High pressure homogenization increases antioxidant capacity and short-chain fatty acid yield of polysaccharide from seeds of Plantago asiatica L.

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ABSTRACT

Physiological properties of homogenized and non-homogenized polysaccharide from the seeds of Plantago asiatica L., including antioxidant capacity and short-chain fatty acid (SCFA) production, were compared in this study. High pressure homogenization decreased particle size of the polysaccharide, and changed the surface topography from large flake-like structure to smaller porous chips. FT-IR showed that high pressure homogenization did not alter the primary structure of the polysaccharide. However, high pressure homogenization increased antioxidant capacity of the polysaccharide, evaluated by 4 antioxidant capacity assays (hydroxyl radical-scavenging, superoxide radical-scavenging, 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH)-scavenging and lipid peroxidation inhibition). Additionally, the production of total SCFA, propionic acid and n-butyric acid in ceca and colons of mice significantly increased after dieting supplementation with homogenized polysaccharide. These results showed that high pressure homogenization treatment could be a promising approach for the production of value-added polysaccharides in the food industry.

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1. Introduction

Polysaccharides have been widely used in food processing and preparation as stabilizers, thickeners, texturizers or emulsifiers (Huck-Iriart, Pizones Ruiz-Henestrosa, & Candal, 2012). An emulsion is a mixture of two immiscible liquids in which one phase is dispersed through the other as small, discrete droplets. Polysaccharides as emulsifiers can assist in obtaining a fine dispersion and in maintaining the homogeneous mixture through homogenization (Lapasin & Pricl, 1995). Many studies have shown that viscosity, rheological and gelling properties of polysaccharides could be influenced by the high pressure homogenization process (Pouliot, Britten, & Latreille, 1990; Silvestri & Gabrielson, 1991). In addition, it was also reported that high pressure homogenization could reduce the particle size of polysaccharides and induce some degradation, but it did not destroy the primary structure of polysaccharides (Chen et al., 2012; Floury, Desrumaux, Axelos, & Legrand, 2002). Concerning the effects of high pressure homogenization on physiological properties of polysaccharides, Lyons and Deidra Shannon (2011) reported that the antimicrobial properties of chitosan could be improved after being treated by high pressure homogenization. However, no study has been conducted to examine the effects of high pressure homogenization on several other properties of polysaccharides, such as their antioxidant capacity or their influence on the production of total SCFA, propionic acid and n-butyric acid in ceca and colon once ingested. Antioxidant capacity of polysaccharides is considered as one of the important physiological properties, for it could help prolong the shelf life of food products (Gan & Latiff, 2011). Short-chain fatty acid production of polysaccharides is also increasingly attracting attention, because it is beneficial for human intestinal health (Huang, Chu, Dai, Yu, & Chau, 2012). Researchers showed that antioxidant activity of the polysaccharides may be influenced by the reduction of the particle size (Pérez-Jiménez et al., 2008), and the particle size of the polysaccharides (determining the surface available to bacteria) may also influence fermentation and thus affect the SCFA production (Guillon, Auffret, Robertson, Thibault, & Barry, 1998). Thus, our hypothesis is that the antioxidant activity and SCFA production of the polysaccharides may be influenced by high pressure homogenization treatment, resulting in the particle size reduction. Polysaccharides could be extracted from many kinds of herbal or plant materials. Some Plantago plants, such as Plantago africa L., Plantago psyllium L., Plantago ovata Forsk (isabgul), Plantago indica L. and Plantago major L., are often used in traditional medicine throughout the world because the soluble fibres in their seeds are able to improve some intestinal functions (Mahady, Fong, & Farnsworth, 1999; Samuelsen, 2000). Our research group has recently isolated a pure and homogeneous polysaccharide from the seeds of P. asiatica L. with a molecular weight of 1894 kDa (Yin, Nie, Zhou, Wan, & Xie, 2010). The polysaccharide was found to...
be a highly branched heteroxylan which consisted of a $\beta$-1, 4-linked Xylp backbone with side chains attached to O-2 or O-3. The side chains consisted of $\beta$-T-linked Xylp, $\alpha$-T-linked Araf, $\alpha$-T-linked GlcAp, $\beta$-Xylp-(1→3)-$\alpha$-Araf and $\alpha$-Araf-(1→3)-$\beta$-Xylp (Yin, Lin, Li, Wang, Cui, Nie, & Xie, 2012). In addition, our recent studies have also shown that this polysaccharide may induce maturation of murine dendrite cells, and may have antioxidant activity and promote defaecation (Huang, Xie, Yin, Nie, & Xie, 2009; Tang, Huang, Yin, Zhou, Xie, & Xie, 2007; Wu, Tian, Xie, & Li, 2007).

In the study, the effect of high pressure homogenization on the polysaccharide from the seeds of *P. asiatica* L. was evaluated by using dynamic light scattering, environmental scanning electron microscopy and FT-IR spectroscopy. In addition, the effects of high pressure homogenization on some properties of the polysaccharide, such as antioxidant activity and SCFA production, were for the first time examined.

2. Materials and methods

2.1. Materials and reagents

The seeds of *P. asiatica* L. were purchased from Ji'an, Jiangxi Province, China and dried in the sun before use. Highly pure SCFAs were used to prepare the standard solutions for gas chromatography (GC) determination. Acetic acid ($\geq$ 99.5% purity) and n-valeric acid ($\geq$ 99.5% purity) were obtained from Merck (Darmstadt, Germany). Propionic acid ($\geq$ 99.5% purity) was purchased from Janssen Chimica (Belgium), while i-butyric acid ($\geq$ 99.5% purity), n-butyric acid ($\geq$ 99% purity), i-valeric acid ($\geq$ 99% purity) and 4-methylvaleric acid (internal standard) ($\geq$ 99% purity) were purchased from Sigma (St. Louis, MO, USA). All other reagents used were of analytical grade and purchased from Shanghai Chemicals and Reagents Co. (Shanghai, China).

2.2. Animals

 Kunming mice, weighing 20.0 ± 2.0 g [Grade II, Certificate Number SCXK (gan) 2006-0001], were purchased from Jiangxi College of Traditional Chinese Medicine, Jiangxi Province, China. In this study, 36 mice were used in total. Mice were randomly divided into three treatment groups and in each treatment group there were 12 mice. All animals used in this study were cared for in accordance with the Guidelines for the Care and Use of Laboratory Animals, published by the United States National Institute of Health (NIH, Publication No. 85-23, 1996), and all procedures were approved by the Animal Care Review Committee (Animal application approval number 0064257), Nanchang University, China.

2.3. Polysaccharide preparation

Polysaccharide from *P. asiatica* L. seeds was prepared using our published method (Yin et al., 2010). Briefly, the seeds of *P. asiatica* L. (50 g) were defatted with 1 l of ethanol at room temperature for 24 h under stirring, and then extracted with 500 ml of doubly distilled water at 100°C for 2 h. The residue was re-extracted. The combined aqueous extract, a gel of high viscosity, was centrifuged (4800 × g, 10 min), prefilled through a cotton cloth bag and concentrated in a rotary evaporator under reduced pressure at 55°C, to yield *P. asiatica* L. water extract. The filtrate was mixed with 1.5 g/l of papain and heated in water at 60°C for 2 h. The resulting aqueous solution was extensively dialyzed against doubly distilled water for 72 h and precipitated by adding 4 volumes of anhydrous ethanol at 4°C for 12 h. After centrifugation the precipitate was washed with anhydrous ethanol, dissolved in water and lyophilized to yield the polysaccharide (soluble fibre content >90.0%).

2.4. High pressure homogenization treatment

The polysaccharide solution (2.5 mg/ml) was dispersed in deionized water and stirred gently at room temperature to achieve complete solubilization. High pressure homogenization was performed using a M-100EH-30 microfluidizer (Microfluidics Co., Newton, USA) at 160 MPa for 5 passes. The experiment was conducted three times with different solutions of polysaccharide in order to verify the repeatability of the homogenization process. Parts of the solutions, after high pressure homogenization, were lyophilized for the Fourier transform infrared (FT-IR) and scanning microscopy analyses.

2.5. Average particle size (PS) and distribution determination

The average particle size and distribution of the polysaccharide in solutions were determined by Nicomp 380/LS Zeta potential/Particle sizer (PSS Nicomp, Santa Barbara, USA), based on dynamic light scattering (DLS). The solutions were diluted to a concentration of 0.5 mg/ml with deionized water, and all measurements were carried out at 25°C (Chen et al., 2012).

2.6. Scanning microscopy analysis

Polysaccharide samples were taken after freeze-drying, and samples were prepared by sticking the polysaccharide onto double-sided adhesive tape attached to a circular specimen stub. The samples were viewed using an environmental scanning electron microscope (ESEM) (Quanta 200F, FEI Deutschland GmbH, Kassel, Germany) at 30 kV voltage and 3.0 spot size. Low vacuum mode was used for the ESEM.

2.7. FT-IR spectroscopy

The FT-IR spectra of polysaccharide samples were recorded on a Nicolet 5700 spectrometer (Thermo Co., Madison, USA). The dried samples were ground with KBr powder (spectroscopic grade) and pressed into pellets for spectra measurement in the frequency range of 4000–400 cm⁻¹. The data was collected and plotted as transmittance (%) in function of the wave number (cm⁻¹) and analyzed with Ominic 7.2 software.

2.8. Antioxidant activity assays

2.8.1. Hydroxyl radical-scavenging activity

The hydroxyl radical-scavenging ability was measured, using a modified method of Halliwell, Gutteridge, and Aruoma (1987). A reaction mixture was prepared by adding 0.1 ml of EDTA (1 mM), 0.01 ml of FeCl₃ (10 mM), 0.1 ml of H₂O₂ (10 mM), 0.36 ml of deoxyribose (10 mM), 1.0 ml of polysaccharide samples (0.1–10 mg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid (1 mM) in sequence. The mixture was then incubated at 37°C for 1 h. 1.0 ml of the incubated mixture was mixed with 1.0 ml of 10% trichloroacetic acid and 1.0 ml of 0.5% thiobarbituric acid (in 25 mM NaOH containing 0.025% butylated hydroxyl anisole) to develop the pink chromogen measured at 532 nm. The hydroxyl radical-scavenging activity of the polysaccharide samples was reported as the percentage of inhibition of deoxyribose degradation and was calculated according to the following equation:

\[
\% \text{ inhibition} = \left( \frac{A_0 - A_t}{A_0} \right) \times 100
\]

where $A_0$ was the absorbance of the control (blank, without polysaccharide samples) and $A_t$ was the absorbance in the presence of the polysaccharide samples. All of the tests were carried out in
triplicate and IC\textsubscript{50} values were expressed as means \pm standard deviation (SD). Ascorbic acid was used as a positive control.

2.8.2. Superoxide radical-scavenging activity

This activity was measured using nitro blue tetrazolium (NBT) reagent as described by Sabu and Kuttan (2002). The method is based on generation of superoxide radical O\textsubscript{2}\textsuperscript{−} by auto-oxidation of hydroxylamine hydrochloride in the presence of NBT, which gets reduced to nitrite. Nitrite, in the presence of EDTA, gives a colour that was measured at 560 nm. Test solutions of polysaccharide (0.1–10 mg/ml) were taken in a test tube. To this, a reaction mixture consisting of 1 ml of (50 mM) sodium carbonate, 0.4 ml of (24 mM) NBT and 0.2 ml of 0.1 mM EDTA solution was added to the test tube and an immediate reading was taken at 560 nm. 0.4 ml of (1 mM) of hydroxylamine hydrochloride was added to initiate the reaction. The reaction mixture was then incubated at 25 °C for 15 min and the reduction of NBT was measured at 560 nm. Decreased absorbance of the reaction mixture indicates increased superoxide anion-scavenging activity. All the samples were treated in a similar manner, absorbance was recorded and the percentage of inhibition was calculated according to the following equation:

\[
\% \text{ inhibition} = \frac{(A_0 - A_t)}{A_t} \times 100
\]

where \(A_0\) was the absorbance of the control (blank, without polysaccharide samples) and \(A_t\) was the absorbance in the presence of the polysaccharide samples. All the tests were performed in triplicate and IC\textsubscript{50} values were obtained. Ascorbic acid was used as a positive control.

2.8.3. DPPH radical-scavenging activity

DPPH radical-scavenging activity of polysaccharide samples was measured according to our published method (Chen, Xie, Nie, Li, & Wang, 2008). The 0.2 mM solution of DPPH in 95% ethanol was prepared daily before UV measurements were taken. One millilitre of the polysaccharide samples of different quantities (0.1–10 mg) in water was thoroughly mixed with 2 ml of freshly prepared DPPH, and 2 ml of 95% ethanol. The mixture was shaken vigorously and left to stand for 30 min in the dark. The absorbance of the supernatant obtained after centrifugation was then measured at 517 nm.

The DPPH radical-scavenging ability was calculated using the following equation:

\[
I\% = \left[ 1 - \left( \frac{A_t - A_c}{A_t} \right) \right] \times 100\%
\]

where \(A_t\) is the absorbance of DPPH solution without polysaccharide sample (2 ml DPPH + 3 ml of 95% ethanol), \(A_c\) is the absorbance of the test polysaccharide sample mixed with DPPH solution (1 ml of polysaccharide sample + 2 ml of DPPH + 2 ml of 95% ethanol) and \(A_j\) is the absorbance of the polysaccharide sample without DPPH solution (1 ml of polysaccharide sample + 4 ml of 95% ethanol). All the tests were performed in triplicate and IC\textsubscript{50} values were obtained. Ascorbic acid was used as a positive control.

2.8.4. Inhibition of lipid peroxidation

This test was conducted using the method of Zhang, Yu, Zhou, and Xiao (1996) with some modifications. Briefly, an equal volume of egg yolk was added to 0.1 M phosphate-buffered saline (PBS, pH 7.45). The mixture was stirred magnetically for 10 min and then diluted with 24 volumes of PBS. The yolk homogenate (1 ml), polysaccharide samples (0.5 ml, 0.1–10 mg/ml), PBS (1 ml) and 25 mM Fe\textsubscript{SO\textsubscript{4}} (1 ml) were mixed in a tube and shaken at 37 °C for 15 min. The reaction was stopped by the addition of trichloroacetic acid and the mixture was centrifuged. Then 1 ml of 8 g/l thiobarbituric acid solution was added to 3 ml of the supernatant. This solution was heated at 10 °C for 10 min, after which its absorbance at 532 nm was measured. The ability to inhibit lipid peroxidation was calculated as follows:

\[
\text{Inhibitory effect(\%)} = \left( \frac{B_0 - B}{B_0} \right) \times 100
\]

where \(B_0\) is the absorbance of the control and \(B\) is the absorbance in the presence of polysaccharide samples. All the tests were performed in triplicate and IC\textsubscript{50} values were obtained. Ascorbic acid was used as a positive control.

2.9. Changes in SCFA production of polysaccharide treated by high pressure homogenization

2.9.1. Animal experiment design

Six-week-old male Kunming mice (20.0 ± 2.0 g) were individually housed in stainless steel cages in a room with controlled temperature (25 ± 0.5 °C), relative humidity (50 ± 5%) and 12 h/12 h light/dark cycle. All mice were fed with the same amount of basal diet (Supplementary Table 1) which was prepared according to the published formula (Adachi et al., 2004; Berggren, Björck, Nyman, & Eggum, 1993), and water was provided ad libitum. Mice were randomly divided into three groups: (1) non-treated polysaccharide group: mice were given oral administration of non-treated polysaccharide at the dose of 0.1 g/kg body weight (BW); (2) homogenization-treated polysaccharide group: mice were given oral administration of homogenization-treated polysaccharide at the dose of 0.1 g/kg BW; (3) control group: mice were given distilled water of the same volume as the mean volume of the polysaccharide groups. Non-treated polysaccharide and homogenization-treated polysaccharide were dissolved in distilled water before administration. Each group had 12 mice, and each mouse was housed in one cage.

All the mice were given oral administration of polysaccharide or water (control) by gavage at about 9:00 am everyday for 4 weeks. In addition, we determined the body weight of each mouse everyday before gavage and changed the volume of the polysaccharide in order to make sure the mice were given polysaccharide at the dose of 0.1 g/kg BW by gavage everyday. Throughout the experiment, the animals' general health status and body mass were observed twice daily. At the end of the designated experimental period, the mice were sacrificed, and the liver, kidney, spleen, heart, lung, intestine, cecum and colon were excised and immediately weighed. After that, the ceca and colons were aseptically removed, immediately, and placed on an ice-cold plate, longitudinaly opened and the cecal and colonic contents were collected for further use.

2.9.2. Measurement of cecal and colonic SCFA concentration

A portion (0.1 g) of the sample (ecal or colonic content) was rapidly put into a round-bottomed stopped tube in an ice-cold water bath. One millilitre of deionized water was added to the tube and mixed intermittently on a vortex-mixer for 2 min. The tube was allowed to stand in the ice-cold water bath for 20 min and then centrifuged at 4800g for 20 min at 4 °C. The supernatant was transferred to another round-bottomed stopped tube. This process was repeated once. The supernatant was analyzed by injection into the chromatographic system.

Chromatographic analysis was carried out according to our published method (Hu, Nie, Min, & Xie, 2012), using an Agilent 6890N GC system equipped with a flame ionization detector (FID) and an N10149 automatic liquid sampler (Agilent, USA). A GC column (HP-INNOWAX, 190901N-213, J & W Scientific, Agilent Technologies Inc., USA) of 30 m × 0.22 mm ID coated with 0.50 µm film thickness was used. Nitrogen was supplied as the carrier gas at a flow rate of 19.0 ml/min with a split ratio of 1:10. The initial oven temperature was 100 °C and it was kept there for 0.5 min, and then raised to 180 °C by 4 °C/min. The temperatures of the FID and injection port were 240 °C. The flow rates of hydrogen and synthetic air were 30 and 300 ml/min, respectively. The injected sam-
ple volume for GC analysis was 0.2 μl, and the running time for each analysis was 20.5 min. The independently replicated determinations were performed three times for standard solutions and each sample. Data handling was carried out with a HP ChemStation Plus software (A.09.xx, Agilent). Mean values and standard deviations were calculated from triplicate determinations. Results were expressed as means ± standard deviation.

2.10. Statistical analysis

All the experiments were done in triplicate. Results were expressed as means ± standard deviation (SD). Data were evaluated by 1-way analysis of variance, using SPSS 10.0 software (Version 16.0, Chicago, United States). The difference between different groups was evaluated by SNK test. The level of significance was set at \( p < 0.05 \).

3. Results and discussion

3.1. Particle size and surface topography

The particle size and distribution of the polysaccharide from *P. asiatica* L. seeds, before and after being treated by high pressure homogenization, are shown in Fig. 1. After high pressure homogenization, the distribution profile of particle size shifted left and narrowed (a typical Gaussian distribution), indicating that the particle size of the polysaccharide decreased. The size of the polysaccharide decreased from 4758.7 ± 153.8 nm to 226.3 ± 4.7 nm after high pressure homogenization.

In addition, as shown by ESEM, the non-treated polysaccharide was relatively tidy, with flake-like lamella (Fig. 2A). However, after high pressure homogenization, many pores appeared in the polysaccharide sample (Fig. 2B). The original flake-like structure was totally changed into smaller fragments after homogenization. Interestingly, it was found that Fig. 2A and B were similar to the microstructure of pectin, with and without high pressure homogenization, respectively (Chen et al., 2012).

It was reported that the complexes could be broken into microfragments by homogenization and thus produce spherical particles with a very low diameter (Chen et al., 1989). Homogenization could result in a change in the degree of aggregation of polymer, as well as irreversible disruption of the polymer (Lagoueyte & Paquin, 1998). These results were similar to ours.

3.2. FT-IR spectroscopy

The chemical structures of the non-treated and homogenization-treated polysaccharide samples were analyzed by FT-IR
time at the best possible quality. The producer will benefit from the longer shelf-life to develop the market over greater distances. Natural antioxidant compounds could help prolong the shelf-life of food, and might also be beneficial for human health to some extent. In this study, antioxidant activity of the polysaccharide samples was evaluated using four different assays. The IC_{50} values of different polysaccharide samples are summarized in Table 1.

Hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in the living cells (Hochstein & Atallah, 1988). As shown in Table 1, homogenization-treated polysaccharide had much more scavenging power for the hydroxyl radical than had non-treated polysaccharide (p < 0.05).

Superoxide anion plays an important role in the formation of reactive oxygen species, such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, and induces oxidative damage in lipids, protein, and DNA (Pietta, 2000). In this study, the IC_{50} value for superoxide radical-scavenging ability of non-treated polysaccharide (2421 ± 2 µg/ml, Table 1) was higher than that for homogenization-treated polysaccharide (2271 ± 3 µg/ml) (p < 0.05).

The model of scavenging the stable DPPH radical is a widely used method for evaluating the free radical-scavenging ability of natural compounds. The effect of antioxidants on DPPH radical-scavenging was thought to be due to their hydrogen-donating ability (Chen et al., 2008). The DPPH-scavenging activity of the non-treated polysaccharide, expressed in terms of IC_{50}, was 503 ± 8 µg/ml (Table 1), with a lower DPPH-scavenging power than that of homogenization-treated polysaccharide (384 ± 5 µg/ml) or ascorbic acid (205 ± 3 µg/ml) (p < 0.05).

Lipid peroxidation (oxidative degradation of polyunsaturated fatty acid in the cell membranes) generates a number of degradation products, such as malondialdehyde (MDA), which is found to cause cell membrane destruction and cell damage, leading to liver injury, atherosclerosis, kidney damage, ageing, and susceptibility to cancer (Rice-Evans & Burdon, 1993). The IC_{50} value (µg/ml), related to the lipid peroxidation inhibitory capacity assay, showed that the lipid peroxidation inhibitory capacity of non-treated polysaccharide (1703 ± 8 µg/ml) was only about 60% of the homogenization-treated polysaccharide (1076 ± 8 µg/ml, Table 1).

In these four antioxidant activity assays, except for the DPPH-scavenging assay, the non-treated polysaccharide and homogenization-treated polysaccharide both have good solubility and no aggregates formed. Possibly some small aggregates were formed after addition of 95% ethanol in the DPPH-scavenging assay. However, these small aggregates could be removed by centrifugation before absorbance determination; thus they did not influence the accuracy of the absorbance. The decrease of absorbance was obvious, in the presence of polysaccharides, compared to the control. In addition, the procedures for DPPH-scavenging assay have been successfully used in our previous studies (Chen et al., 2008; Yin et al., 2010).

The antioxidant capacity results indicated that the antioxidant capacity of the polysaccharide from P. asiatica L. was improved after the high pressure homogenization treatment. It is reported that one of the antioxidant mechanisms of polysaccharides may be due to the hydrogen supplied from some substituent groups in the polysaccharides (such as ferulic acids), which combine with radicals and form a stable radical to terminate the radical chain reaction (Chen et al., 2008). The other possibility is that the polysaccharide itself combines with metal ions or free radicals that are necessary for the radical chain reaction, so the reaction is terminated (Yin et al., 2010). In this study, the antioxidant capacity of original polysaccharide from P. asiatica L. may be attributed to both of them. On the one hand, the polysaccharide may have strong ability to chelate metal ions or free radicals, as inferred from...
On the other hand, there are some ferulic acids present in the molecule of the polysaccharide (Yin et al., 2012), which have been proved to be hydrogen-donors and have obvious antioxidant ability (Graf, 1992). After high pressure homogenization, the particle size of the polysaccharide was significantly reduced (Fig. 1), resulting in an increase of total particle surface area for combining with metal ions or free radicals. Hence, the antioxidant capacity of the polysaccharide was improved after high pressure homogenization.

In addition, it was reported that the viscosity of the polysaccharide may influence its chelation ability towards metal ions or free radicals, and polysaccharides with lower viscosity exhibited higher antioxidant ability due to their greater chelation capacity (Kamil, Jeon, & Shahidi, 2002). In this study, it was found that the viscosity

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC50 (μg/ml)</th>
<th>Hydroxyl radical-scavenging</th>
<th>Superoxide radical-scavenging</th>
<th>DPPH radical-scavenging</th>
<th>Lipid peroxidation inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treated polysaccharide</td>
<td>3084 ± 2a</td>
<td>2421 ± 2a</td>
<td>503 ± 8a</td>
<td>1703 ± 8a</td>
<td></td>
</tr>
<tr>
<td>Homogenization-treated polysaccharide</td>
<td>2394 ± 3b</td>
<td>2271 ± 3b</td>
<td>384 ± 5b</td>
<td>1076 ± 8b</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>220 ± 3c</td>
<td>304 ± 5c</td>
<td>205 ± 3c</td>
<td>440 ± 3c</td>
<td></td>
</tr>
</tbody>
</table>

* The IC50 value is expressed as μg/ml and represents the concentration of sample that is required for 50% inhibition of hydroxyl radical, superoxide radical, DPPH radical, and lipid peroxidation. A lower IC50 value indicates a higher antioxidant activity. Each value in the table was obtained by calculating the average of three determinations ± standard deviation.

b Mean values in the same column with different letters are significantly different (Tukey test, p < 0.05).

c Ascorbic acid was used as a positive control.

Table 2

<table>
<thead>
<tr>
<th>SCFA (μg/g of cecal content)</th>
<th>Control group</th>
<th>NTP groupa</th>
<th>HTP groupb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>2525 ± 46a</td>
<td>3026 ± 51b</td>
<td>3043 ± 41b</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>1022 ± 24a</td>
<td>1108 ± 28b</td>
<td>1499 ± 17c</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>1386 ± 21a</td>
<td>2101 ± 33b</td>
<td>2604 ± 25c</td>
</tr>
<tr>
<td>i-Butyric acid</td>
<td>117 ± 11a</td>
<td>105 ± 19a</td>
<td>112 ± 12a</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>73 ± 6a</td>
<td>74 ± 7a</td>
<td>72 ± 9a</td>
</tr>
<tr>
<td>i-Valeric acid</td>
<td>125 ± 15a</td>
<td>120 ± 11a</td>
<td>123 ± 12a</td>
</tr>
<tr>
<td>Total SCFA</td>
<td>5248 ± 132a</td>
<td>6534 ± 144b</td>
<td>7453 ± 152c</td>
</tr>
</tbody>
</table>

* NTP group, non-treated polysaccharide group.

b HTP group, homogenization-treated polysaccharide group.

c Each value is the mean ± standard deviation (n = 12); means in the same line not sharing a common letter are significantly different (p < 0.05).

Table 3

<table>
<thead>
<tr>
<th>SCFA (μg/g of colonic content)</th>
<th>Control group</th>
<th>NTP groupa</th>
<th>HTP groupb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>2332 ± 57a</td>
<td>3074 ± 61b</td>
<td>3090 ± 49b</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>949 ± 39a</td>
<td>1064 ± 27b</td>
<td>1468 ± 40c</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>1167 ± 21a</td>
<td>1509 ± 36b</td>
<td>2206 ± 30c</td>
</tr>
<tr>
<td>i-Butyric acid</td>
<td>171 ± 14a</td>
<td>164 ± 15a</td>
<td>160 ± 20a</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>62 ± 6a</td>
<td>58 ± 6a</td>
<td>60 ± 10a</td>
</tr>
<tr>
<td>i-Valeric acid</td>
<td>146 ± 11a</td>
<td>144 ± 13a</td>
<td>146 ± 18a</td>
</tr>
<tr>
<td>Total SCFA</td>
<td>4827 ± 147a</td>
<td>6013 ± 142b</td>
<td>7130 ± 156c</td>
</tr>
</tbody>
</table>

* NTP group, non-treated polysaccharide group.

b HTP group, homogenization-treated polysaccharide group.

c Each value is the mean ± standard deviation (n = 12); means in the same line not sharing a common letter are significantly different (p < 0.05).
of the polysaccharide decreased after high pressure homogenization treatment, which was consistent with another report (Chen et al., 2012). Thus, the higher antioxidant ability of the homogenization-treated polysaccharide may also be attributed to its lower viscosity compared to the non-treated polysaccharide. Furthermore, the process of homogenization may lead the polysaccharide molecule being dispersed more homogeneously, which resulted in the ferulic acid present in the molecule also being dispersed more homogeneously. This could mean that the ferulic acid provides hydrogen more effectively and performs antioxidant activity better. For the above reasons, the antioxidant capacity of the polysaccharide could be improved after high pressure homogenization treatment.

3.4. SCFA production of polysaccharide

3.4.1. General health status and organ weight of mice

Throughout the experimental period, no noticeable behavioral or activity changes were observed in the mice, and no treatment-related illness or death occurred. The growth of mice appeared normal throughout the experimental period, and no mouse experienced diarrhea or constipation. After 4 weeks, the average body weight gain was 18.6 g per mouse, and the average dietary intake was 213.5 g per mouse. There was no observable difference in the animals’ body mass and hair lustre between the polysaccharide-treated groups and control group.

Supplementary Table 2 summarizes the effects of the polysaccharide-administration on the weights of various mouse organs. Organ weights were not different amongst the groups, except for the cecal weight and colon weight. The cecum and colon of the polysaccharide-treated groups were all significantly heavier than those of the control group (p < 0.05). This phenomenon has generally been observed when mice were fed with soluble dietary fibre and other carbohydrates (Arjmandi, Craig, Nathani, & Reeves, 1992; Moundras, Behr, Demigné, Mazur, & Rémésy, 1994). There was no significant difference in mouse cecum and colon weight between the homogenization-treated polysaccharide group and the non-treated polysaccharide group (p > 0.05).

3.4.2. Cecal and colonic SCFA concentration of mice

In mice, the cecum and colon are sites of vigorous microbial activity where polysaccharides undergo fermentation, yielding SCFA. SCFA was reported to provide more than 70% of the oxygen consumed by human cecal and colonic tissue. Acetate is oxidized by brain, heart and peripheral tissues; propionic acid affects the liver and cholesterol metabolism; butyric acid serves as an energy source for bacteria and the surface availability for enzymes, and thus the polysaccharides are more extensively degraded (Guillon et al., 1998). In the present study, the particle size of the polysaccharide from P. asiatica L. decreased significantly after high pressure homogenization (Fig. 1). Meanwhile, the surface topography of the polysaccharide was changed from large flake-like structure to smaller porous chips, which increased the porosity of the polysaccharide (Fig. 2). Therefore, the accessible surface area of the polysaccharide for the cecal and colonic microbiota to contact was increased, and the glucose, xylose and arabinose in the polysaccharide could be more easily utilized by the microbiota after high pressure homogenization.

4. Conclusions

The effect of high pressure homogenization on the physiological properties of polysaccharide, such as antioxidant capacity and SCFA production, was for the first time investigated. It was found that the particle size of polysaccharide from the seeds of Plantago asiatica L. decreased and the surface topography of the polysaccharide changed from large flake-like structure to smaller porous chips after high pressure homogenization. The antioxidant capacity and SCFA production of the polysaccharide were improved by the homogenization treatment. Our results showed that high pressure homogenization treatment could be a promising approach for the production of value-added polysaccharides in the food industry.

5. Conflict of interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

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References


