h. After that, the suspension was centrifuged at 3000 *g* for 20 min. The supernatant was discarded, and the weight of the wet sediment was recorded. The wet sediment was then dried at 105°C for 2 h to obtain its dry weight, and the WRC was calculated based on the equation shown below:

$$\text{WRC (g/g)} = (\text{Hydrated weight after centrifugation} - \text{dry weight}) / \text{Dry weight}$$

2.11. Amylose content determination

Amylose content was quantified according to the following procedures. Briefly, a portion of dried chestnut starch sample (100 mg) in 1 mL of ethanol was mixed with 10 ml of 1 N NaOH solution. After 10 h, the volume was made up to 100 ml with distilled water. An aliquot of 2.5 mL of the solution was added into 20 mL of distilled water, and three drops of phenolphthalein were added. Afterwards, 0.1 N HCl was added drop by drop until the pink color disappeared. Iodine reagent (1 mL) was then added and the volume was made up to 50 mL with distilled water. The absorbance was read at 590 nm using a spectrophotometer. Iodine reagent (1 mL) diluted into 50 mL of distilled water was used as the blank. The amount of amylose present in the sample was calculated with the following equation:

$$\%\text{amylose} = \text{Amylose concentration (mg/mL)} * 100\text{mL}/\text{sample weight (mg)} * 100\%$$

2.12. Pasting properties testing

The pasting properties of chestnut starches were analyzed using a Rapid Visco Analyzer (Brookfield Engineering Labs Inc, Middleboro, U.S.A), following a method defined by Reddy, Luan, Xu [16]. Starch sample (3 g) was mixed with 25 mL of distilled water and was tested under the programmed heating and cooling cycle. The starch slurries were held at 50°C for 1 min, heated to 95°C at a rate of 12°C/min and held for 2.5 min. Then the slurries were cooled to 50°C at the same rate and allowed to stay at 50°C for 2 min. The paddle speed was 960 rpm for the first 10 s, and then 160 rpm for the rest of the experiment. Parameters recorded were pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BD), final viscosity (FV), and setback viscosity (SB).

2.13. Fourier transforms infrared (FTIR) spectroscopy analysis

The FTIR spectra of the starches were detected by a FT-IR spectrophotometer (NICOLET iS50 FI-TR, New York, USA) using ATR (Attenuated Total Internal Reflectance) method. Two to three milligrams of each starch sample were placed on the diamond crystal plate with pressure applied, and the measurement started after pressing the ATR touch button. The spectra were obtained from 4000 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1}.

2.14. X-ray diffraction analysis

The X-ray diffraction patterns of the starch samples was determined using an X-ray diffractometer (Rigaku Dmax/2400, Shibuya-ku, Japan). The scanning region of diffraction was 1.5406 Å with a target voltage of 40 kV and a current of 100 mA. The scanning speed was 6°C/min and the step size was 0.02°. The diffraction pattern graphs were obtained using the Origin software (Origin 8.0, OriginLab Inc, U.S.A.). The type of chestnut starch was analyzed based on the occurrence of prominent peaks at certain diffraction angles.

2.15. Thermal properties analysis

Thermal transitions and the gelatinization properties of starches were measured by a differential scanning calorimeter (Q2000, TA Instruments-Waters LLC, New Castle, DE, U.S.A.). Each starch sample (3 mg, dry basis) was weighed and mixed with 7 μL of distilled water in a DSC pan to make starch slurry, which was equilibrated at 4°C for 2 h. The scanning temperature was 20-120°C and the heating rate was 10°C/min.

2.16. Statistical analysis

All of the experiments were conducted in triplicates and the results were expressed as mean value ± standard deviation with one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was conducted to determine the significant differences among the mean values ($p < 0.05$). A Pearson correlation analyses of several parameters of the starch samples were done. These analyses were accomplished with SPSS 17.0 software.

3. Results and discussion

3.1. Moisture content and particle size distribution of chestnut starches

The moisture contents as well as the mean and median values of particle size of the isolated starches from different cultivation regions in China are shown in **Supplemental Table 1**. The moisture contents of chestnut starches from 21 regions ranged from 11.3% to 16.9%, among which the one from Tai'an (Shandong) exhibited the highest moisture content, while the lowest was presented in the starch of chestnut from Haikou (Hainan). As presented in the table, all isolated starches showed a bimodal particle size distribution, which means there were a peak distribution of particle size below the medium value while another peak above that. As all of the starches were from the same chestnut variety, *C. mollisima* Blume, they presented a similar and wide range of particle size distribution, ranging from approximately 1 to 500 μm, with only a few exceptions. Starches isolated from chestnuts grown in Shangluo (Shaanxi), Liuzhou (Guangxi), and Haikou (Hainan) had relatively smaller range of particle size distribution, and the mean values were also much lower than others, indicating there were no particles with diameter larger than 301, 345, and 200 μm for these 3 chestnut starches, respectively. The mean values of the chestnut starches isolated ranged from 21.5 to 111.7 μm, while most of them fell between 60 and 90 μm. Among them, chestnut starch from Yangjiang (Guangdong) exhibited the highest mean particle size (111.7 μm), as well as the median value (96.6 μm); while that from Haikou (Hainan) presented the lowest mean (21.5 μm) and median value (10.3 μm). It was also found that the average particle size of starches isolated from *Maoli* was smaller than those of *Banli* and *Youli*.

According to Corriea, Cruz-Lopes, and Beirão-da-Costa [17], the granule size distribution of chestnut starch can be affected by the isolation method used. In their study, chestnut starch consisted of mainly small and medium granules, and it was indicated that the more moderate the isolation method was used, the wider range of starch granule size was presented. In this study, the starches were isolated by sodium bisulfite and ethanol, which was more moderate than alkali extraction and enzymatic methods, so less granules were lost or destroyed during isolation, thus presenting a larger range of particle size distribution.

The size of starch granules can affect the digestibility and mouth feel when comes to their utilization. Generally, starch granules with smaller particle size presented greater digestibility, which is mainly because the smaller size granules have relatively larger surface area and are more readily digested by enzymes [18]. In addition, previous study reported that smaller-sized starch granules can provide a better mouth feel, which can serve as better choices for lipid substitute [19]. Therefore, it is expected that the starches isolated from chestnuts grown in Haikou (Hainan), Liuzhou (Guangxi), and Shangluo (Shaanxi), exhibiting small mean particle size and narrow size distribution, may provide better digestibility and mouth feel. In addition, among the starches with smaller granule sizes, most were isolated from *Maoli* (such as the ones from Haikou and Shangluo), indicating that *Maoli* starches presented generally higher digestibility and better mouth feel.

3.2. Morphological characteristics of chestnut starches

The morphology of starches isolated from chestnuts grown in different regions was analyzed using SEM, as shown in **Fig. 1**. It can be seen that starches isolated from chestnuts grown in different cultivation regions exhibited similar granule shape due to their same botanical origin but had various sizes. Most starch granules exhibited oval to spherical shape, with some elliptical ones scattered among the granules. All the exposed surfaces of starch granules presented smooth surfaces and edges, without any serrations or indentations. The shape of starch granules varied within each SEM image, and also exhibited differences among starches isolated from chestnuts from different cultivation regions. Starches isolated from chestnuts grown in Beijing (a), Jixian (b), Qinhuangdao (d), Wuxi (k), and Guilin (p) exhibited relatively uniform distribution of granule size, while for the rest of chestnut starches, the size of starch granules showed obvious variation, with diameters ranged from less than 1 μm to larger than 20 μm. For the overall granule size of the chestnut starches, those from Qinhuangdao (d), Tai'an (f), Shangluo (h), and Guilin (p) presented more small granules (with diameter less than 5 μm) than larger ones, which was relatively identical to the results tested by particle size analyzer. According to Reddy, Luan, and Xu [16], generally the morphological characteristics of starch granule are related to the physiological properties of its botanical source, including the amylose content, swelling power, light transmittance, and water absorption capacity.

3.3. Color values of chestnut starches

The color parameters include *L**, *a**, *b**, and W values of isolated chestnuts starches from 21 regions are shown in **Supplemental Table 2**. As one of the physical properties of starches, color value plays an important role in indicating quality and purity, as well as in many food applications. The color values (parameters *L**, *a**, *b**, W) of the 21 isolated chestnut starches exhibited significant difference ($p < 0.05$). The overall results indicated high lightness of all the starch samples, which were all above 90 with only one exception, the starch isolated from Tai'an (Shandong), which presented a *L** value of 84.91, exhibiting the lowest lightness. The reason of this exceptionally low lightness may be due to the slight degradation of the chestnut starch because of its higher moisture content, which introduced some extra pigments from the growth of microorganisms. Except for that of Tai'an (Shandong), the range of *L** value of other 20 chestnut starches were between 97.21 and 102.50, with the highest one from Linyi (Shandong) and the lowest from Wuxi (Jiangsu). According to Reddy, Luan, and Xu [16], higher *L** values of isolated starches reflect the lightness and purity of starches. Besides, the highest white value could improve the usage of the starch in plenty of food applications where quality in color is recommended. As shown in **Supplemental Table 2**, the starch isolated from chestnut grown in Zhaotong (Yunnan) exhibited the highest whiteness, with a W value of 97.02, followed by that from Xinyang (Henan), with a W value of 96.84; while the chestnut starch from Tai'an (Shandong) presented the lowest

W value, which was the same as that of lightness.

For the a^* value, it was observed that all the chestnut starches exhibited an a^* value above 0, indicating a tendency to red. The highest one was the chestnut starch from Jixian (Tianjin), which was 1.87, exhibiting a higher red tendency; while the lowest one was from Xinyang (Henan), which was 0.26. The values of b^* obtained were all below 0, showing a blue tendency. The highest b^* value was exhibited in the chestnut starch isolated from Tai'an (Shandong), which was -0.09; and the lowest one was from Linyi (Shandong), which was -8.83, indicating the lowest and the greatest tendency to blue, respectively. Since the two isolated starches were from the same province, the reason that such variation was exhibited may still owe to the degradation of the chestnut starch from Tai'an (Shandong), which could present pigments from sources other than the starch itself. Moreover, in addition to the genetic makeup of starches, the ageing of chestnut samples during storage prior to the extraction and isolation step of starch could also affect the color characteristics [20].

3.4. Physiochemical properties of chestnut starches

The basic physiochemical properties of the isolated chestnut starches include light transmittance, syneresis, swelling power (SP), water solubility index (WSI), and water retention capacity (WRC). The light transmittance of each chestnut starch solution was determined every 24 h, as shown in **Supplemental Table 3**. The transparency of starch is related to the degree of rearrangement of starch molecules after being heated. The transparency is high when the degree of rearrangement is low, indicating fewer retro-gradation [21]. Also, larger amount of straight-chain amylose normally results in a de-crease in transparency of starch paste, while more amylopectin can increase that. As was shown in the table, for almost all chestnut starches, the highest light transmittance appeared at 0 h and then gradually decreased after setting for 24 h, 48 h, and 72 h, primarily due to a result of retrogradation. The range of light transmittance at the beginning (0 h) was between 1.50 and 1.95, without much significant difference. Starch isolated from chestnut grown in Nanping (Fujian) presented the highest light transmittance at 0 h, which was 1.95, which still held a relatively high transmittance value after standing for 48 h and 72 h. For the rest of samples the transmittance values became quite close as the standing time increased.

The syneresis, WSI, SP, and WRC of each chestnut starch are shown in **Table 2**. Syneresis is the water separation from a starch gel upon standing at cold storage. The syneresis rate of all the chestnut starches ranged between 37.06 % (the chestnut starch from Fuzhou) and 56.40 % (the one from Zhaotong), presenting a significant difference ($p < 0.05$). According to Hermansson and Svegmark [22], higher amylose content can result in more interaction with amylopectin chains, causing more gel shrinkage, which might lead to higher syneresis.

WRC values were in the range of 0.82 to 2.48 (g/g) for the starches isolated from chestnuts grown in different regions. According to Nawab, Alam and Hasnain [23], the availability of water binding sites is increased as more hydroxyl groups are exposed with the rise in temperature. Starches with higher water retention can be applied to enhance functional properties of certain food products, which can retain a slower water intake and prevent lump formation when mixing with other food powder.

SP and WSI are characteristics analyzed to understand the interaction between the water molecules within the crystal domains of starch and the starch chains during heating [24]. As seen in **Table 2**, the SP was in the range of 12.55 to 23.49 (g/100g), whereas the WSI range from 3.20% to 27.65%. Both the SP and WSI of starches isolated from chestnuts varied significantly ($p < 0.05$) among different cultivation regions. Moreover, SP is related to the pasting characteristics of starches, especially to trough, final and setback viscosity [14]. Commonly, the swelling of starch granules depends on the quantity of amylose and particle size distribution, and can be influenced by the structure of amylopectin [25]. Solubility represents the amount of solubilized starch molecules at certain temperature. The variation in WSI could be largely due to the different distribution of starch chain length and granule size [16].

3.5. Amylose content of chestnut starches

The amylose contents of the isolated chestnut starches are presented in **Table 2**. Linear non-branched chain of glucose, like amylose, can absorb iodine within its helical coils to produce a blue colored complex which is measured calorimetrically, and consequently, inflate the iodine affinity and the apparent amylose content of the starch [26]. Amylose can also form a V-helix complex with the hydrocarbon portion of monoglycerides and fatty acids [27]. In this study, endogenous lipids were not removed, and thus it may lead to an underestimation of amylose content. It was found that amylose content of the 21 chestnut starches ranged between 29% (the one from Xinyang, Henan) and 42% (the one from Liuzhou, Guangxi) with significant difference ($p < 0.05$) observed among the samples. According to Tester, Karkalas, and Qi [28], regular starches contain approximately 20-30% amylose, waxy starches less than 10% amylose and high-amylose starches more than 40% amylose. Therefore, most starch samples isolated from Chinese *C. mollisima* exhibited a higher amylose content than regular starches. High amylose content can induce an increase in gelatinization temperature due to retarded swelling, as well as thus to affect the pasting properties of starches.

3.6. Pasting properties of chestnut starches

The pasting properties of the 21 chestnut starches are shown in **Table 3**, including the parameters of pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BD), final viscosity (FV), and setback viscosity (SB). As one of the most important characteristics of starches, viscosity can provide essential information about the cooking behaviors of starches during the heating and cooling cycles [16]. As the starch-water dispersion is heated under shear above its gelatinization temperature, the phase transition from order to disorder state takes place and the viscosity of the system increases dramatically, yielding the starch paste [9, 16]. The properties of the starch paste are affected by the quantity and length of amylose chain, as well as the size and distribution of branched chains of amylopectin [16]. Furthermore, the pasting profile of starches serves as an effective method for relating starch functionality with its structural features and assessing various potential applications for its usage in the industry as a thickener or binder [9].

As **Table 3** shows, significant differences ($p < 0.05$) were observed among PT, PV, TV, BD, FV, and SB of the starch samples. For all the starches analyzed, there was a gradual increase in viscosity with the temperature increase in the initial phase, which can be explained by the loss of free water and decreased flow of water due to the increased volume of swollen starch granules that occupy more space. PT is the temperature at which the viscosity starts to rise, or the starch begins the gelatinization. The PT of the isolated starches ranged between 68.67 °C (the starch from Kunming, Yunnan) and 77.76 °C (the one from Yangjiang, Guangdong). According to Singh et al. [25], a higher PT indicates a higher resistance to swelling. Therefore, the starches isolated from chestnuts grown in Guangdong (S20), Jiangsu (S11), Zhejiang (S13), and Henan (S9) presented higher resistance compared to others, most of which were *Youli*. The starch isolated from chestnut produced in Yangjiang (Guangdong) also presented a high amylose content.

The PV of the isolated chestnut starches ranged from 1120.67 cP to 4422.67 cP, with the lowest one from Zhaotong (Yunnan), and the highest one from Liuzhou (Guangxi). The PV value of most starch samples were in the range between 3000 and 4000 cP. Commonly, PV refers to the swelling peak of the starch, which may be influenced by its composition and structure, and is also associated with the circumstances whereby the starch granules maintain stable between the status of swelling and rigidity. Results showed the starch isolated from chestnut produced in Zhaotong (Yunnan) had a very high amylose content (40.75%), which presented the lowest peak viscosity. This was in accordance with the study of Andrabi, Wani, Gani, Hamdani, and Masoodi [29], in which it was indicated that starches with high amylose contents would present lower PV because of the restricted swelling of starch granules. Moreover, the peak viscosity often plays an important role in the quality of end-product, providing an indication of the viscous load when the food ingredients are about to be mixed [30]. Also, PV and its corresponding temperature are indicative for the water binding capacity of starches [31].

The trough viscosity (TV) of isolated starches ranged from 195.33 cP (the starch from Zhaotong, Yunnan) to 3442.33 cP (the one from Shangluo, Shaanxi), which represented the reduction in viscosity of starches after PV due to the largely breakdown of starch granules by high temperature. For the breakdown viscosity, it is the result of the dissociation and recombination of swollen starch granules due to the continuous shear stress at high temperatures, which can be used to predict the capacity of starch pastes used as thickeners to resist high shear conditions [32]. The range of BD of the isolated starches was from 453.33 cP (the one from Nanping, *Youli*) to 2015.67 cP (the one from Liuzhou, *Banli*), and the low BD of chestnut starch from Nanping (Fujian) indicated that this starch was more resistant to the effect of sheer during heating. Moreover, it can also be seen that high breakdown values were associated with high peak viscosities, which are related to the degree of swelling of the starch granules.

Final viscosity (FV) of the isolated starches increased due to the cooling effect, and the values ranged between 314.00 cP (the one from Zhaotong) and 4600.33 cP (the one from Shangluo). It can be inferred that the chestnut starch from Shangluo (Shaanxi) might have the highest tendency to retrograde than others. The setback viscosity was a measure of syneresis of starch upon cooling, which can be obtained based on the FV and TV. It is generally used as a measure to indicate the gelling ability or retrogradation tendency of the starch, which can also be indicative of good cooking quality [33]. The SB values of isolated starches ranged from 118.67 cP (the one from Zhaotong) to 1559.33 cP (the one from Qinhuangdao, Hebei), which also had certain relationship with the amylose content. Moreover, SB of starches can also show the interaction among leached amylose chains during the cooling cycle and the existence of intact granules in the amylose network [34].

3.7. Structural properties of chestnut starches assessed by FTIR spectroscopy

The FTIR spectra of all starches extracted from 21 regions are divided into five groups, based on the different climate zones they are in, as shown in **Supplemental Fig. 1**. The FTIR spectra are similar for all the chestnut starch samples, which exhibited major absorption bands at the wavelengths around 522-524 cm^{-1}, 572-573 cm^{-1}, 761-762 cm^{-1}, 853-859 cm^{-1}, 927-928 cm^{-1}, 995-997 cm^{-1}, 1077-1078 cm^{-1}, 1149-1150 cm^{-1}, 1338-1360 cm^{-1}, 1417 cm^{-1}, 1640-1649 cm^{-1}, 2929-2931 cm^{-1}, and 3286-3309 cm^{-1}, indicating the polysaccharide nature of the starches. No distinctive differences were found among the vibration bands of O-H, C-H, and C-O in the chestnut starches, which were due to the similarity in chemical structures of the starches, resulting in the similar absorption peaks.

For the broad band in the wavelength region of 3000-3650 cm^{-1}, the peaks at 3286-3309 cm^{-1} indicated the complex vibration stretching contained free, inter- and intra-molecular hydroxyl (-OH) groups [17]. The absorption peaks at 2929-2931 cm^{-1} were contributed by the -CH stretching associated with the ring methine hydrogen atoms. The absorptions at 1640-1649 cm^{-1} were due to the H-O-H bending vibration of bound water, a typical band in the spectra of starch and its derivatives [35]. There were small absorption peaks at around 1417 cm^{-1}, which were related to the angular deformation of C-H; and the peaks at 1338-1360 cm^{-1} were due to the twitching of CH_2 [36]. For the absorption peaks at 1149-1150 cm^{-1}, they were attributed to the coupling of C-O, C-C stretching, and peaks at 1077-1078 cm^{-} represented the C-H-O bending in the starch molecules [16], and have also been used to express the amorphous state in starch [37]. In addition, according to Yu, Wang, and Ma [38], the sharp absorption peaks at 995-997 cm^{-1} were due to the C-O bending in the C-O-C structure in starches, the wave number of which can be influenced by the time of storage of the starches. The peaks at 927-928 cm^{-1} indicated the skeletal mode vibrations of α-1,4 glycosidic linkage, and those at 853-859 cm^{-1} were due to the C-H bending and CH_2 deformation. For the peaks at 761-762 cm^{-1}, C-C stretching was exhibited; while for the absorption bands below the region of 580 cm^{-1}, skeletal modes of pyranose ring were indicated [39].

3.8. Structural properties of starches assessed by X-ray diffraction analysis

The results of the X-ray diffraction of the isolated chestnut starches are classified into the same five groups, as shown in **Fig. 2**. The starch samples were marked as S1 - S21 and divided into **Fig. 2A** – **Fig. 2E** under the same principle as above. Generally, starch granules present semi-crystalline structures and can be identified through X-ray diffraction patterns. Based on their characteristics and distinct XRD patterns, native starches can be categorized into A, B, and C types. Among them, the A-type exhibits the characteristics of most starches of cereal origin; the B-type mostly includes potato, root starches and amylomaize starches; and the C-type characterizes the starches of smooth pea and various edible beans [31].

According to Gernat, Radosta, Damaschun, and Schierbaum [40], chestnut starch presents a C-type XRD pattern, which is a mixture of A and B type granules with varying proportions. For the B-type pattern, it was characterized by peaks at diffraction angles at around 5.6°, 15°, 17°, and 20°, with a double peak at 22-24°. Some typical B-type starches include food legume starches and potato starches [41]. While for the pure A-type starches, such as maize and wheat starches, they do not present the 2θ peak at diffraction angle of 5.6°, but exhibit a shoulder at 18°, and a unique peak at around 23° instead of the doublet peak at 22-24°. Also, an increase is presented in the relative intensity of the band at 15° [42]. Further, the C-type pattern exhibits broad peaks at around 17° and 23°, with some minor peaks at 5.6° and 15°, which is often classified into C_a, C_b, and C_c types based on their resemblance to A and B-type, or between the two [40].

For the starch samples isolated from chestnuts grown in 21 regions, it can be seen that most of them exhibited a similar XRD pattern, with obvious peaks at 5.6°, 15°, 17°, and broad peaks at 22-24°. Most of the starches could be classified as C_b-type starches, as there were a small peak at 20° and doublet peak at 22-24°, including starches S1-S8, S10, S12, S14, S15, S16, S18, S19, and S21 (containing *Banli, Youli*, and *Maoli*). While for the rest of the starch samples (S9, S11, S13, S17, and S20, with no *Youli* included), they presented as C_a-type starches, as the peak at 5.6° was not as intense as that of the C_b pattern, and there was no obvious peak at 20°; also, a single peak was exhibited at around 23° instead of a doublet one. According to Yang, Jiang, Prasad, Gu, and Jiang [10], the presence of a peak at the diffraction angle of near 20° indicates the occurrence of crystalline amylose-lipid complexes, which means the C_b-type starches presented this characteristic.

3.9. Thermal properties of chestnut starches

The gelatinization temperatures, including onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy of gelatinization (ΔH) of chestnut starches from the 21 regions are illustrated in **Table 4**. Starch granules undergo significant structural and morphological changes when heated in excess of water, including the loss of crystallinity due to the dissociation of amylopectin double helix, the starch swelling due to water absorption, and the leaching of amylose to the water phase. These series of changes are generally referred to as starch gelatinization, which occurs in a temperature range depend on the botanical origin of different starches [9]. As shown in **Table 4**, the lowest onset temperature (T_o) was 59.6°C for the starch from Liuzhou (Guangxi) (*Banli*); while the highest one was 68.9°C from Xinyang (Henan) (*Youli*). The lowest peak temperature (T_p) was 64.0°C, occurred in the starch from Kunming (Yunnan) (*Banli*); while the highest one was 74.8°C from Yangjiang (Guangdong) (*Youli*). The lowest conclusion temperature (T_c) was 68.9°C for the starch from Guilin (Guangxi) (*Banli*); while the highest one was 81.2°, which also occurred in the starch from Yangjiang (*Youli*). Compared to the previous research on the gelatinization temperatures of chestnut starch isolated from *C. mollissima* [10], the T_o, T_p, and T_c values were mostly consistent with those reported in the article, which were 64.3, 68.7 and 76.5°C, respectively.

In addition, a higher T_o indicates the starch crystallites have a higher degree of stability and the amount of structural defects is less. Also, high gelatinization temperature values indicate more energy is required for the initiation of starch gelatinization; and the more difficult to gelatinize, the more crystalline the starch granules are [43]. Therefore, it can be found that the starch from *Youli* presented an overall higher gelatinization temperature, which indicated it is more stable in structure and more suitable for high-heat cooking such as stir frying. The typical ones included those starches isolated from chestnuts produced in Yangjiang (Guangdong) (S20) and Xinyang (Henan) (S9), with relatively higher values of T_o, T_p, and T_c presented. According to Singh et al. [25], the variation in starch gelatinization temperatures may be due to multiple factors such as the quantity of amylose, the structure of amylopectin, molecular structure and weight distribution of starch granules, as well as granular architecture includes size and shape.

The enthalpy of gelatinization (ΔH) reflects the loss of double helix order instead of crystalline order. The ΔH values of isolated starches ranged between 0.65 and 5.52 J/g, which differed significantly between the highest value of chestnut starch from Linyi, Shandong (*Maoli*) and the lowest one from Huairou, Beijing (*Banli*). The enthalpy showed lower values than that reported in the previous study conducted by Yang et al. [10]. According to Lazaridou and Biliaderis [44], variation in ΔH indicates the difference in required energy for disrupting hydrogen bonds within the crystalline zones of starch, and the higher ΔH value of gelatinization suggested that a better crystalline region was generated during the hardening process of heating, which may be due to the degradation of endo-amylase. Also, a stronger interaction of carbohydrate and water as well as a better organized microstructure lead to a higher ΔH value [45].

3.10. Correlation among the properties of chestnut starches

The Pearson correlation analyses of major parameters of the chestnut starches are shown in **Supplemental Table 4**. As seen from the highlighted *p*-values, SP of the chestnut starch granules was negatively correlated with the enthalpy of gelatinization (ΔH) ($r = -0.435$, $p < 0.05$). This negative correlation indicated that granules hold more water within swollen starch molecules require less energy for disrupting hydrogen bonds during the gelatinization process. Syneresis property was significantly positively correlated to the granule size of starches ($r= 0.593$, $p< 0.05$), indicating that smaller starch granules usually lead to slower exudation of water from starch gel.

For the pasting properties, there was a significant positive correlation between the pasting temperature and peak temperature in the DSC ($r = 0.859$, $p = 0$), which was also reported by Liu et al. [7]. Pasting TV, FV, and SV all had significant positive correlations with peak viscosity ($p = 0$ or 0.001), and they were also significantly positively correlated with each other ($p = 0$). This correlation indicated that higher content of dissociation and recombination of swollen starch granules under high temperature or shear stress could result in more reduction in viscosity. In addition, TV, FV, and SV were negatively correlated with WSI ($p < 0.05$).

4. Conclusions

In this study, starches from chestnuts produced in 21 different regions in China were isolated, characterized and compared. Significant differences ($p < 0.05$) were observed in their color values, physio-chemical properties, pasting and thermal properties, depending on their botanical origins; while the particle size distribution pattern, functional group composition assessed by FTIR, and the structural properties assessed by XRD presented similar patterns, due to their same variety. The amylose content of the chestnut starches ranged between 29% and 42%, higher than that of regular starches. Chestnut starches from Zhaotong, Nanping, and Anqing presented lower peak viscosities due to restricted swelling; while starches from Liuzhou, Huairou, and Jixian exhibited high viscous loads when used to mix with food ingredients. Both the pasting and thermal properties showed that starches isolated from *Youli* presented higher resistance to the sheer force effect and swelling during heating, indicating *Youli* was more stable in structure and more suitable for high-heat cooking like stir-frying. The FTIR spectra showed the polysaccharide nature of the starch samples. All the chestnut starches were C-type starches based on their XRD patterns, among which most were classified into the C_b-type, which generally had higher crystallinity than the C_a-type ones. Moreover, the high amounts of starch present in the chestnut fruits and their functional properties examined made chestnut a possible alternative source of starch. Although only applied in laboratory scale, the low cost of reagents and simple isolation procedures could be easily applied at industrial scale with proper adaptation. Therefore, the potential utilization of starches isolated from chestnuts produced in different regions in China would be relevant for both domestic and industrial applications.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1. List of 21 chestnut samples

Sample No.	Cultivar	Variety	Common name	Size	Temperature zone	Picture
1	Huairou, Beijing	*Castanea mollisima* Blume	*Banli*	Medium+	Warm-temperature zone	
2	Jixian, Tian-jin	*Castanea mollisima* Blume	*Youli*	Small+	Warm-temperature zone	
3	Tangshan, Hebei	*Castanea mollisima* Blume	*Banli*	Medium+	Warm-temperature zone	
4	Qinhuang-dao, Hebei	*Castanea mollisima* Blume	*Banli*	Medium+	Warm-temperature zone	
5	Xingtai, He-bei	*Castanea mollisima* Blume	*Banli*	Medium	Warm-temperature zone	
6	Tai'an, Shan-dong	*Castanea mollisima* Blume	*Maoli*	Small+	Warm-temperature zone	
7	Linyi, Shan-dong	*Castanea mollisima* Blume	*Maoli*	Small	Warm-temperature zone	
8	Shangluo, Shaanxi	*Castanea mollisima* Blume	*Maoli*	Small+	Warm-temperature zone	
9	Xinyang, He-nan	*Castanea mollisima* Blume	*Youli*	Medium+	North subtropical zone	
10	Anqing, An-hui	*Castanea mollisima* Blume	*Banli*	Medium-	North subtropical zone	

11	Wuxi, Jiangsu	*Castanea mollisima* Blume	*Banli*	Medium+	North subtropical zone	
12	Xiangyang, Hubei	*Castanea mollisima* Blume	*Banli*	Medium	North subtropical zone	
13	Wenzhou, Zhejiang	*Castanea mollisima* Blume	*Youli*	Medium-	Mid subtropical zone	
14	Nanping, Fujian	*Castanea mollisima* Blume	*Youli*	Medium	Mid subtropical zone	
15	Fuzhou, Jiangxi	*Castanea mollisima* Blume	*Maoli*	Small	Mid subtropical zone	
16	Guilin, Guangxi	*Castanea mollisima* Blume	*Banli*	Medium-	Mid subtropical zone	
17	Liuzhou, Guangxi	*Castanea mollisima* Blume	*Banli*	Medium	Mid subtropical zone	
18	Kunming, Yunnan	*Castanea mollisima* Blume	*Banli*	Medium+	Mid subtropical zone	
19	Zhaotong, Yunnan	*Castanea mollisima* Blume	*Maoli*	Small	Warm-temperature zone	
20	Yangjiang, Guangdong	*Castanea mollisima* Blume	*Youli*	Large	South subtropical zone	
21	Haikou, Hainan	*Castanea mollisima* Blume	*Maoli*	Medium	Tropic zone	

Table 2. Physiochemical properties of chestnut starches

Starch sample No.	Region	Syneresis (%)	WSI	SP	WRC	Amylose Content
1	Huairou, Beijing	42.43 ± 0.04^{j}	10.52 ± 0.06^{f}	19.98 ± 0.03^{g}	0.92 ± 0.02^{j}	34.17 ± 0.48^{cde}
2	Jixian, Tianjin	39.97 ± 0.03^{k}	10.28 ± 0.13^{f}	20.69 ± 0.05^{e}	0.91 ± 0.03^{j}	31.63 ± 0.07^{ghi}
3	Tangshan, Hebei	49.56 ± 0.04^{c}	9.26 ± 0.08^{g}	17.79 ± 0.04^{k}	0.93 ± 0.02^{j}	31.77 ± 0.11^{fghi}
4	Qinhuang Island, Hebei	42.76 ± 0.06^{i}	3.91 ± 0.36^{n}	16.23 ± 0.11^{o}	1.03 ± 0.02^{fgh}	33.57 ± 1.10^{cdef}
5	Xingtai, Hebei	50.64 ± 0.08^{b}	4.27 ± 0.12^{m}	15.83 ± 0.07^{p}	1.25 ± 0.03^{d}	33.75 ± 0.89^{cde}
6	Tai'an, Shandong	35.23 ± 0.05^{p}	7.89 ± 0.08^{h}	19.08 ± 0.03^{h}	2.48 ± 0.14^{a}	34.76 ± 0.12^{c}
7	Linyi, Shandong	38.20 ± 0.06^{m}	4.31 ± 0.14^{m}	17.10 ± 0.05^{m}	1.00 ± 0.03^{ghi}	34.11 ± 0.15^{cde}
8	Shangluo, Shaanxi	44.23 ± 0.09^{fg}	4.03 ± 0.10^{mn}	15.59 ± 0.03^{q}	1.08 ± 0.04^{efg}	37.13 ± 0.89^{b}
9	Xinyang, Henan	44.45 ± 0.08^{f}	25.32 ± 0.12^{b}	23.49 ± 0.10^{a}	1.47 ± 0.03^{b}	29.42 ± 0.63^{j}
10	Anqing, Anhui	39.10 ± 0.05^{l}	5.28 ± 0.10^{j}	17.96 ± 0.03^{j}	1.09 ± 0.02^{ef}	32.75 ± 0.47^{defg}
11	Wuxi, Jiangsu	44.47 ± 0.06^{f}	6.43 ± 0.20^{i}	18.57 ± 0.07^{i}	1.06 ± 0.03^{efgh}	32.98 ± 1.06^{cdefg}
12	Xiangyang, Hubei	44.08 ± 0.06^{g}	10.98 ± 0.13^{e}	20.32 ± 0.06^{f}	1.05 ± 0.04^{efgh}	31.66 ± 1.48^{ghi}
13	Wenzhou, Zhejiang	36.29 ± 0.09^{o}	5.20 ± 0.21^{j}	17.55 ± 0.07^{l}	0.82 ± 0.02^{k}	32.40 ± 1.86^{efgh}
14	Nanping, Fujian	45.55 ± 0.07^{e}	3.20 ± 0.13^{o}	13.68 ± 0.04^{r}	1.23 ± 0.01^{d}	33.11 ± 1.71^{cdefg}
15	Fuzhou, Jiangxi	37.06 ± 0.59^{n}	3.77 ± 0.31^{n}	17.68 ± 0.10^{kl}	0.99 ± 0.03^{hij}	33.97 ± 0.64^{cde}
16	Guilin, Guangxi	43.73 ± 0.06^{h}	4.42 ± 0.19^{kl}	16.41 ± 0.08^{n}	1.03 ± 0.03^{fgh}	30.12 ± 1.20^{ij}
17	Liuzhou, Guangxi	26.87 ± 0.08^{r}	16.30 ± 0.18^{d}	22.39 ± 0.10^{c}	0.92 ± 0.05^{j}	41.52 ± 0.66^{a}
18	Kunming, Yunnan	42.90 ± 0.09^{i}	5.45 ± 0.37^{j}	20.23 ± 0.19^{f}	0.94 ± 0.03^{ij}	32.38 ± 1.88^{efgh}
19	Zhaotong, Yunnan	56.40 ± 0.10^{a}	27.65 ± 0.13^{a}	21.53 ± 0.10^{d}	1.21 ± 0.04^{d}	40.75 ± 0.30^{a}
20	Yangjiang, Guangdong	48.76 ± 0.09^{d}	4.69 ± 0.12^{k}	12.55 ± 0.03^{s}	1.11 ± 0.03^{e}	34.34 ± 0.78^{cd}
21	Haikou, Hainan	29.40 ± 0.13^{q}	18.49 ± 0.16^{c}	22.59 ± 0.09^{b}	1.34 ± 0.03^{c}	30.80 ± 1.57^{hij}

Mean ± SD is reported; different lower case superscript letters in the same column indicate significant differences ($p < 0.05$, n = 3)

Table 3. Pasting properties of 21 chestnut starches

Starch sample No.	Region	PT (°C)	PV (cP)	TV (cP)	BD (cP)	FV (cP)	SB (cP)
1	Huairou, Beijing	71.82 ± 0.03^{h}	4375.33 ± 50.81^{ab}	3057.33 ± 85.33^{cd}	1318.00 ± 40.63^{c}	4447.00 ± 80.57^{b}	1389.67 ± 24.11^{b}
2	Jixian, Tianjin	69.47 ± 0.06^{k}	4297.33 ± 51.16^{b}	3236.00 ± 50.69^{b}	1061.33 ± 8.39^{g}	4230.67 ± 55.59^{c}	994.67 ± 5.13^{l}
3	Tangshan, Hebei	73.46 ± 0.04^{e}	4188.67 ± 47.35^{i}	2718.00 ± 36.04^{e}	470.67 ± 11.72^{mn}	3852.67 ± 45.06^{g}	1134.67 ± 9.02^{ghi}
4	Qinhuangdao, Hebei	71.00 ± 0.05^{i}	3706.00 ± 40.58^{fg}	2957.33 ± 79.26^{d}	748.67 ± 39.53^{l}	4516.67 ± 56.19^{ab}	1559.33 ± 25.48^{a}
5	Xingtai, Hebei	71.84 ± 0.04^{h}	3567.00 ± 63.22^{h}	2698.67 ± 36.01^{e}	868.33 ± 28.36^{j}	3911.67 ± 18.61^{fg}	1213.00 ± 17.69^{de}
6	Tai’an, Shandong	72.60 ± 0.05^{g}	2217.67 ± 54.98^{j}	1327.00 ± 44.19^{i}	890.67 ± 11.02^{ij}	2148.00 ± 54.03^{l}	821.00 ± 14.18^{m}
7	Linyi, Shandong	73.61 ± 0.06^{e}	3200.33 ± 60.93^{i}	2259.33 ± 31.09^{g}	941.00 ± 92.00^{h}	3420.33 ± 32.50^{j}	1161.00 ± 3.00^{fgh}
8	Shangluo, Shaanxi	73.08 ± 0.43^{f}	3951.33 ± 63.10^{d}	3442.33 ± 37.69^{a}	509.00 ± 26.15^{m}	4600.33 ± 6.81^{a}	1158.00 ± 44.03^{fgh}
9	Xinyang, Henan	75.78 ± 0.06^{c}	3790.00 ± 26.06^{ef}	2340.67 ± 29.05^{fg}	1449.33 ± 4.51^{b}	3724.33 ± 38.62^{h}	1383.67 ± 13.61^{b}
10	Anqing, Anhui	72.63 ± 0.07^{g}	3155.00 ± 34.51^{i}	1906.67 ± 40.50^{h}	1248.33 ± 6.03^{d}	2995.33 ± 42.10^{k}	1088.67 ± 3.51^{ij}
11	Wuxi, Jiangsu	76.25 ± 0.40^{b}	3864.33 ± 58.01^{de}	2746.00 ± 54.67^{e}	1118.33 ± 11.06^{f}	4039.33 ± 56.31^{de}	1293.33 ± 3.06^{c}
12	Xiangyang, Hubei	71.84 ± 0.05^{h}	3548.67 ± 85.98^{h}	2697.33 ± 70.07^{e}	851.33 ± 18.15^{j}	3957.00 ± 66.12^{ef}	1259.67 ± 5.51^{cd}
13	Wenzhou, Zhejiang	75.89 ± 0.05^{c}	3550.00 ± 48.81^{h}	2321.33 ± 56.04^{fg}	1228.67 ± 7.51^{d}	3523.33 ± 47.44^{i}	1202.00 ± 9.00^{ef}
14	Nanping, Fujian	75.02 ± 0.07^{d}	3118.33 ± 41.50^{i}	2665.00 ± 46.00^{e}	453.33 ± 4.51^{n}	4068.00 ± 29.46^{d}	1403.00 ± 17.78^{b}
15	Fuzhou, Jiangxi	69.58 ± 0.11^{k}	3972.33 ± 84.29^{cd}	3109.00 ± 80.32^{c}	863.33 ± 4.04^{j}	4135.00 ± 32.05^{cd}	1026.00 ± 105.67^{kl}
16	Guilin, Guangxi	70.31 ± 0.1^{j}	3751.00 ± 39.04^{f}	3027.33 ± 39.50^{cd}	723.67 ± 2.08^{l}	4131.33 ± 42.67^{cd}	1104.00 ± 4.58^{ij}
17	Liuzhou, Guangxi	72.52 ± 0.07^{g}	4422.67 ± 105.70^{a}	2407.00 ± 104.79^{f}	2015.67 ± 8.08^{a}	3475.33 ± 78.09^{ij}	1068.33 ± 28.45^{jk}
18	Kunming, Yunnan	68.67 ± 0.06^{l}	3550.67 ± 60.62^{h}	2743.33 ± 77.00^{e}	807.33 ± 16.80^{k}	4135.00 ± 71.55^{cd}	1391.67 ± 6.35^{b}
19	Zhaotong, Yunnan	73.50 ± 0.05^{e}	1120.67 ± 77.50^{k}	195.33 ± 83.00^{j}	925.33 ± 5.51^{hi}	314.00 ± 72.75^{m}	118.67 ± 12.42^{n}
20	Yangjiang, Guangdong	77.76 ± 0.46^{a}	3607.67 ± 88.64^{gh}	2429.33 ± 82.10^{f}	1178.33 ± 11.06^{e}	3556.33 ± 91.58^{i}	1127.00 ± 12.29^{hi}
21	Haikou, Hainan	70.22 ± 0.03^{j}	4067.67 ± 77.53^{c}	3003.33 ± 80.65^{cd}	1064.33 ± 4.16^{g}	4188.00 ± 88.54^{c}	1184.67 ± 10.97^{efg}

Mean ± SD is reported; different lower case superscript letters in the same column indicate significant differences ($p < 0.05$, n = 3).

Table 4. Thermal properties of chestnut starches

Starch sample No.	Region	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
1	Huairou, Beijing	62.84 ± 0.18^{ef}	67.31 ± 0.26^{g}	71.68 ± 0.31^{j}	0.65 ± 0.00^{n}
2	Jixian, Tianjin	61.45 ± 0.23^{j}	65.49 ± 0.17^{j}	69.87 ± 0.11^{mn}	2.45 ± 0.01^{l}
3	Tangshan, Hebei	62.58 ± 0.14^{fg}	67.29 ± 0.18^{g}	71.85 ± 0.18^{j}	3.44 ± 0.01^{ghi}
4	Qinhuangdao, Hebei	59.85 ± 0.13^{m}	64.15 ± 0.13^{m}	70.51 ± 0.17^{l}	5.32 ± 0.01^{b}
5	Xingtai, Hebei	63.09 ± 0.10^{de}	67.58 ± 0.09^{f}	72.08 ± 0.08^{i}	4.03 ± 0.00^{d}
6	Tai'an, Shandong	63.88 ± 0.06^{c}	67.88 ± 0.04^{e}	71.66 ± 0.10^{j}	1.96 ± 0.00^{m}
7	Linyi, Shandong	62.87 ± 0.09^{ef}	67.36 ± 0.06^{g}	73.24 ± 0.08^{g}	5.52 ± 0.05^{a}
8	Shangluo, Shanxi	61.90 ± 0.06^{hi}	67.33 ± 0.08^{g}	71.83 ± 0.09^{j}	3.62 ± 0.00^{efg}
9	Xinyang, Henan	68.90 ± 0.08^{a}	73.98 ± 0.05^{b}	78.56 ± 0.10^{c}	3.07 ± 0.06^{jk}
10	Anqing, Anhui	63.27 ± 0.07^{d}	67.70 ± 0.09^{ef}	72.98 ± 0.08^{h}	4.19 ± 0.00^{d}
11	Wuxi, Jiangsu	62.03 ± 0.10^{h}	69.46 ± 0.09^{d}	79.19 ± 0.11^{b}	2.37 ± 0.00^{l}
12	Xiangyang, Hubei	62.48 ± 0.09^{g}	66.61 ± 0.09^{h}	71.42 ± 0.09^{k}	3.03 ± 0.00^{k}
13	Wenzhou, Zhejiang	62.84 ± 0.14^{ef}	70.61 ± 0.11^{c}	77.35 ± 0.10^{d}	3.63 ± 0.01^{efg}
14	Nanping, Fujian	61.62 ± 0.66^{ij}	66.23 ± 0.06^{i}	73.42 ± 0.09^{g}	4.23 ± 0.01^{d}
15	Fuzhou, Jiangxi	60.97 ± 0.14^{k}	64.90 ± 0.14^{k}	69.68 ± 0.19^{n}	4.44 ± 0.00^{c}
16	Guilin, Guangxi	59.69 ± 0.12^{m}	64.48 ± 0.12^{l}	68.94 ± 0.12^{o}	3.58 ± 0.00^{fgh}
17	Liuzhou, Guangxi	59.60 ± 0.16^{m}	66.26 ± 0.01^{i}	76.57 ± 0.13^{e}	3.26 ± 0.01^{ij}
18	Kunming, Yunnan	60.34 ± 0.14^{l}	64.00 ± 0.12^{m}	69.07 ± 0.15^{o}	3.73 ± 0.01^{ef}
19	Zhaotong, Yunnan	64.41 ± 0.01^{b}	69.32 ± 0.11^{d}	75.26 ± 0.07^{f}	3.40 ± 0.52^{hi}
20	Yangjiang, Guangdong	63.92 ± 0.10^{c}	74.83 ± 0.14^{a}	81.16 ± 0.13^{a}	4.11 ± 0.00^{d}
21	Haikou, Hainan	59.72 ± 0.06^{m}	65.06 ± 0.14^{k}	70.06 ± 0.11^{m}	3.82 ± 0.01^{e}

Mean ± SD is reported; different lower case superscript letters in the same column indicate significant differences ($p < 0.05$, n = 3).