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Crystalline, thermal and textural characteristics of starches isolated from chestnut (*Castanea mollissima* Bl.) seeds at different degrees of hardness

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ABSTRACT

The crystalline, thermal and textural properties of chestnut starches (CSs I–V) at 0%, 25%, 50%, 75% and 100% degrees of hardening (DHs) were investigated in this work. CS I had a structure of C-type crystallinity. With the increase of DH, more B-type crystalline regions and amylose–lipid complexes were formed. The gelatinisation temperature of CS and melting temperature of amylose–lipid complexes were not significantly changed as DH increased. However, a significant ($P < 0.05$) increase of ΔH was observed for CS V. The texture analysis indicated that CS V had a lower firmness, adhesiveness and a higher cohesiveness than CS I. The correlation test confirmed the significant correlations between ΔHs of gelatinisation and melting of amylose–lipid complexes, firmness, adhesiveness, cohesiveness and DH. All the results indicated that degradation of CS should be one of important mechanisms for the hardening of chestnut, especially in the late period of hardening.

1. Introduction

The chestnut has a growing history of over 2000 years in China with an important role in the economy. It is also one of the oldest edible seeds in northern hemisphere, which was consumed as extensively as the potato in the past (Ferreira-Cardoso, Rodrigues, Gomes, Sequeira, & Torres-Perreira, 1999). Literature suggests that chestnut is a good source of bioactive substances including lectin, cysteine proteinase inhibitor and quercetin (Wang & Ng, 2003). It also comprises of considerable levels of vitamins, fibres, essential fatty acids and minerals (Borges, de Carvalho, Correia, & Silva, 2007). There is increasing evidence that shows that the consumption of chestnuts has become more important in human nutrition due to the health benefits provided by the antioxidants present (Blomhoff, Carlsen, Andersen, & Jacobs, 2006).

Hardening is a common physiological disorder during the storage of chestnut. It deteriorates the edible quality significantly. Therefore, an investigation into the hardening mechanism will be useful for avoiding quality deterioration of chestnuts and other foods which have the same behaviour. The loss of moisture and disruption of cell wall during the storage, are two processes which are responsible for the hardening of chestnut (Yu, Tan, Zhou, & He, 2008). Polysaccharides play an important role in the growth and development of living organisms (Yang et al., 2009), while starch is one of the principal forms. Starch is organised in a concentric alternate semi-crystalline and amorphous layers as granules of various sizes in the plant cell (Svihus, Uhlen, & Harstad, 2005). It is the main nutrient in the chestnut fruit with a content range from 50% to 80% (Miguelez, Bernárdez, & Queijeiro, 2004). During the hardening of chestnut, the loss of moisture and disruption of the cell wall might further affect the physical and chemical properties of the chestnut starch (CS). It will be an interesting attempt to investigate the change in physical property of CS at different degrees of hardening (DHs). The results can explain the microenvironmental change during hardening and elucidate the possible mechanism of chestnut hardening from starch. Until now, relevant literature regarding this topic is very limited. Therefore, the objective of this work was to extract starches from chestnuts at different DHs and to determine their crystalline, thermal and textural properties. These results could disclose the property change of CS during hardening and explain the hardening mechanism from the view of starch.

2. Materials and methods

2.1. Plant materials

Chinese chestnut (*Castanea mollissima* Bl.) seeds at different DHs were donated by Guangzhou University (Guangzhou, China). These fruits were peeled manually and cut into half. The colour of cross-section was recorded for the evaluation of DH. A well-trained panel, consisting of six persons, was employed to make the evaluation of DH. Five DH levels were set as 0 (no hardened

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area), 25 (one-quarter hardened area of the cross-section), 50 (half hardened area of the cross-section), 75 (three-quarter hardened area of the cross-section) and 100% (fully hardened area). The pulp was pulverised by a miller (LK-200A, Wenlin Chuangli Medicine mechanics Company, Wenlin, China) and screened through a 100-mesh iron sieve.

2.2. Chemicals

Sodium sulphite and sodium chloride was obtained from Guangzhou Reagent Co. (Guangzhou, China). The water was prepared by Milli-Q synthesis system (Millipore, Guangzhou, China).

2.3. Extraction of CS

The extraction of CS was according to the method of Singh and Singh (2001) with minor modifications. The chestnut powder (10 g) was added into 100 ml of distilled water with 0.5% (w/w) sodium sulphite to avoid browning. The slurry was filtered through 200-mesh stainless seive. The residue left on the seive was washed with water, for three times. The filtrates were combined and precipitated over night at 4 °C. The supernatant was discarded and the crude starch was cleaned with distilled water and subjected to above precipitation program for three times. The CS was collected and dried at 40 °C in an oven (NJ101, Wanneng Heating Instrument Company, Nanjing, China). The starch extracted from chestnut at 0%, 25%, 50%, 75% and 100% DHs were CSs I–V, respectively.

2.4. Analysis of crystallinity

The crystallinity of CS was analysed by the method of Jouquand, Ducruet, and Bail (2006). CSs I–V were equilibrated above a saturated sodium chloride solution at 25 °C. The wide angle X-ray diffraction pattern was measured by an automatic X-ray diffractometer (D/max-IIIA, Rigaku, Tokyo, Japan). A voltage of 35 kV and electric current of 30 mA were set. Cu Kα-radiation (λ = 1.54 nm) was selected using a quartz monochromator. The diffraction intensity in the range of 4–30° 2θ was monitored.

2.5. Analysis of thermal property

The measurement of thermal property was carried out according to the method of Yang, Jiang, Zhao, Shi, and Wang (2008). A Diamond differential scanning calorimeter (PerkinElmer, Massachusetts, USA) was used for analysis. CSs I–V were mixed with fivefold distilled water, respectively. The mixture (10 mg) was weighed and then filled in an aluminium pan. The pan was sealed hermetically and heated from 30 to 120 °C at a rate of 10 °C/min. The *To*, *Tp*, *Tc* and ΔH were recorded. *To*, *Tp*, *Tc* are the onset, peak and conclusion temperatures during the gelatinisation of starch or the melting of amylose–lipid complexes. ΔH represents the required energy for disrupting hydrogen bonds within the crystalline zones.

2.6. Analysis of textural characteristics

Measurement of force in compression was performed with the method of Zhao, Zhao, Yang, and Cui (2009). A TA-XT 2i texturometer (Stable Microsystems, Surrey, UK) was used to determine the textural characteristics of CSs I–V at room temperature (25 °C). Two grams of CS were added into water to a final concentration of 6% (w/w). The slurry was gelatinised from room temperature to 95 °C at 5 °C/min with agitation by a magnetic stirrer, then holding for 15 min, finally cooling naturally to room temperature, and holding for 1 h. A cylinder probe with a diameter of 6 mm was used to penetrate into the sample to a depth of 20 mm at a rate of 1.0 mm/s and the force exerted on the probe was automatically recorded. Seven parameters (firmness, adhesiveness, springiness, cohesiveness, gumminess and chewiness) were recorded. The compression was repeated twice to generate a force–time curve. Firmness is the height of the first peak; Adhesiveness is calculated by the negative area of the curve during retraction of the probe. Springiness is the ratio between the recovered height after the first compression and the original gel height. Cohesiveness is the ratio between the area under the second peak and that under the first peak. Gumminess is determined by multiplying firmness and cohesiveness. Chewiness is determined by multiplying gumminess and springiness. All the tests were done in triplicates.

2.7. Statistical analyses

Data were expressed as mean ± standard deviation of three replicated determinations. One way of variance analysis was applied for determining significant difference at $P < 0.05$. Statistical analysis software SPSS Version 10.0 (SPSS Inc., Chicago, Illinois, USA) was used to analyse the correlation coefficient.

3. Results and discussion

3.1. X-ray diffraction pattern of CS

Starch granule is generally classified into three types (A, B and C) based on the X-ray diffraction pattern given by the amylopectin crystalline structure (Kubo et al., 2008). Cereal starch often has an A-type X-ray diffraction pattern, while tuber starch has a B-type and bean starch gets a C-type pattern, a joint presences of A- and B-types (Cairns, Bogracheva, Ring, Hedley, & Morris, 1997). The amylopectin fraction of starch accounts for its crystallinity. The starch with longer amylopectin branches shows B-type diffraction behaviour, while short branch length concides with A-type crystallinity (Hizukuri, 1986). Moreover, the branch pattern also affects the crystallinity type (Jane, Wong, & McPherson, 1997).

As shown in Fig. 1, CS I displayed strong reflections at 15.0, 17.1, 17.9 and 23.0° 2θ which were typical peaks of A-type diffraction pattern. A weak diffraction peak was detected at 5.6° 2θ and the 17.1° 2θ peak was somewhat more intense than the 17.9° 2θ neighbour. These behaviours indicated the occurrence of B-type crystalline regions in CS I (Vermeylen, Goderis, Reynaers, & Delcour, 2004). Therefore, the diffraction pattern of CS I was C-type. The additional peak at 20.0° 2θ indicated the crystalline amylose–lipid complexes. This peak has also been observed earlier for starches of

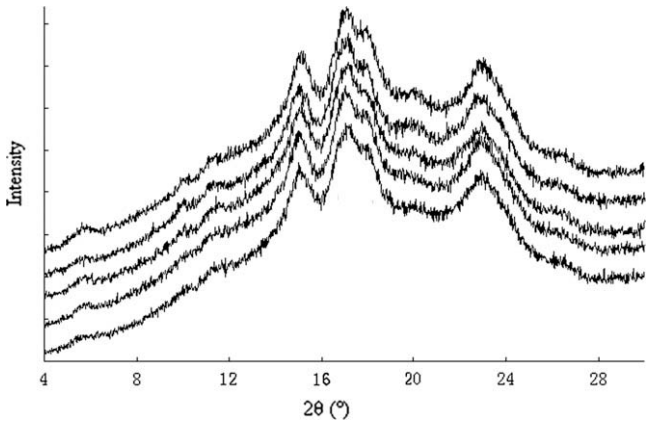

Fig. 1. X-ray diffractograms of CSs I–V at different DHs. From bottom up, they are CSs I, II, III, IV and V, respectively.

several maize genotypes, like dull, sugary and amylose extender by Zobel (1988). With the increase of DH, the diffraction peaks at 15.0°, 17.1°, 17.9° and 23.0° 2θ showed no apparent change. However, obvious increases of 5.6° and 20.0° 2θ peaks could be observed as the DH increased. The increase of 5.6° 2θ peak represented the transformation of A-type or amorphous region into B-type crystalline regions. The increase of 20.0° 2θ peak indicated more crystalline amylose–lipid complexes were generated. It is hypothesised that the break of glycosidic linkages in amylose and amylopectin by endo-amylases renders them sufficient mobility to become organised in more perfect or larger crystalline regions. This behaviour is analogous to the effect of mild acid hydrolysis. Waigh, Perry, Riekel, Gidley, and Donald (1998) have suggested that decoupling of double helices from the amylopectin promotes the formation of larger and/or more perfect crystallites. Therefore, we can postulate that the hardening of chestnut is accompanied by the formation of more B-type crystalline regions and crystalline amylose–lipid complexes. In turn, these behaviours result in the quality deterioration of chestnut.

3.2. Thermal properties of CSs I–V

The thermal properties of CSs I–V were determined by differential scanning calorimetry (DSC) in the range of 30–120 °C. Table 1 shows the *To*, *Tp*, *Tc* and ΔH values of CSs I–V, including the endothermal characteristics of gelatinisation of starch and melting of amylose–lipid complexes. For the starch of fresh chestnut (CS I), the *To*, *Tp* and *Tc* values of gelatinisation were 64.3, 68.7 and 76.5 °C, respectively. The ΔH of gelatinisation was determined to be 11.6 J/g. A small endothermal peak with a ΔH value of 0.05 J/g was detected for the melting of amylose–lipid complexes in CS I. Due to the small endotherm for the melting of amylose–lipid complexes, only *Tp* values are listed in Table 1. The *Tp* value of CS I was 106.1 °C. After measurement of CSs at different DHs, it was clear that no significant ($P > 0.05$) change in *To*, *Tp* and *Tc* values of gelatinisation and melting of amylose–lipid complexes was detected, when comparing CS II–V with CS I. The ΔH values of gelatinisation or melting of amylose–lipid complexes were also not significantly ($P > 0.05$) different between CS I–IV. However, a significant ($P < 0.05$) improvement of ΔH values for both gelatinisation (15.1 J/g) and melting of amylose–lipid complexes (0.2 J/g) were detected when comparing CS V with other CS samples.

Due to the semi-crystalline nature of starch, glass transition of the amorphous region and melting of crystalline region occur during the thermal treatment of starch/water system (Maache-Rezzoug, Zarguili, Loisel, Queveau, & Buléon, 2008). DSC is an effective technique for analysing the melting characteristics of crystallites (Garcia et al., 1996). The melting temperature of starch slurry depends on the water content. When the water content is greater than 60% (w/w), only one endotherm is observed in the DSC thermogram and the melting temperature refers to gelatinisation temperature of starch. When a lower water content is chosen, multiple transition endotherms occur which reflect the melting and recrystallisation behaviours during thermal treatment (Biliaderis, Page, Maurice, & Juliano, 1986). In this work, the water content of starch/water system for DSC analysis was 83.3%, which was higher than 60%. Therefore, only one endotherm was observed for gelatinisation of CS. The *To*, *Tp* and *Tc* values reflected the gelatinisation characteristics of CS. The *Tp* value of CS was little higher than potato starch (ca. 66 °C) (Liu, Tarn, Lynch, & Skjodt, 2007) and much higher than wheat starch (ca. 60 °C) (Wickramasinghe, Miura, Yamauchi, & Noda, 2005).

Hardening is a physiological disorder of chestnut. It concerns the degradation of cell wall. As the major component of chestnut fruit, the characteristics of starch might be impacted during the degradation of cell wall. From the DSC thermograms of CSs I–V, the gelatinisation temperature showed no significant change with the increase of DH. However, CS V with DH of 100% had a significantly higher ΔH values of gelatinisation and melting of amylose–lipid complexes than CSs I–IV. It is known that ΔH indicates the required energy for disrupting hydrogen bonds within the crystalline zones (Lazaridou & Biliaderis, 2004). A stronger carbohydrate/water interaction and better organised microstructure lead to a higher ΔH value (Chung, Lee, & Lim, 2002). The higher ΔH value of gelatinisation for CS V suggested that a better crystalline region was generated during the hardening, which might be due to the degradation of endo-amylase. The improved ΔH value for the melting of amylose–lipid complexes represented a better organised and/or more regions had developed. Moreover, no significant difference was observed for DSC endotherms between CSs I–IV. It was presumed that the degradation of starch only took place at the late period of hardening.

3.3. Textural characteristics of CSs I–V

The textural characteristics of CSs I-V gels were determined by a texture analyser. The results of firmness, adhesiveness, springiness, cohesiveness, gumminess and chewiness are shown in Table 2. CS I was the starch extracted from fresh chestnut. It had a firmness of 0.52 g, adhesiveness of −24.09 g × s, springiness of 0.78 mm, cohesiveness of 0.13 g, gumminess of 0.13 g and chewiness of 0.11 g × mm. The increase of DH was accompanied with changes in these parameters. The firmness values of CSs IV (0.39 g) and V (0.38 g) were significantly ($P < 0.05$) lower than those of CSs I–III. Moreover, CSs IV and V had significantly ($P < 0.05$) lower adhesiveness values (−8.35 and −7.43 g × s) than CSs I–III. Only CS V showed a significantly ($P < 0.05$) higher cohesiveness value (0.38) than CSs I–IV. However, no significant ($P > 0.05$) difference for springiness, gumminess and chewiness was observed between CSs I–V. These results indicated that at the beginning period of hardening, the textural characteristics of CS was kept unchanged, comparing with native starch (SC I). However, at the late period of hardening, when the DH was up to 100%, changes in some textural characteristics occurred, like the decrease or increase of firmness, adhesiveness or cohesiveness.

The mechanical characteristics of starch gel are influenced by rheological properties of amylose matrix, volume fraction and rigidity of gelatinised starch granules, and interactions between

Table 1
To, *Tp*, *Tc* and ΔH values of gelatinisation and melting of amylose–lipid complexes.

CS	Gelatinisation				Melting of amylosel–lipid complexes	
	To (°C)	*Tp* (°C)	*Tc* (°C)	ΔH (J/g)	*Tp* (°C)	ΔH (J/g)
CS I	64.3 ± 0.2a	68.7 ± 0.4a	76.5 ± 0.9a	11.6 ± 0.5a	106.1 ± 0 .4a	0.05 ± 0.01a
CS II	64.6 ± 0.5a	69.1 ± 0.7a	76.5 ± 0.5a	11.3 ± 0.6a	106.1 ± 0.4a	0.05 ± 0.01a
CS III	63.9 ± 0.6a	68.4 ± 0.5a	76.8 ± 0.6a	11.2 ± 0.6a	105.8 ± 0.6a	0.05 ± 0.01a
CS IV	64.4 ± 0.3a	69.0 ± 0.6a	77.0 ± 0.5a	10.8 ± 0.4a	105.9 ± 0.2a	0.07 ± 0.01a
CS V	64.5 ± 0.5a	69.1 ± 0.6a	76.5 ± 0.5a	15.1 ± 0.9b	107.2 ± 0.5a	0.20 ± 0.03b

The values that have different letters in the same column are significantly ($P < 0.05$) different.

Table 2
Textural characteristics of CSs I–V*.

	Firmness (g)	Adhesiveness (g × s)	Springiness (mm)	Cohesiveness	Gumminess (g)	Chewiness (g × mm)
CS1	0.52 ± 0.01a	−24.09 ± 0.17a	0.78 ± 0.18a	0.26 ± 0.05a	0.13 ± 0.02a	0.11 ± 0.04a
CS2	0.58 ± 0.08a	−26.00 ± 1.27a	0.90 ± 0.01a	0.25 ± 0.01a	0.14 ± 0.02a	0.13 ± 0.02a
CS3	0.52 ± 0.01a	−13.52 ± 0.52b	0.87 ± 0.09a	0.24 ± 0.01a	0.12 ± 0.01a	0.12 ± 0.01a
CS4	0.39 ± 0.04b	−8.35 ± 1.04c	0.94 ± 0.03a	0.23 ± 0.01a	0.09 ± 0.02a	0.09 ± 0.01a
CS5	0.38 ± 0.02b	−7.43 ± 0.70c	0.90 ± 0.03a	0.38 ± 0.03b	0.14 ± 0.02a	0.13 ± 0.01a

* The values that have different letters in the same column are significantly ($P < 0.05$) different.

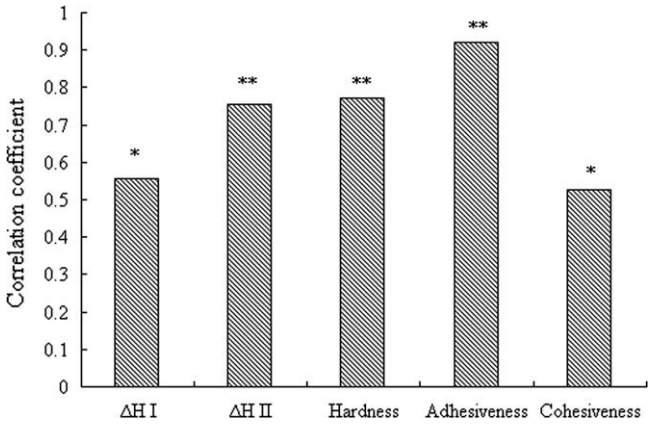

Fig. 2. Correlation coefficient between DH and other determined parameters. ΔH I is the ΔH of starch gelatinisation; ΔH II is the ΔH for the melting of amylose–lipid complexes. The correlation coefficient between DH and firmness is shown as an absolute value. $^{*}P < 0.05$; $^{**}P < 0.01$.

dispersed and continuous phase of gel, which further depend on the structure of amylose and amylopectin (Sandhu & Singh, 2007). Firmness represents the force necessary to attain a given deformation. The gel firmness is mainly affected by retrogradation of starch gel, which is associated with the syneresis of water and crystallisation of amylopectin, leading to a harder gel (Miles, Morris, Orford, & Ring, 1985). Starch exhibiting a harder gel tends to have a higher amylose content and longer amylopectin chains (Mua & Jackson, 1997). From the results obtained in this work, a decrease of firmness was found for CSs IV and V, comparing with CSs I–III, which indicated a correlation between DH and firmness. Degradation of cell wall is one of pathways involved in the hardening of chestnut. It is possible that this degradation induces the activation of endo-amylases, which further decomposes the starch in the cell. This could be the possible mechanism resulting in the decrease of firmness of CS.

Cohesiveness is the extent to which a material can be deformed before rupturing (Szczesniak, 2002). Adhesiveness is generally considered a surface characteristic. It positively relies on the combined role of adhesive and cohesive forces (Adhikari, Howes, Bhandari, & Truong, 2001). An increase of cohesiveness and a decrease of adhesiveness was detected for CS V at 100% DH. This suggested that the decrease of adhesive force was more significant than the increase of cohesive force. The apparent change in physical properties of starch granule should be attributed to the changes in chemical structure and microenvironment. The degradation of amylose and amylopectin by endo-amylases to some extent during hardening might be responsible for the changes in textural properties.

Springiness represents the extent of gel height recovery which is also called as elasticity (Choi & Kerr, 2003). It was found to have no significant change between CSs at different DHs, which indicated that the degradation of amylose and amylopectin was not significant. From the calculation equation, gumminess is the multiplication of firmness and cohesiveness. Chewiness is a parameter to stimulate the energy required for masticating a semi-solid sample to a steady state of swallowing, which is calculated as the multiplication of gumminess and springiness. Therefore, it is easy to understand that no significant change had taken place.

3.4. Correlation test

Correlation test is important to confirm the correlation between two variables. To confirm the significant correlation between hardening and physical properties of CS, the correlation coefficients between ΔHs of gelatinisation and melting of amylose–lipid complexes, firmness, adhesiveness, cohesiveness and DH were calculated. As shown in Fig. 2, the results indicated that the change in ΔHs of gelatinisation (0.557, $P < 0.05$) and melting of amylose–lipid complexes (0.755, $P < 0.01$) were significantly correlated with DH. Significant positive correlations were determined for adhesiveness (0.922, $P < 0.01$) and cohesiveness (0.525, $P < 0.05$) while significant negative correlation was obtained for firmness (−0.773, $P < 0.01$). These results proved that the change in physical properties of CS was one of the mechanisms involved in the hardening of chestnut.

4. Conclusions

Determinations of crystalline, thermal and textural properties could well reflect the changes in physical property of CSs at different DHs. The CS I had strong reflections at 15.0°, 17.1°, 17.9° and 23.0° 2θ and a weak diffraction peak at 5.6° 2θ. With the increase of DH, two stronger peaks at 5.6° and 20.0° 2θ were observed, which represented increases of B-type crystalline regions and crystalline amylose–lipid complexes, respectively. The gelatinisation temperature of CS and melting temperature of amylose–lipid complexes were not changed significantly as the DH increased. However, a significant increase of ΔH value was observed for CS V, comparing with CSs I–IV. The test of textural characteristics indicated that CS V had a lower firmness, adhesiveness and a higher cohesiveness than CS I. The change of physical structure, like the increases of B-type crystalline regions and crystalline amylose–lipid complexes in CS V, might be responsible for the changes in textural characteristics. The calculation of correlation coefficient confirmed the significant correlation between ΔHs of gelatinisation and melting of amylose–lipid complexes, firmness, adhesiveness, cohesiveness and DH. All the results indicated that degradation of CS should be one of mechanisms for the hardening of chestnut, especially in the late period of hardening. Further investigation of the structural change in CSs at different DHs could be carried out as future work, to better explain the mechanism of chestnut hardening.

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