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Structural changes in polysaccharides isolated from chestnut (*Castanea mollissima* Bl.) fruit at different degrees of hardening

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ABSTRACT

Hardening is an important physiological disorder of chestnut fruit during storage. In this study, polysaccharides of chestnut (PCs) at 0%, 50% and 100% degrees of hardening (DH) were extracted and then sequentially fractionated using 40% and 80% ethanol in order to obtain PCs I and II. For fresh chestnut, arabinose, fructose, glucose and galactose were the main monosaccharides of PCs I and II. Significantly positive correlations ($P < 0.01$) existed between xylose, galactose, arabinose contents and DH, while significantly negative correlations ($P < 0.01$) were present between glucose, fructose contents and DH for both PCs I and II. The appearance in hardening of chestnut fruit was accompanied with decreases in the contents of 1,3-fructose, 1,3- and 1,6-glucose, and increases of 1,4-arabinose, 1,6-galactose and 1,3-xylose. The results confirmed that cell wall degradation of chestnut induced the structural changes in PCs I and II, which further led to the occurrence of hardening.

1. Introduction

Chestnut is an important edible fruit in northern hemisphere, which has been consumed as extensively as potato in the past (Ferreira-Cardoso, Rodrigues, Gomes, Sequeira, & Torres-Perreira, 1999). It has a long growing history of over 2000 years in China with an important role in the economy. Literatures suggest that chestnut is a good source of bioactive substances including lectin, cysteine, proteinase inhibitor and quercetin (Wang & Ng, 2003). It also contains considerable vitamins, fibres, essential fatty acids and minerals (Borges, de Carvalho, Correia, & Silva, 2007; Borges, Gonçalves, de Carvalho, Correia, & Silva, 2008). Increasing evidences show that the consumption of chestnut become more important in human nutrition due to the health protection provided by the antioxidants (Blomhoff, Carlsen, Andersen, & Jacobs, 2006).

Chestnut hardening is a commonly physiological disorder during storage and deteriorates seriously edible quality. It can be observed by the colour of chestnut. The formation of lime-white region indicates the occurrence of hardening. Loss of moisture and disruption of cell walls during storage are two possible processes responsible for the hardening of chestnut (Yang, Jiang, Prasad, Gu, & Jiang, in press). However, understanding of this disorder is still unclear and requires to be investigated. It is well known that polysaccharides are the most important component of cell wall (Yang, Jiang, Wang, Zhao, & Sun, 2009a; Zhao, Yang, Yang, Jiang, & Zhang, 2007). The disruption of cell wall during the storage of chestnut might affect the compositions and structural characteristics of polysaccharides. Elucidation of the structural changes in polysaccharides of chestnut (PC) during storage will be helpful to understand the occurrence of hardening. Therefore, the objective of this work was to extract polysaccharides from chestnut at various degrees of hardening (DH) and then to identify their structures. The changes in monosaccharide composition and glycosidic linkage of PC were also determined to further evaluate the hardening disorder of chestnut during storage.

2. Materials and methods

2.1. Plant materials

Chinese chestnut (*Castanea mollissima* Bl.) fruits at different degrees of hardening 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 of six persons was employed for the evaluation. Three DH levels were set as 0%, 50% and 100%. DH of 0% was defined that no lime-white region was observed from the cross-section of chestnut, while DH of 50% was that half area of the cross-section appeared as lime-white, DH of 100% was that the

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whole cross-section appeared as lime-white. These chestnut fruits were pulverized by a miller and then screened through a 100-mesh iron sieve.

2.2. Chemicals

Ethanol, phenol and sulphuric acid were obtained from Guangzhou Reagent Co. (Guangzhou, China). Standards of xylose, arabinose, glucose, galactose, fructose and rhamnose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the other chemicals used were of analytical grade.

2.3. Extraction and quantification of PC

PC was extracted with hot water by the method of Yang et al. (2009b). The fine chestnut seed powder (5 g) was extracted for 120 min with 100 ml of distilled water in a 150-ml conical flask submerged in a water bath at 55 °C. The extract was filtered through a Whatman No. 1 paper (Whatman Plc., Shanghai, China) and then concentrated to 25 ml using a vacuum rotary evaporator (BC-R203, Shanghai Biochemical Equipment Co., Shanghai, China) at 65 °C. The proteins in the extract were removed by Sevag reagent (Navarini et al., 1999). Then, anhydrous ethanol was added into the extract to obtain a final concentration of 40% and maintained overnight at 4 °C to precipitate large molecular-weight polysaccharides (PC I), which was then obtained after centrifugation at 4000*g* for 15 min. Ethanol was then added into the supernatant to obtain a final concentration of 80%. The above programme was repeated to obtain small molecular-weight polysaccharides (PC II).

The content of polysaccharides was determined by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and expressed as glucose equivalent. Protein content was calculated from nitrogen content ($N \times 6.25$), which was determined by a PE-2400 series II automatic elemental analyser (Perkin-Elmer, Waltham, Massachusett, USA). The moisture and ash contents of PCs I and II were also determined according to the method of Singthong, Ningsanond, and Cui (2009).

2.4. Analysis of monosaccharide composition

PCs I or II (10 mg) was hydrolysed for 4 h with 10 ml of 2 M trifluoroacetic acid at 100 °C (Erbing, Jansson, Widmalm, & Nimmich, 1995). Derivatisation of the released monosaccharides was then carried out using trimethylsilylation reagent according to the method of Guentas et al. (2001). The trimethylsilylated derivatives were loaded onto a HP-5 capillary column and then determined by a flame ionization detector. The following programme was used for gas chromatography analysis: injection temperature, 230 °C; detector temperature, 230 °C; column temperature programmed from 130 to 180 °C at 2 °C/min, holding for 3 min at 180 °C, then increasing to 220 °C at 10 °C/min and finally holding for 3 min at 220 °C. N_2 was used as the carrier gas and maintained at 40.0 ml/min. The speeds of air and H_2 were 400 and 40 ml/min, respectively. The split ratio was set as 10:1. Inositol was used as the internal standard.

2.5. Methylation analysis

Methylation of PCs I or II was carried out by the method of Needs and Sevendran (1993) with minor modification. Five milligrams of sample was weighted precisely and then dissolved in 5.0 ml of dimethyl sulfoxide before 200 mg of NaOH was added. The mixture was treated for 10 min at 120 W by ultrasonic wave using an ultrasonic cleaner (KQ-300DE, 40 kHz, Kunshan Ultrasonic Equipment Co., Kunshan, China). After 1 h of incubation at room temperature (25 °C), 1.5 ml of methyl iodide were added for methylation. The sample solution was kept for 1 h in dark before 4 ml of distilled water was used to decompose the remained methyl iodide. The methylated polysaccharides were extracted with 3×2 ml of chloroform and then dried at a low pressure by a rotary evaporator (RE52AA, Yarong Instrument Co., Shanghai, China). After hydrolysis with 10 ml of 2 M trifluoroacetic acid, the PC hydrolysates were dissolved in 4 ml of 1% (w/w) NaOH. Twenty milligrams of $NaBH_4$ was added to reduce the uronic acid and hemiacetal bond. After 30 min of incubation at 40 °C, one hundred microlitres of glacial acetic acid were used to terminate the reduction. The sample was dried under low pressure, and then acetylated with 2 ml of acetic anhydride and 2 ml of pyridine. The reaction was kept at 100 °C for 1 h. Two millilitres of distilled water was used to hydrolyse the remained acetic anhydride. The acetylated derivatives were extracted with 4 ml of methylene chloride. A gas chromatography/mass spectrometer (GCMS-QP 2010, Shimadzu, Kyoto, Japan) was used to analyse the glycosidic linkage. The acetylated derivatives were loaded into a HP-1 capillary column. The temperature programme was set as follows: the initial temperature of column was 150 °C, increased to 180 °C at 10 °C/min, then from 180 to 260 °C at 15 °C/min, holding for 5 min at 260 °C, with an injection temperature of 220 °C. The ion source of mass spectrometer was set at 200 °C. One microlitre of sample was injected, with a split ratio of 50:1.

2.6. Statistical analysis

Data were expressed as mean ± standard deviation of three replicated determinations. One way of variance analysis was applied for determining the 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 between relative molar percentage of monosaccharide, glycosidic linkage and DH.

3. Results and discussion

3.1. Chemical compositions of PCs I and II

Ethanol precipitation is a useful method for fractionating water-soluble polysaccharides in terms of molecular weight (Yang, Zhao, Shi, Yang, & Jiang, 2008). The water-soluble polysaccharides with high molecular weight are precipitated in ethanol with low concentration, while those having low molecular weight can only be precipitated by high-concentration ethanol. In this work, hot water extraction was used to prepare water-soluble polysaccharides from chestnut with different DHs (0%, 50% and 100%). Fractionation by ethanol solution led to the precipitation of PCs I and II. Table 1 shows the chemical compositions of PCs I and II. The protein contents of all the PC fractions were close to 2.0%. Peña-Méndez, Hernández-Suárez, Díaz-Romero, and Rodríguez-Rodríguez (2008) have determined the chemical compositions of various chestnut cultivars and have found the total proteins account for approximately 3% of the fresh weight of chestnut. During the extraction, proteins are usually co-precipitated with the PC fraction in ethanol solution. The Sevag reagent effectively removed most of the proteins from the PC fraction. This explains that only few proteins were detected in PCs I and II. Small amount of ash was also detected in the PC fraction. The ash content of PC I at 0% DH was 2.2%, while that of PC II at 0% DH was 3.1%. The mineral content of chestnut ranged from 1% to 3% on dry weight basis, mainly including K, Mg, P and Ca (Miguelez, Bernárdez, & Queijeiro, 2004). Furthermore, the carbohydrate contents of PC I (or PC II) at DHs of 0% and 50% were not significantly different ($P > 0.05$).