Food Chemistry 112 (2009) 428–431

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

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

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ARTICLE INFO

Article history:
Received 24 January 2008
Received in revised form 4 May 2008
Accepted 28 May 2008

Keywords:
Longan
Polysaccharide
Cellulose
Lignin

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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doi:10.1016/j.foodchem.2008.05.097

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

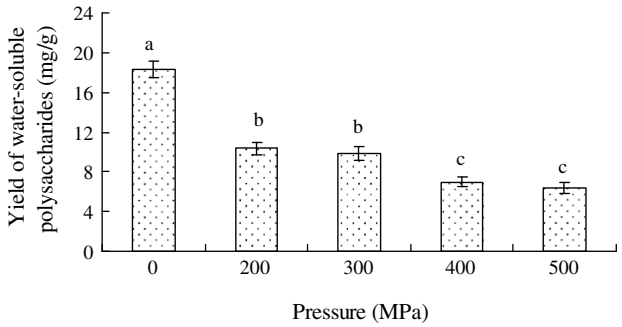

Fig. 1. Effect of ultra-high pressure treatment on the yield of water-soluble polysaccharides of longan fruit pericarp. Each data point is a mean ± standard deviation of three replicated determinations. The columns having the same letter were not significantly different ($P > 0.05$).

the structural modification of water-soluble polysaccharides. Fernández García, Butz, and Tauscher (2001) have reported a similar effect of ultra-high pressure treatment on the extractability of carotenoids from tomato pulp tissues, in which the high pressure-treated tomato pulp had a lower carotenoid recovery, compared with the non-treated pulp when petroleum ether was used as extraction solvent. Butz et al. (2002) have suggested that ultra-high pressure treatment can modify the structure of macromolecules, e.g. by dissociation of the complex macromolecular system and unfolding of protein chains. Therefore, a structural rearrangement of water-soluble polysaccharides of longan fruit pericarp could be induced by the ultra-high pressure treatment in this work, resulting in a reduced accessibility of water-soluble polysaccharides to the extraction solvent (water).

3.2. Alkali-soluble polysaccharide yield

The alkali-soluble polysaccharide yield of longan fruit pericarp with or without ultra-high pressure treatment is present in Fig. 2. The alkali-soluble polysaccharide yield of longan fruit pericarp extracted at ambient pressure was 20.4%, which indicated that alkali-soluble polysaccharides were important components of longan fruit pericarp. After treatment with an ultra-high pressure of 500 MPa, the alkali-soluble polysaccharide yield decreased to 20.1%. However, no significant ($P > 0.05$) difference was found between the ultra high pressure-treated and non-treated samples, which suggested that ultra-high pressure treatment could not significantly change the alkali-soluble polysaccharide yield. Alkali-soluble polysaccharide consists mainly of hemicellulose and small amounts of pectin in the plant cellular walls and constructs a firm network with cellulose (Hilz, Bakx, Schols, & Voragen, 2005). The hemicellulose is less susceptible to degradation than are other polysaccharides. Pectinmethylesterase and polygalacturonase are responsible for the redistribution of water- and alkali-soluble polysaccharides, which are pectinmethylesterase and polygalacturonase, respectively (Wennberg & Nyman, 2004). The two enzymes can be inactivated by ultra-high pressure treatment, while the inactivation of the two enzymes might explain the insignificant effect of the ultra-high pressure treatment on the alkali-soluble polysaccharide yield.

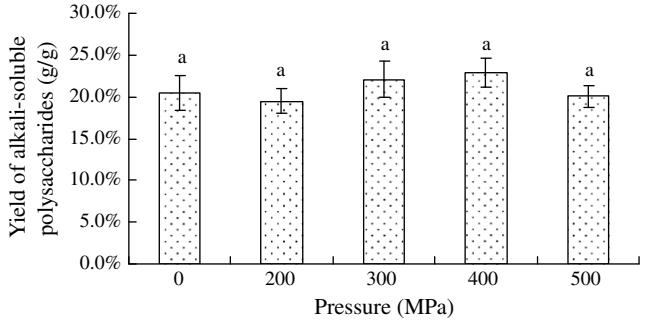

Fig. 2. Effect of ultra-high pressure treatment on the yield of alkali-soluble polysaccharides of longan fruit pericarp. Each data point is a mean ± standard deviation of three replicated determinations. The columns having the same letter were not significantly different ($P > 0.05$).

3.3. Cellulose yield and DH

The cellulose yields, with or without ultra-high pressure treatment, are shown in Fig. 3. The cellulose yield without ultra-high pressure treatment was 73.2%, while yields with ultra-high pressure treatment at 200, 300, 400 and 500 MPa were 63.4%, 64.8%, 71.0% and 70.2%, respectively. By the analysis of variance, no significant differences at $P < 0.05$ were found between the ultra-high pressure-treated and non-treated samples. Furthermore, the DH of cellulose is shown in Fig. 4. The DH of the control sample was 26.6%. Treatment with 500 MPa gave the highest DH (29.4%), significantly ($P < 0.05$) higher than the control. The DHs of cellulose treated at 200, 300 and 400 MPa were 25.5%, 25.9% and 24.8%, respectively.

Ultra-high pressure can affect the hydrophobic and ionic bonds within or between macromolecules, but does not obviously influence other stronger bonds, like hydrogen bonds (Montero, Fernández-Díaz, & Gómez-Guillén, 2002). Cellulose is a linear natural polymer with anhydroglucose units linked by β-1,4-glycosidic bonds, and is confirmed by the presence of three hydroxyl groups at C-2, C-3 and C-6 and the formation of strong inter- and intramolecular hydrogen bonds (Kadla & Gilbert, 2000). These hydrogen

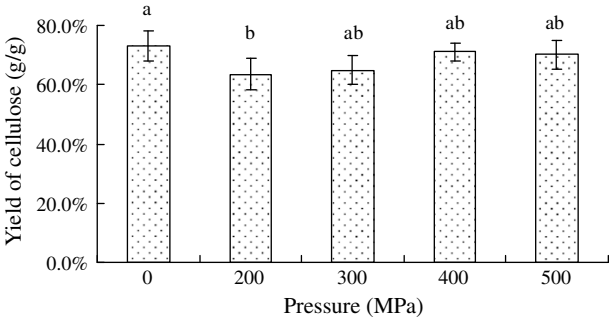

Fig. 3. Effect of ultra-high pressure treatment on the yield of cellulose of longan fruit pericarp. Each data point is a mean ± standard deviation of three replicated determinations. The columns having the same letter were not significantly different ($P > 0.05$).

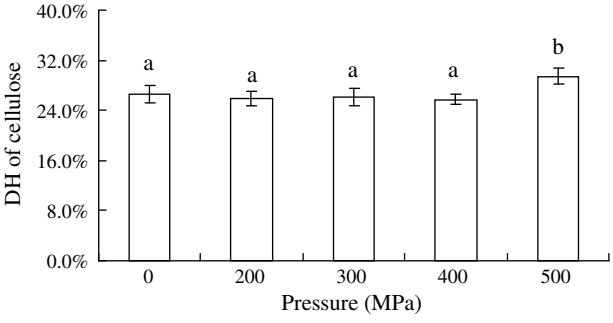

Fig. 4. DHs of cellulose prepared under different ultra-high pressures. Each data point is a mean ± standard deviation of three replicated determinations. The columns having the same letter were not significantly different ($P > 0.05$).

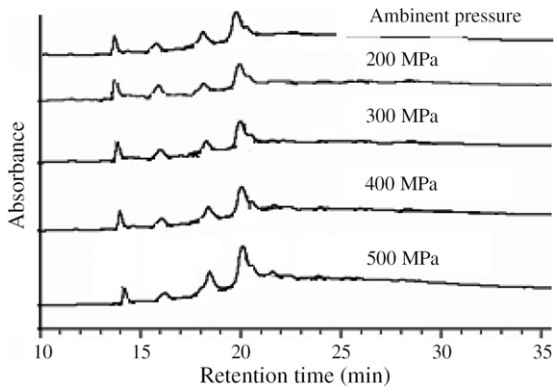

Fig. 5. High performance liquid chromatogram of lignin composition under different ultra-high pressures.

bonds are critical to the formation of the predominant double-chain monoclinic structure in cellulose of higher plants (Sugiyama, Persson, & Chanzy, 1991). This work indicates that ultra-high pressure treatment did not have any significant effect on cellulose structure.

3.4. Lignin compositions

In this study, cellulose was dilignified by acetic acid and sodium chloride, resulting in the formation of aldehydes and phenolic acids. Fig. 5 shows the profile of lignins after degradation and oxidation by high performance liquid chromatography. Four dominant peaks were recorded as the main products, but no significant change in these peaks was observed for the samples, with or without ultra-high pressure treatment.

Lignins are difficult to extract as a pure material, and they are present with cellulose and hemicellulose to various extents (Singh et al., 2005). The components of lignins are phenylpropene and phenylethyl units, bonded by ether and carbon–carbon linkages (da Cunha, Serve, Gadel, & Blazi, 2001). As described above, the covalent bond of the macromolecule cannot be modified by ultra-high pressure treatment, which may account for no change in the composition of lignins.

4. Conclusions

A negative relationship was observed between pressure and water-soluble polysaccharide yield. The lowest yield (6.4 mg/g) was obtained when a pressure of 500 MPa was used. No significant difference at $P < 0.05$ in the yields of alkali-soluble polysaccharides and cellulose was found between the ultra-high pressure-treated and non-treated samples. The DH of non-treated cellulose was 26.6%. The highest cellulose DH was 29.4% when 500 MPa was used, significantly ($P < 0.05$) higher than that of the control. The lignins after degradation and oxidation were analysed by high performance liquid chromatography. Four main peaks were recorded in the chromatogram at 280 nm, which showed that ultra-high pressure treatment could not result in change in the composition of lignins. However, the structural identification of polysaccharides after ultra-high pressure treatment is necessary in further research to understand the mechanism of the process.

Acknowledgements

The financial support provided by the National Natural Science Foundation of China (Grant Nos. 30425040 and 30700557), China Postdoctoral Science Foundation (No. 20070420802), International Foundation for Science (No. F/4451-1) and 11th-five-year National Key Technology R&D Program of China (Nos. 2006BAD27B03 and 2006BAD27B04) was appreciated.

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