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Ultra-high pressure treatment effects on polysaccharides and lignins of longan fruit pericarp

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ABSTRACT

Longan fruit pericarp was subjected to ultra-high pressure treatment. The yields of water-soluble polysaccharides, alkali-soluble polysaccharides and cellulose were comparatively analysed before and after ultra-high pressure treatment. A negative relationship was observed between pressure and water-soluble polysaccharide yield. The lowest yield (6.4 ± 0.6 mg/g) was obtained at 500 MPa. No significant differences ($P > 0.05$) in alkali-soluble polysaccharide and cellulose yields was found between the ultra-high pressure-treated and non-treated samples (control). Furthermore, a similar phenomena was observed for cellulose. The degrees of hydrolysis (DH) of control and 500 MPa-treated cellulose were 26.6% and 29.4%, respectively, and there was a significant difference ($P < 0.05$) between them. The degradation and oxidation of lignins were analysed using high performance liquid chromatography, and four main peaks appeared. A comparative profile suggested that ultra-high pressure treatment could not result in a change in the lignin composition.

1. Introduction

Longan (*Dimocarpus longan* Lour.) is an important subtropical fruit in Asia and other countries (Jiang, Zhang, Joyce, & Ketsa, 2002). It has also been used as a Chinese traditional medicine since ancient times. Great attention has been paid to this fruit for its many health effects (Yang, Zhao, Shi, Yang, & Jiang, 2008). Polysaccharides (e.g. hemicellulose and cellulose) and lignins in longan fruit pericarp tissues might be responsible for these effects. Water-soluble and alkali-soluble polysaccharides are important components of cellular walls, next to cellulose. In recent years, there has been an increasing interest in utilising these polysaccharides from plant sources in medicine and cosmetics. The health effects of plant polysaccharides in human diet include anti-cancer effects, immuno modulation, anti-bacterial and anti-cardiovascular disease effects (Deters, Lengsfeld, & Hensel, 2005; Sonoda et al., 1998).

Lignin is a complex polymer that occurs predominantly in the xylem of most plants, forming approximately 1/3 of the terrestrial woody biomass (Donaldson, 2001). Lignin biosynthesis starts from lignin monomers (coniferyl alcohol, sinapyl alcohol, and *p*-coumaryl alcohol). Peroxidase and oxidase are involved in the early and late stages of lignification (Sterjiades, Dean, Gamble, Himmelsbach, & Eriksson, 1993). As an aromatic macromolecule, lignin provides strength and rigidity to cell walls by acting as a glue between the polysaccharide filaments and fibres (Hofrichter, 2002). Due to the bond types and their heterogeneity, lignin cannot be cleaved by hydrolytic enzymes. During industrial applications, e.g. the kraft pulping process, the objective is to remove lignin for separating cellulosic fibres from each other and producing pulp suitable for paper making (Chakar & Ragauskas, 2004). However, the conventional method is treating the material at 170 °C for 2 h, which is energy-consuming. Therefore, it is interesting to find an efficient alternative to remove lignin easily.

Cellulose is the major component of plant materials. The annual world biosynthesis production is calculated to be 10^{11} tonnes (Sun, Sun, Zhao, & Sun, 2004). Due to its unusual physicochemical properties, cellulose can be employed as a food matrix, dietary fibre, filter membrane, ultra-strength paper and fine fibre network with coating, thickening and suspending functions (Kent, Stephens, & Westland, 1991; Yoshinaga, Tonouchi, & Watanabe, 1997). In recent years, cellulose has proved to be usable as an artificial skin for temporary covering of wounds because of its high mechanical strength, substantial permeability for liquid and gas and low irritation of skin (Klemm, Schumann, Udhardt, & Marsch, 2001). Hydrolysis of cellulose, to produce soluble sugars, has been investigated for its potential in providing abundant food and energy resources. However, commercial application of acid or enzymatic hydrolysis has been limited by low efficiency and high cost (Gan, Allen, & Taylor, 2003). It would be interesting to find a novel way to modify the structures of cellulose and lignin, to facilitate a more extensive hydrolysis.

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Ultra-high pressure treatment is widely used in food and medicine. Application of this technique for extraction of bioactive substances from plant materials yields some advantages, such as short extraction time, mild extraction condition, high extraction yield and less impurity (Zhang, Bi, & Liu, 2006), but ultra-high pressure treatment can lead to dissociation or denaturation of some macromolecules during extraction. Gómez-Guillén, Giménez, and Montero (2005) have indicated that high pressure at above 150 MPa can induce protein denaturation by disturbing the balance of non-covalent interactions within or between proteins. Therefore, it is worthwhile to investigate the application of ultra-high pressure treatment for modifying structures of cellulose and lignin, and then facilitate their hydrolysis. However, few publications about the hydrolysis of cellulose and lignin by ultra-high pressure treatment are available. In this work, longan fruit pericarp was treated with ultra-high pressure and used to preparing water-soluble polysaccharides, alkali-soluble polysaccharides, lignin and cellulose. The DHs (degrees of hydrolysis) of cellulose and lignin, before and after ultra-high pressure treatment, were comparatively analysed.

2. Materials and methods

2.1. Materials

Fresh fruits of longan (*Dimocarpus longan* Lour. cv. Shixia) were purchased directly from a commercial market of Guangzhou. Fruits were selected for uniformity of shape and colour and then separated. Finally, the fruit pericarp were separated manually and dried by a GLZY-0.5B freeze-dryer (Pudong Freeze Dryer Co., Shanghai, China).

2.2. Chemicals

Phenol, ethanol, sodium chloride and concentrated sulphuric acid were purchased from Guangzhou Reagent Co. (Guangzhou, China). Acetonitrile and acetic acid were from Sigma Chemical Co. (St. Louis, MO, USA). Other reagents were of analytical grade.

2.3. Treatment with ultrahigh pressure

The dry longan fruit pericarp was pulverised in a mill (DFT-50, Lingda Mechanics Co., Zhejiang, China) and screened through a 60-mesh sieve. Ten grammes of the dried pericarp powder were exactly weighed and mixed with 150 ml of distilled water. A plastic bag was employed to hold the distilled water solution, then sealed hermetically and finally submitted to ultra-high pressure treatment using an ultra-high pressure machine (Kefa Food Equipment Co., Baotou, China). Various pressures above ambient pressure (control), 200, 300, 400 and 500 MPa were used in this study, respectively. All the experiments were performed at 25 °C. After the treatment, pressure was increased from the ambient pressure to the designated value, it was maintained constantly for 30 min and then released rapidly to the ambient pressure.

2.4. Isolation of water-soluble and alkali-soluble polysaccharides and cellulose

After treatment with ultra-high pressure, water-soluble polysaccharides were obtained according to the method of Zhao, Yang, Yang, Jiang, and Zhang (2007). The extract was filtered through Whatman No. 1 filter paper and the filtrate was then concentrated to 25 ml using a rotary evaporator at 65 °C under vacuum. The residues were twice subjected to the above-mentioned extraction for 2 h at 50 °C and at ambient pressure. All of these filtrates were combined. The proteins in the filtrate were removed using the Sevag reagent (Navarini et al., 1999). After removal of the Sevag reagent, 100 ml of anhydrous ethanol were added; the mixture was then placed in a beaker overnight at 4 °C to precipitate polysaccharides. Water-soluble polysaccharides were obtained after centrifugation at 3860*g* for 15 min. Before the extraction of alkali-soluble polysaccharides, 200 ml of absolute ethanol were used to remove ethanol-soluble substances from the residues. The operation to remove ethanol-soluble substances was conducted three times. Then, 200 ml of 8% NaOH were added to the residues for extracting alkali-soluble polysaccharides for 2 h at 50 °C, in triplicate. After filtration, the extract was adjusted to pH 7.0 with HCl, then dialysed for 24 h against distilled water, and finally concentrated at 65 °C under vacuum. A fourfold volume of absolute ethanol was added to precipitate alkali-soluble polysaccharides at 4 °C. The alkali-soluble polysaccharides were obtained by following the above-mentioned programme. Two hundred millilitres of distilled water and 1.0 g of sodium chlorite were added to the residues for delignification. Glacial acetic acid was added to the extract to regulate the pH to 3.5–4.0. The delignification was carried out three times at 80 °C for 2 h. The cellulose residues were dried at 50 °C in the oven. The filtrates were combined and analysed for lignin composition by high performance liquid chromatography (Waters 2695 Separation Module, Waters, MA, USA). A Pinnacle II C18 stainless steel column (250 × 4.6-mm internal diameter) (Restek, PA, USA) was used. Elution conditions were as follows: 1 ml/min of flow rate; solvent A, water/acetic acid (98:2, v/v); solvent B, acetonitrile/water/acetic acid (80:19:2, v/v/v); isocratic for 4 min with 1% B, from 1% to 60% B for 30 min and from 60% to 100% B for 4 min. The profile was recorded at 280 nm, using a Waters 2487 dual λ absorbance detector (Waters, Massachusetts, USA). The yields of water-soluble polysaccharides, alkali-soluble polysaccharides and cellulose were calculated by the gravimetric method.

2.5. Acid hydrolysis of cellulose

Five microgrammes of cellulose were weighed precisely. Ten millilitres of 2 M trifluoroacetic acid were added. The hydrolysis was performed for 3 h at 100 °C. The phenol–sulphuric acid method was employed to determine water-soluble saccharide content with some modifications (Dubois et al., 1956). One millilitre of sample was mixed with 1 ml of 5% (w/w) phenol and 5 ml of sulphuric acid at room temperature (25 °C). After standing for 30 min, the absorbance was recorded at 490 nm, using a UV-2102 PC UV–visible spectrophotometer (Unico, Shanghai, China). Glucose was used to make a standard curve. The content of water-soluble saccharides was expressed as glucose equivalents. The DH was calculated as follows:

$$\mathrm{DH}\ (\%) = \frac{\text{Weight of water} - \text{soluble saccharides}}{\text{Weight of cellulose}} \times 100$$

3. Results and discussion

3.1. Water-soluble polysaccharide yield

Fig. 1 shows water-soluble polysaccharide yield of longan fruit pericarp prepared under various ultra-high pressures. At ambient pressure, the water-soluble polysaccharide yield was 18.3 ± 0.8 mg/g. After treatment with ultra-high pressure, the water-soluble polysaccharide yield decreased, with a negative relationship between ultra-high pressure and the water-soluble polysaccharide yield. The lowest yield (6.4 ± 0.6 mg/g) was obtained when 500 MPa of ultra-high pressure was applied.

Application of ultra-high pressure could lead to a decreased yield of water-soluble polysaccharides, which might be due to