Changes in the properties of *Radix Aconiti Lateralis Preparata* (Fuzi, processed aconite roots) starch during processing

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Abstract *Radix Aconiti Lateralis Preparata* (Fuzi) is an important, toxic traditional Chinese medicine that has been widely used in clinical practice. Due to the toxicity of its raw materials, it needs to be processed before application. The changes in the physicochemical properties of Fuzi starch during processing were evaluated by scanning electron microscopy, X-ray diffraction and differential scanning calorimetry. The results showed the following: morphological properties changed from spherical to irregular and polygonal particles, while the particle size increased significantly; amylose content and solubility decreased significantly; swelling power and water-binding capacity increased significantly; the X-ray diffraction peak disappeared, and the crystallinity decreased; and the gelatinization temperature and enthalpy decreased significantly. The properties of Fuzi starch were similar to those of pregelatinized starch. These results indicated that Fuzi starch undergone repeated processes of gelatinization and aging, which destroyed the original crystal structure of the starch.

Keywords *Radix Aconiti Lateralis Preparata* · *Aconitum carmichaeli* Debx. · Starch · Processed · Physicochemical characterization

Abbreviations

AM	Amylose
TCM	Traditional Chinese medicine
AOAC	Association of Official Analytical Chemists
WBC	Water-binding capacity

Introduction

Radix Aconiti Lateralis Preparata (also known as Fuzi, aconite root, monkshood root), is the processed root of *Aconitum carmichaeli* Debx. (Ranunculaceae) and is produced mainly in southern China (e.g., Sichuan Province). As an important traditional Chinese medicine (TCM) and a healthcare product, Fuzi has been proved to effectively restore vigor, prevent fainting and restore energy for more than 2000 years (Commission 2015; Murayama et al. 1991; Nyirimigabo et al. 2015; Singhuber et al. 2009). In modern clinical practice, Fuzi has been extensively used to treat hypotension, shock, rheumatic heart disease and coronary heart disease (Murayama et al. 1991; Zhao et al. 2006). However, the raw *Aconitum* root is toxic to the nervous system, circulatory system and digestive system, and should pass through complex processing before application (Chan 2009; Hikino et al. 1977; Jaiswal et al. 2013). Research has shown that Fuzi has a high starch content, and starch is the main constituent of aconite (Xia et al. 2011). During processing, the properties of starch change, which has a significant impact on the quality, storage, and application of Fuzi. Therefore, elucidating the changes in the physicochemical and functional

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properties of starch during the processing of Fuzi is of great value to understand the processing technology of Fuzi.

However, previous research on the processing of Fuzi has focused mainly on the attenuation of toxicity and the maintenance of its curative properties (Liu et al. 2017; Zhao et al. 2014). Although the properties of Fuzi and Aconitum starch were compared by Xia et al. (2011), the changes in starch properties during processing are still not very clear. Additionally, the study of starch properties has concentrated mainly on starch modification and comparison of quality characteristics of starch from different raw materials (Sharma et al. 2015; Xia et al. 2013). Reports on the changes in starch during plant tissue processing are rare.

To clarify the changes of starch properties during Fuzi processing, this work studied the physicochemical, morphological, thermal and crystalline properties of starch from Fuzi samples taken during various processing stages and investigated the variation of starch properties during processing from several aspects. This study provides a technical reference for the theoretical development of aconite processing.

Materials and methods

Materials

Aconitum carmichaeli Debx. was purchased from Sichuan Jiangyou xingling Fuzi Medicinal Corp. (Jiangyou, Sichuan province, China) and identified by Dr. Qingwan Huang, Pharmacy College, Chengdu University of Traditional Chinese Medicine, China.

Processing procedure

Aconitum carmichaeli Debx. was processed into Fuzi, according to the Chinese Pharmacopoeia (Commission 2015).

Step 1 Fresh *Aconitum* was soaked in brine (the main component is calcium chloride, concentration greater than 25%) for 7 days then boiled in water for 20 min.

Step 2 Boiled *Aconitum* was soaked in the water: brine (3:1) mixture overnight. The soaked *Aconitum* was sliced to 0.3–0.5 cm pieces and rinsed with water until *Aconitum* slices changed from yellow to white. Then, *Aconitum* was removed, placed into the same amount of water and rinsed for 2 days.

Step 3 Rinsed *Aconitum* was steamed at 120 °C for 40 min until the surface was shiny. Steamed *Aconitum* was dried by sunlight or by oven-drying.

Isolation of starch

Fifty gram samples taken from different processing stages were crushed and ground with 100 mL of water. The slurry was sieved through a 100-mesh sieve and centrifuged at $3000\times g$ for 10 min to remove the supernatant and the mucus layer on the surface of the precipitate. The precipitate was rinsed repeatedly five to seven times in distilled water by cycles of centrifugation and decanting until the supernatant was transparent. The settled layer was quickly frozen in liquid nitrogen and freeze-dried. The dried samples were subsequently milled and sieved through a 100-mesh sifter.

Physicochemical properties analysis of starch

The starch content of the samples was measured through an Association of Official Analytical Chemists (AOAC) method 996.11 (Latimer 2012). The AM content of the starch isolated from Fuzi was determined by iodine colorimetry using the procedure described by Williams et al. (1970). Swelling power and solubility were determined according to the method of Leach (1959). The WBC of starches was determined using the method described by Medcalf and Gilles (1965).

Scanning electron microscopy (SEM)

The morphology of the starch samples was analyzed under an Inspect F50 scanning electron microscope (FEI, Hillsboro, USA) at an accelerating potential of 10 kV and image capture at $2000\times$ magnification.

Starch particle size analysis

The starch granule size distribution was determined using a Mastersizer 2000 laser diffractometer (Malvern Instruments Ltd., Malvern, UK) at room temperature. A total of 0.02 g of starch was suspended in distilled water and fed directly into the instrument's mixing cell to reach an approximate 2% obscuration level.

X-ray diffraction (XRD)

XRD analysis of the starch samples was conducted using a D8 ADVANCE diffractometer (Bruker, Karlsruhe, Germany) with Ni-filtered Cu-K α radiation (40 kV, 30 mA). The operating conditions were as follows: diffraction angle 2θ range: 4° – 60° ; scanning speed: $3^{\circ}/\text{min}$; step size: 0.02° (Xia et al. 2013). The degree of relative crystallinity of the samples was estimated following the method of Nara and Komiya (1983).

Differential scanning calorimetry (DSC)

The thermal characteristics of the starch samples were measured using a DSC8000 DSC (Perkin-Elmer Co. Norwalk). Each starch sample (2.5 mg, dry weight) was loaded onto an aluminum pan, and 10.0 μL of distilled water was added. The pan was sealed and allowed to stand for 1 h at room temperature. Starch samples were scanned from 20 to 140 $^{\circ}\text{C}$ at a heating rate of 10 $^{\circ}\text{C}/\text{min}$ with an empty pan as a reference. The DSC curve was recorded during the heating process and was calculated based on the onset temperature (T_o), peak temperature (T_p), completion temperature (T_c), as well as the enthalpy change (ΔH) (Wang et al. 2006).

Statistical analysis

All measurements were done in triplicate. Statistical analysis was conducted using one-way ANOVA followed by Duncan's shortest significant range test for multiple mean comparisons by using SPSS 19.0 (SPSS, Chicago, USA). Differences were considered statistically significant at $P < 0.05$.

Results and discussion

Morphological properties and particle size analysis

SEM analysis of the morphological properties of Fuzi starch at each processing step showed very significant differences in the starch granules at different processing stages of the starch (Fig. 1). Most of the natural *Aconitum*

starch granules appeared spherical, similar to the morphology of the starch granules of potato and other rhizomes (Fig. 1a). The Fuzi starch granules swelled and deformed to form lumps in step 1 (Fig. 1b), which may be due to the soaking and cooking processes that caused the Fuzi starches to gelatinize and expand, thus creating an amorphous appearance. At steps 2 and 3, Fuzi starch granules showed a dense, rock-like, irregular morphology (Fig. 1c, d), which may be attributed to aging and recrystallization caused by soaking and rinsing (step 2), as well as gelatinization caused by high-pressure steaming followed by drying (step 3). The morphological changes during processing suggest that the morphology of the natural *Aconitum* starch was completely disintegrated, and the natural crystal structure was completely destroyed, while the resulting starch formed irregular fragment-shaped particles. The morphological property of Fuzi starch was similar to that of the pregelatinized starch (Yan and Zhengbiao 2010).

The average particle size of the samples is shown in Table 1, which demonstrates that the differences in Fuzi starch particle size and diameter distribution at the different processing stages were very significant. The average particle size gradually increased during processing, and the diameter range also gradually increased. Similarly, the unevenness of the particles also increased. These results also reflect that Fuzi starch cannot be dispersed into uniform granules after processing. The variation in starch particle size may be related to the mechanical crushing process during starch preparation, which was confirmed by SEM.

Fig. 1 SEM of starch isolated from Fuzi at each processing stage. **a** *Aconitum*, $\times 2000$; **b** processing step 1, $\times 2000$; **c** processing step 2, $\times 2000$; **d** processing step 3, $\times 2000$

Table 1 Particle size of starch from Fuzi at each processing stage

Samples	Particle size (μm)	D(10) (μm)	D(50) (μm)	D(90) (μm)
<i>Aconitum</i>	25.84 ± 0.25^d	4.25 ± 0.39^c	20.11 ± 0.26^d	55.36 ± 0.31^d
Step 1	64.44 ± 1.56^c	13.38 ± 1.23^b	53.34 ± 1.13^c	130.28 ± 1.76^c
Step 2	117.81 ± 3.45^b	21.64 ± 2.78^a	95.50 ± 3.34^b	247.38 ± 3.12^b
Step 3	139.42 ± 3.51^a	24.64 ± 3.62^a	113.81 ± 3.42^a	296.47 ± 3.78^a

Data were presented as mean $\pm$ SEM ($n = 3$). Data in the same column followed by different superscript letters represent a significant difference at $P < 0.05$

Physicochemical characteristics of Fuzi starches

The measurements of starch content, AM content, swelling power, solubility and WBC of Fuzi starch at each processing stage are summarized in Table 2. The starch content of Fuzi from each processing stage was similar. However, the AM contents changed significantly from step 1 to step 2, with values of 34.3 and 26.7%, respectively, which showed that the rinsing procedure caused a large amount of AM loss. At the same time, protein content significantly decreased from 5.6 to 0.5%, after each successive processing stage. It is similar to the change in protein content of maize starch during commercial wet milling (Singh et al. 2014). The swelling power and WBC increased significantly, while the solubility decreased significantly during the processing. The degree to which starch swells and dissolves in water is affected by its morphology, particle size, crystalline properties, the amylopectin (AP) molecular structure and the AM/AP ratio (Hoover 2001; Singh and Singh 2001). According to Kaur et al. (2002), potato starch of larger particle size and irregular shape has a high swelling power and low solubility. These changes may have resulted from AM content reduction and changes in particle size, a morphological feature of Fuzi starch granules. The dramatic increase in WBC also implies a loose association between amylose and amylopectin molecules in Fuzi starch granules (Soni et al. 1987)).

Crystalline properties

The X-ray diffraction peak intensity and the full width at half maximum (FWHM) reflected the degree of crystallinity of the particles, the degree of amorphousness and the lattice-like changes (Ryland 1958). XRD patterns of the Fuzi starch from each processing stage are presented in Fig. 2. According to the X-ray diffraction pattern, starch can be divided into A, B, C and V types. Compared with the standard diffraction patterns (Krueger et al. 1987; Lee and Osman 1991), *Aconitum* starch displayed a typical B-type pattern, with strong reflection at 2θ 5.8°, 17.2°, 22.1°, and 24.3° and some weak peaks at approximately 2θ 15.2° and 20.3°, similar to potato and other rhizome starch (Buléon et al. 1987). However, the XRD patterns of Fuzi starch changed significantly during processing. The intensity of the diffraction peak was obviously weakened in processing step 1, indicated that the starch crystals were destroyed, and a certain degree of gelatinization occurred after soaking and boiling. Subsequently, the diffraction intensity was enhanced in processing step 2, which shows the starch aging and recrystallization appeared after soaking and rinsing for a long period of time. However, the diffraction peak disappeared in processing step 3, and the degree of crystallinity was seriously reduced. This result implies that high-pressure steaming results in starch gelatinization, and the starch crystalline structure was completely destroyed, thereby transforming the starch into an amorphous structure. Ultimately, the XRD spectra of Fuzi starch were similar to those of pregelatinized starch

Table 2 Starch contents, AM contents, protein contents, swelling power, solubility and WBC of starch isolated from Fuzi at each processing stage

Samples	Starch (%)	AM (%)	Protein (%)	Swelling power (%)	Solubility (%)	WBC (%)
<i>Aconitum</i>	75.3 ± 1.0^a	35.4 ± 0.6^a	5.6 ± 0.6^a	98.5 ± 4.4^c	14.9 ± 0.2^b	83.1 ± 0.9^b
Step 1	76.7 ± 1.1^a	34.3 ± 0.4^a	3.3 ± 0.5^b	66.4 ± 5.6^d	44.4 ± 2.2^a	82.4 ± 2.2^b
Step 2	77.8 ± 0.8^a	26.7 ± 0.8^b	0.7 ± 0.2^c	292.4 ± 6.2^b	6.8 ± 0.4^d	212.8 ± 5.3^a
Step 3	77.4 ± 1.2^a	25.8 ± 0.7^b	0.5 ± 0.1^c	320.3 ± 6.7^a	9.2 ± 0.3^c	215.7 ± 6.7^a

Data were presented as mean $\pm$ SEM ($n = 3$). Data in the same column followed by different superscript letters represent a significant difference at $P < 0.05$

Fig. 2 XRD spectra of starch isolated from Fuzi at each processing stage

(Nakorn et al. 2009) and maize starch during commercial wet milling (Singh et al. 2014). In addition, the degree of crystallinity of *Aconitum* starch started at 34.2%, and then gradually decreased to 22.3% (step 1), 26.7% (step 2) and 13.0% (step 3) after processing.

Thermal properties

Starch gelatinization and aging will cause starch crystals to disintegrate and recrystallize, resulting in changes in the crystal structure and the degree of crystallinity of starch (Zobel et al. 1988). The starch gelatinization process requires additional energy to destroy these crystal structures (Chung and Lim 2006). Therefore, DSC analysis of the heat-induced phase transitions of the starch/water system recorded an endothermic peak, and the peak area is affected by the number of crystals and the structural complexity.

According to the DSC thermograms, the gelatinization onset (T_o), peak (T_p) and completion (T_c) temperatures and gelatinization enthalpy (ΔH) of samples from each processing stage are summarized in Table 3. There were significant changes in the thermal properties of Fuzi starch from each processing stage. The decreasing gelatinization transition temperature and enthalpy in step 1 and step 3 reflect lower crystallinity and more disordered molecules in the starch granules after boiling and steaming. The increasing gelatinization transition temperatures and enthalpy in step 2 suggest that starch aging and recrystallization appeared after soaking and rinsing. These changes may make starch granules more gelatinized, resulting in earlier and lower gelatinization temperature (Tester and

Table 3 Thermal properties of starch from Fuzi at each processing stage

Samples	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
<i>Aconitum</i>	40.9 ± 0.2^a	77.2 ± 0.2^a	98.7 ± 0.3^a	1.77 ± 0.08^a
Step 1	33.1 ± 0.1^c	65.9 ± 0.3^c	96.5 ± 0.4^c	0.97 ± 0.05^c
Step 2	39.8 ± 0.3^b	74.9 ± 0.3^b	97.6 ± 0.4^b	1.49 ± 0.06^b
Step 3	33.4 ± 0.2^c	61.6 ± 0.2^d	89.0 ± 0.3^d	0.71 ± 0.06^d

Data were presented as mean $\pm$ SEM ($n = 3$). Data in the same column followed by different superscript letters represent a significant difference at $P < 0.05$

Morrison 1990). These results were in agreement with the degree of crystallinity found in XRD.

It has been reported that the difference in gelatinization temperature may be due to differences in amylose content, size, form and distribution of granules, as well as the molecular architecture of the crystalline region (Jane et al. 1999; Stevens and Elton 1971; Yuan et al. 1993). In addition, changes in enthalpy value have been attributed to the granule shape, the percentage of large and small granules and the presence of phosphate esters (Stevens and Elton 1971; Yuan et al. 1993). As processing progressed, Fuzi starch showed lower crystallinity, AM content and protein content, and a more disordered structure. The energy required to destroy the organized helical complex structure formed by the interactions between AM–AM and AM–AP chains during gelatinization was greatly reduced (Jane et al. 1999; Jayakody and Hoover 2002). In addition, according to Singh et al. (2014), the starch with higher content of protein had more stable complex lipids structure. During Fuzi processing, a significant decrease in protein content inhibits the formation of this stable composite structure. Therefore, Fuzi starch has a much smaller gelatinization enthalpy than that of *Aconitum* starch.

Conclusion

During processing, the properties of Fuzi starch changed significantly, including morphological property changes from spherical to irregular and polygonal particles, significant particle size increase, significant AM content, protein content and solubility decreases, significant swelling power and WBC increases, X-ray diffraction peak disappearance and crystallinity decrease, and significant gelatinization temperature and enthalpy decreases. Fuzi starch showed similar properties to those of pregelatinized starch. These results indicate that Fuzi starch undergoes the repeated processes of gelatinization and aging, which destroy the original crystal structure of the starch. These

changes of starch properties are conducive to reduce the loss of active ingredients of Fuzi during storage and clinical application. At the same time, these changes in starch granules can be used for the identification of Fuzi based on starch structure characteristics.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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