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# Isolation, chemical characterization, and immunomodulatory activity of naturally acetylated hemicelluloses from bamboo shavings*#

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**Abstract:** Bamboo shavings, the outer or intermediate layer of bamboo stems, are the bulk of by-products produced in bamboo processing. In this study we investigated the isolation, chemical characterization, and immunostimulatory activity in vitro of the hemicelluloses from bamboo shavings. Shavings were first pretreated by steam explosion. The optimal pretreatment was found to be steam explosion at 2.2 MPa for 1 min. Following this pretreatment, the yield of hemicelluloses reached (2.05±0.22)% (based on the dry dewaxed raw materials), which was 5.7-fold higher than that of untreated samples. Bamboo-shavings hemicellulose (BSH) was then prepared by hot water extraction and ethanol precipitation from the steam-exploded shavings. Purification of BSH by anion-exchange chromatography of diethylaminoethanol (DEAE)-sepharose Fast Flow resulted in a neutral fraction (BSH-1, purity of 95.3%, yield of 1.06%) and an acidic fraction (BSH-2, purity of 92.5%, yield of 0.79%). The weight-average molecular weights ($M_w$) of BSH-1 and BSH-2 were 12 800 and 11 300 g/mol, respectively. Chemical and structural analyses by Fourier transform infrared spectroscopy (FT-IR), 1D ($^1$H and $^{13}$C) and 2D (heteronuclear single quantum correlation (HSQC)) nuclear magnetic resonance (NMR) spectra revealed that BSH-1 was *O*-acetylated-arabinoxylan and BSH-2 was *O*-acetylated-(4-*O*-methylglucurono)-arabinoxylan. BSH-1 had a higher content of acetyl groups than BSH-2. For the immunomodulatory activity in vitro, BSH and BSH-2 significantly stimulated mouse splenocyte proliferation while BSH-1 had no effect; BSH, BSH-1, and BSH-2 markedly enhanced the phagocytosis activity and nitric oxide production of the murine macrophage RAW264.7 in a dose-dependent manner. Our results suggest that the water-extractable hemicelluloses from steam-exploded bamboo shavings are naturally acetylated and have immunostimulatory activity.

**Key words:** Bamboo shavings; Steam explosion pretreatment; Naturally acetylated hemicelluloses; Isolation; Chemical characterization; Immunomodulatory activity

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## 1 Introduction

Hemicelluloses, the second most abundant constituent of lignocellulosic biomass, are polysaccharides in plant cell walls that are characterized by being neither cellulose nor pectin and by having β-(1→4)-linked backbones with an equatorial configuration (Scheller and Ulvskov, 2010). They may contain pentoses (β-D-xylose and α-L-arabinose), hexoses (β-D-mannose, β-D-glucose, and α-D-galactose), and/or uronic acids (α-D-glucuronic, α-D-4-*O*-methylglucuronic, α-D-galacturonic, and α-D-4-*O*-methyl-galacturonic acids), and the hydroxyl groups of sugars can be partially substituted with acetyl groups (Gírio *et al.*, 2010). Xylans are the most abundant hemicelluloses, which have a wide range of uses, being converted into sugars, bio-fuel (i.e. ethanol), chemicals, and biopolymers (Ebringerova *et al.*, 2005). Hemicelluloses also have the potential to be applied in medical fields

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due to their immunomodulatory activity (Nagata *et al.*, 2001; Cao *et al.*, 2011).

Bamboo, a large perennial plant belonging to Gramineae: Bambusoideae, is widely grown in tropical and subtropical regions. As a fast growing species, bamboo is highly abundant in Asia with $7.6\times10^6$ ha of bamboo forests in China alone (Sella Kapu and Trajano, 2014). Bamboo is considered to be a rapidly renewable and sustainable biomass material (He *et al.*, 2014). Its present applications include not only paper, textile, and construction, but also the food industry and medicine (Peng *et al.*, 2011a). Since the 1970s, scientists have been isolating and characterizing bamboo hemicelluloses. Extractions with alkaline agents or organic solvents (e.g. dimethyl sulfoxide (DMSO)) are the commonly used isolation methods. Maekawa (1976) isolated a bamboo hemicellulose from *Phyllostachys reticulata* C. Koch by NaOH extraction and characterized it as (4-*O*-methylglucurono)-arabinoxylan, with 4-*O*-methyl-D-glucuronic acid (4-*O*-Me-D-GlcA), L-arabinose, and D-xylose in a molar ratio of 1.0:1.3:25.0. He also prepared a xylan containing 6.5% native acetyl groups by extraction with DMSO from bamboo holocellulose in a yield of 3.1% (based on the defatted bamboo meal). Wen *et al.* (2011) obtained seven alkali-soluble hemicelluloses from bamboo (*Bambusa rigida*) with yields of 4.2%–7.2% (based on the dry dewaxed samples). They consisted of a backbone of (1→4)-linked xylose residues having branches of L-arabinose and 4-*O*-Me-D-GlcA. Proper pretreatment is considered to be a key step for the isolation of hemicelluloses. Yang *et al.* (2013) reported an ionic liquid (IL)-based pretreatment method for bamboo. In their study, ball milled bamboo was first dissolved in 1-allyl-3-methylimidazolium chloride ([AMIN]Cl) and regenerated with distilled water, followed by consecutive extraction with 0.5 mol/L NaOH aqueous solution and 70% ethanol containing 1.0 mol/L NaOH to obtain lignin-, hemicellulose-, and cellulose-rich fractions. The isolated hemicelluloses consisted mainly of 4-*O*-methyl-D-glucurono-L-arabino-D-xylan. Steam explosion, as a method for pretreatment of biomass, has been widely used for the breakdown of structural lignocellulosic materials (Song *et al.*, 2014). This method is based on pressurizing and forcing steam into fibrous tissues and cells of a biomass, followed by rapidly releasing the pressure in an explosive decompression step and disrupting molecular interactions between hemicelluloses and other cell wall components (Zhao *et al.*, 2012). It has the advantages of a significantly lower environmental impact, lower capital investment, and the use of less hazardous chemicals (Gong *et al.*, 2012). Recently, Sun *et al.* (2014) developed a two-stage approach using a steam explosion pretreatment followed by sequential alkali and alkali/ethanol extractions to fractionate the hemicelluloses from bamboo (*Phyllostachys pubescens*), with yields of 2.5%–10.4% (based on the dry dewaxed samples). The hemicelluloses obtained were characterized as L-arabino-4-*O*-methyl-D-glucurono-D-xylan, with β-(1→4)-linked xylose residues as the backbone, L-arabinose linked to *O*-2 and *O*-3, and 4-*O*-Me-D-GlcA linked to *O*-2 of the xylopyranose residues (Sun *et al.*, 2014). These studies showed that the yield and characterization of bamboo hemicelluloses are highly dependent on the isolation method adopted. Each method has its own strengths and weaknesses. Alkaline treatment can obtain a relatively high yield of bamboo hemicelluloses, but it deacetylates the hemicelluloses. Extraction with DMSO can retain the native acetyl groups of hemicelluloses, but the long extraction time required, high cost, and potential hazards of handling large volumes of DMSO limit its utilization (Kenealy *et al.*, 2007). Although IL has proved to be an efficient system for hydrolysis of lignocellulosic biomass under mild conditions, the cost of the IL pretreatment still needs to be considered (He *et al.*, 2014). An appropriate method has yet to be reported for obtaining hemicelluloses with higher purity and yield, lower cost, weaker degradation of structure, and less use of hazardous chemicals. In view of the advantages of the steam explosion pretreatment and that there are no reports on the immunomodulatory activity of naturally acetylated hemicelluloses, in this study, we applied a steam explosion pretreatment method followed by hot water extraction, instead of alkali extraction, to isolate bamboo hemicelluloses.

Bamboo shavings, the outer or intermediate layer of bamboo stems, are the bulk of by-products produced in bamboo processing. Annually, large quantities of bamboo shavings are generated in China, most of which are underutilized. In the present work, we aimed to develop a new way for converting the shavings into value-added materials. A steam explosion

pretreatment was applied in the isolation of hemicelluloses from bamboo shavings. The pretreatment condition was optimized, and then the structural features and immunomodulatory activity in vitro of the hemicelluloses obtained were investigated.

## 2 Materials and methods

### 2.1 Materials

Bamboo shavings were obtained from the stems of *P. pubescens* and kindly provided by Zhejiang Suichang Limin Pharmaceutical Enterprise Co., Ltd. (Zhejiang, China). Chemical analyses revealed the composition to be 72.5% holocellulose, 27.5% pentosans, 22.4% Klason lignin, 3.2% extractives, and 1.9% ash (dry basis) according to the method of Leopold (1961) and Technical Association of the Pulp and Paper Industry (TAPPI) standards.

### 2.2 Chemicals and reagents

Diethylaminoethanol (DEAE)-sepharose Fast Flow was from the GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Dextran T-series standards, monosaccharide standards (mannose, ribose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose, xylose, arabinose, fucose, and lactose), 1-phenyl-3-methyl-5-pyrazolone (PMP), α-amylase (from porcine pancreas), concanavalin A (ConA), lipopolysaccharide (LPS), and methylthiazolyldiphenyltetrazolium bromide (MTT) were from the Sigma Chemical Co., Ltd. (St. Louis, MO, USA). RPMI-1640 medium, fetal bovine serum (heat-inactived), penicillin, streptomycin, and phosphate buffer saline (PBS) were from the Bioind Co., Ltd. (Israel). Erythrocyte lysis buffer was from the eBioscience Co., Ltd. (USA). Neutral red dye (for cell culture) was from Shanghai Aladdin Industrial Inc. (Shanghai, China). The nitric oxide (NO) assay kit was from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All chemicals used in the experiments were of analytical grade.

### 2.3 Steam explosion pretreatment of bamboo shavings

Steam explosion was performed on a QBS-200B batch steam explosion apparatus (Hebi Gentle Bioenergy Ltd., China). The apparatus consisted of a stainless steel reactor, a steam generator, a receiver, and a rapid-opening ball valve. The capacity of the reactor was 5.0 L, and the highest pressure was 4.0 MPa (250 °C). About 300 g of dried bamboo shavings were loaded in the reactor per batch. The reactor was heated with saturated steam to reach the controlled pressures of 1.4, 1.8, 2.2, 2.6, and 3.0 MPa (at 195, 207, 217, 226, and 234 °C), respectively. Each pressure was maintained for 1 min. Then the reactor was suddenly depressurized by opening the ball valve. Each exploded feedstock was collected and air-dried. Similarly, each sample was steamed at 2.2 MPa for 0.5, 1, 2, 5, or 10 min. The pH values of water extracts of different steam-exploded samples were measured, with the solid to liquor ratio being 1:10 (w/v).

### 2.4 Preparation of bamboo-shavings hemicellulose

The different steam-exploded samples were dewaxed with toluene-ethanol (2:1, v/v) in a Soxhlet apparatus for 6 h and oven-dried at 60 °C for 16 h. About 50 g of each dewaxed sample was extracted with 750 ml of distilled water at 95 °C for 2 h. The water extract solution obtained was adjusted to about pH 6.9, incubated with 250 U of α-amylase at 50 °C for 15–20 min with continuous stirring, and then boiled for 10 min to inactivate the enzyme. The de-starched solution was concentrated to a volume of 50 ml at a reduced pressure, and then precipitated by the addition of four volumes of ethanol with stirring. After standing overnight at 4 °C, the resulting precipitate was collected, washed thoroughly with 80% (v/v) ethanol, and then redissolved in distilled water. After removing the ethanol by vacuum evaporation, the residual solution was centrifuged. The supernatant was filtered through a 0.22-μm filter (Millipore, USA), dialyzed against distilled water (cut-off weight-averaged molecular weight ($M_w$) 2000 g/mol), and lyophilized to afford the hemicelluloses. The yield of hemicelluloses was expressed as the percentage of the weight of the dry dewaxed sample. The optimal pretreatment conditions were chosen based on the maximal yield. The bamboo-shavings hemicellulose (BSH) used for further studies was prepared under those conditions.

### 2.5 Separation and purification of BSH

Briefly, the aqueous solution of BSH (60 mg/ml, 1 ml) was loaded on an equilibrated column of DEAE-sepharose Fast Flow (2.6 cm×10 cm). Then, the

column was stepwise eluted with pure water and 0.1, 0.3, and 0.5 mol/L of aqueous NaCl solutions at a flow rate of 2 ml/min. The obtained eluate (8 ml/tube) was collected automatically and the carbohydrate elution profiles were determined using the phenol-sulfuric acid method (Dubois *et al.*, 1956). The related fractions were pooled, dialyzed against distilled water (cut-off $M_w$ 2000 g/mol) and lyophilized, affording two hemicellulosic fractions (BSH-1 and BSH-2).

## 2.6 Structural characterization of hemicellulosic fractions

### 2.6.1 Chemical analyses

Total sugar content was determined by the phenol-sulfuric acid colorimetric method using xylose as the standard (Dubois *et al.*, 1956). The Klason lignin content was detected according to the TAPPI standard T 222 om-02. Protein content was measured by Bradford's method with bovine serum albumin as the standard (Bradford, 1976).

The monosaccharide composition was analyzed by liquid chromatography as described previously (Dai *et al.*, 2010; Wang *et al.*, 2014) with some modifications. Briefly, the sample (2 mg) was dissolved in 0.5 ml of distilled water, and then hydrolyzed with 1 ml of trifluoroacetic acid (TFA, 3 mol/L) at 121 °C for 2.5 h. After hydrolysis, the excess TFA was removed by repeated co-distillations with methanol. The dried hydrolysate was reconstituted with 1 ml of distilled water and centrifuged. A 160-μl volume of supernatant was mixed with 200 μl of distilled water, 20 μl of lactose (internal standard, 0.01 mol/L aqueous solution), 20 μl of NaOH (3 mol/L), and 400 μl of PMP (0.5 mol/L methanolic solution). Twenty microliters of the mixed standards (0.01 mol/L aqueous solution) were added to 360 μl of distilled water, 20 μl of NaOH (3 mol/L), and 400 μl of PMP. The mixture was allowed to react at 70 °C for 60 min, and then 20 μl of HCl (3 mol/L) was added. The neutralized solution was extracted with chloroform three times. The aqueous phase was collected and filtered through a 0.22-μm filter before ultra-performance liquid chromatography (UPLC) analysis. PMP-labeled monosaccharides were detected using a Waters Acquity UPLC system (Waters, USA). A ZORBAX Eclipse XDB-$C_{18}$ column (2.1 mm×150 mm, 1.8 μm) (Agilent, USA) was used for separation. The sample (2 μl) was eluted with a mixture of phosphate buffer (25 mmol/L, pH 6.7) and acetonitrile in a ratio of 83:17 (v/v) for 30 min at 35 °C, with a flow rate of 0.22 ml/min. The wavelength of detection was 250 nm.

### 2.6.2 Gel permeation chromatography

The molecular weights of all samples were determined by gel permeation chromatography (GPC). The analysis was carried out using a Waters 515 HPLC system (Waters, USA), equipped with a Waters 2414 refractive index detector (Waters, USA) and a TSK G4000 $SW_{XL}$ column (7.8 mm×300 mm) (Tosoh, Japan). A 50-μl volume of sample was injected and eluted with 0.1 mol/L $NaNO_3$ for 20 min at 40 °C at a flow rate of 0.8 ml/min. Commercially available dextrans were used as standard molecular markers (peak values of the molecular weights ($M_p$) of 5000, 10 500, 43 500, 76 900, and 413 000 g/mol). Data were processed using GPC software (Millennium$^{32}$).

### 2.6.3 FT-IR and NMR spectroscopy

The Fourier transform infrared spectroscopy (FT-IR) spectra of all samples were recorded on a Bruker spectrophotometer from 4000 to 400 $cm^{-1}$ with a resolution of 2 $cm^{-1}$ in the transmission mode. A KBr disc containing a 1% finely ground sample was used for measurement. The solution-state $^1H$ nuclear magnetic resonance (NMR) and $^{13}C$ NMR spectra were recorded on an Agilent 600 MHz DD2 (DirectDrive2) NMR spectrometer. The hemicellulose (15 mg for $^1H$, 80 mg for $^{13}C$) was dissolved in 1 ml of $D_2O$. The chemical shifts were calibrated relative to the signal from $H_2O$ at $\delta$ 4.70 ppm (ppm: part per million) for the $^1H$ NMR spectrum. The $^{13}C$ NMR spectrum was obtained at 25 °C after 30 000 scans, with acetone as the internal standard ($\delta$ 30.50 ppm for carbon). The heteronuclear single quantum correlation (HSQC) spectrum was acquired over a $t_1$ spectral width of 30 165.9 Hz and a $t_2$ width of 9615.4 Hz, and the acquired time (AQ) per scan was 0.153 s. The number of scans (NS) was 128. The delay between transients was 1.0 s, and the delay for polarization transfer was set to correspond to an estimated average $^1H$-$^{13}C$ coupling constant of 146 Hz.

## 2.7 In vitro immunomodulatory activity tests

### 2.7.1 Animals, cell lines, and sample treatments

Specific pathogen free (SPF) female ICR mice (18–22 g) were obtained from the Laboratory Animal

Center of Zhejiang Academy of Medical Sciences (certificate No. SCXK-2008-0033, Hangzhou, China). Animals were housed in polypropylene cages with sawdust bedding in a hygienically controlled environment with a temperature of (24±1) °C, humidity of (50±10)%, and a 12-h light/dark cycle, with free access to water and standard rodent chow. Murine macrophage RAW264.7 (America Type Culture Collection (ATCC), Rockville, MD, USA) was cultured in RPMI-1640 medium supplemented with 10% heat-inactived fetal bovine serum, 100 IU/ml penicillin, and 100 IU/ml streptomycin in a 95% humidified atmosphere containing 5% $CO_2$ at 37 °C. BSH, BSH-1, and BSH-2 were all freshly dissolved in RPMI-1640 complete medium for cell treatments, at concentrations of 0.1, 1, 10, 100, and 1000 μg/ml. The endotoxin levels of BSH, BSH-1, and BSH-2 were less than 0.015 EU/mg (negative) as determined by a *Limulus* amebocyte lysate (LAL) assay.

2.7.2 Splenocyte proliferation assay

Splenocyte proliferation assay was performed using the method described by a previous study (Yu *et al*., 2016) with minor modifications. Spleens were removed under aseptic conditions from the sacrificed ICR mice, and placed in serum-free RPMI-1640 medium, gently homogenised, and passed through a 40-μm nylon cell strainer to obtain single-cell suspensions. After treatment with erythrocyte lysis buffer, the remaining cells were centrifuged at 200*g* for 10 min and washed with PBS three times. The cells were resuspended to a final density of $2.5\times10^6$ cells/ml with RPMI-1640 complete medium (RPMI-1640 medium supplemented with 10% heat-inactived fetal bovine serum, 100 IU/ml streptomycin, and 100 IU/ml penicillin). BSH, BSH-1, and BSH-2 were dissolved in RPMI-1640 complete medium for experiments. Splenocytes (100 μl/well) were seeded in sextuplicates in 96-well plates and cultured with different concentrations of BSH, BSH-1, and BSH-2 (0.1, 1, 10, 100, and 1000 μg/ml) for 68 h. ConA (2.5 μg/ml) and LPS (7.5 μg/ml) were used as the positive controls, while the blank control was treated with RPMI-1640 complete medium only. The proliferation activity of splenocytes was tested by MTT assay according to the method of Mosmann (1983). The optical density (OD) was determined at 570 nm and the stimulation index (SI) was calculated based on the following formula: $SI=(OD_{experimental}-OD_{blank\ control})/OD_{blank\ control}$.

2.7.3 Measurement of phagocytosis of macrophage RAW264.7

The phagocytosis of macrophages was measured using the neutral red uptake assay as described previously (Chen *et al*., 2010) with minor modifications. RAW264.7 cells ($1\times10^5$ cells/well) were incubated in sextuplicates in 96-well plates with RPMI-1640 complete medium (for the blank control group) and various concentrations (0.1, 1, 10, 100, and 1000 μg/ml) of BSH, BSH-1, and BSH-2. LPS (5 μg/ml) was used as the positive control. After incubation for 48 h, the supernatant was removed thoroughly, and 100 μl of 0.075% neutral red was added to each well. After co-incubation with macrophages for another 4 h, the supernatants were removed, and the wells were washed with 200 μl of PBS three times. Cells in each well were treated with 200 μl of lysis buffer (acetic acid: ethanol=1:1 (v/v)). The plates were incubated at 4 °C overnight. The OD determined at 540 nm was taken as a measure of the phagocytosis of the macrophages.

2.7.4 Measurement of NO production from macrophage RAW264.7

RAW264.7 cells were cultured with different concentrations (0.1, 1, 10, 100, and 1000 μg/ml) of BSH, BSH-1, and BSH-2 as described in Section 2.7.3. After 48 h, the NO content in the culture supernatant was measured by commercial assay kits according to the manufacturer's instructions.

### 2.8 Statistical analysis

The data are expressed as mean±standard deviation (SD). Statistical analyses were carried out using SPSS for Windows (Version 17.0). The differences between various groups were tested by one-way analysis of variance (ANOVA) followed by Dunnett's test. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$.

## 3 Results and discussion

### 3.1 Optimization of steam explosion pretreatment

In this study, a steam explosion pretreatment was applied in the preparation of hemicelluloses from

bamboo shavings. The effects of steam pressure and residence time on the yield of hemicelluloses are shown in Fig. 1. When the residence time was fixed at 1 min, the yield of hemicelluloses increased with rising steam pressure to reach a peak at 2.2 MPa, and then decreased (Fig. 1a). With the steam pressure maintained at 2.2 MPa, the yield of hemicelluloses increased with increasing residence time to reach a maximum at 1 min, after which it decreased (Fig. 1b). The results indicated that the yield of hemicelluloses decreased under the more harsh conditions (higher pressure or prolonged time). This may be because a more drastic steam explosion promotes disintegration of acetyl groups from xylan backbone structures and the degradation of monose, producing acetic acid and other acidic compounds (i.e. formic acid and levulinic acid) (Klinke *et al*., 2004). These acidic compounds could facilitate the hydrolysis of hemicelluloses, resulting directly in the decrease in yields. The pH value of water extracts declined with increasing steam pressure or residence time (Fig. 1). Overall, the yield of hemicelluloses achieved a maximum ((2.05±0.22)%) after steam explosion at 2.2 MPa for 1 min, which was 5.7 times higher than the yield of the untreated sample ((0.31±0.04)%). The results suggest that a steam explosion pretreatment may assist the fractionation of hemicelluloses from bamboo shavings.

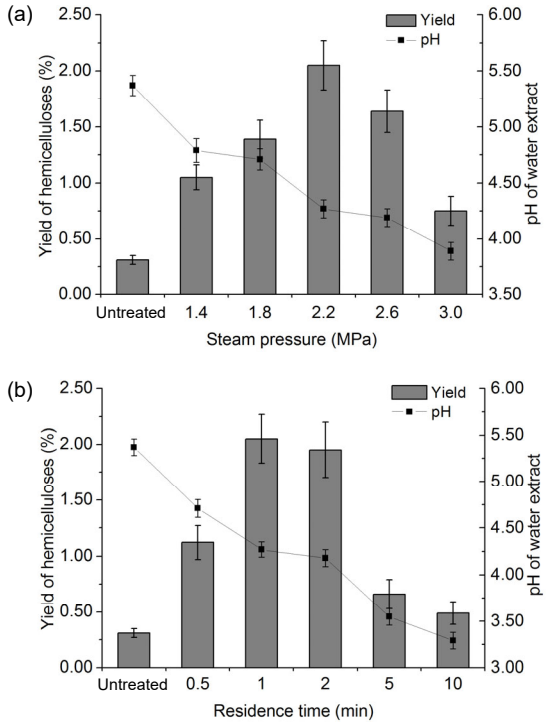


**Fig. 1 Effects of steam pressure and residence time on the yield of hemicelluloses and the pH of water extracts from bamboo shavings**

(a) Steamed at 1.4, 1.8, 2.2, 2.6, and 3.0 MPa for 1 min. (b) Steamed at 2.2 MPa for 0.5, 1, 2, 5, and 10 min. Values are expressed as mean±SD ($n$=3). The yield of hemicelluloses is expressed as the percentage of the weight of the dry dewaxed sample. The solid-to-liquor ratio for water extract was 1:10 (w/v)

### 3.2 Preparation of hemicellulosic fractions

BSH was prepared from bamboo shavings by steam explosion (steam pressure 2.2 MPa, residence time 1 min), hot water extraction, and ethanol precipitation, as described in Section 2.4. The GPC profile of BSH presented as a single peak (data not shown), implying that BSH could not be separated by molecular sieve chromatography. Monosaccharide composition analysis revealed that BSH contained glucuronic acid (Table 1), suggesting that it had acidic components. Therefore, we further separated BSH through a DEAE-sepharose anion-exchange column. Fig. 2a shows that BSH was separated into two sub-fractions: BSH-1, eluted with pure water, and BSH-2, eluted with 0.1 mol/L NaCl. They were assumed to be a neutral fraction and an acidic fraction, respectively. The yields of BSH-1 and BSH-2 were 1.06% and 0.79%, respectively, based on the dry dewaxed raw material (Table 1).

**Table 1 Yields and chemical compositions of BSH, BSH-1, and BSH-2**

| Parameter | BSH | BSH-1 | BSH-2 |
|---|---|---|---|
| Yield (%)[a] | 2.05 | 1.06 | 0.79 |
| Total sugar (%) | 90.2 | 95.3 | 92.5 |
| Klason lignin (%) | 3.5 | 2.4 | 3.6 |
| Protein (%) | 0.8 | 0.3 | 0.5 |
| Starch (%) | n.d. | n.d. | n.d. |
| Monosaccharide composition (%) | | | |
| Xylose (Xyl) | 90.5 | 92.0 | 89.9 |
| Arabinose (Ara) | 2.3 | 2.0 | 2.8 |
| Glucuronic acid (GlcA) | 1.9 | n.d. | 3.0 |
| Glucose (Glc) | 1.7 | 3.4 | n.d. |
| Mannose (Man) | 1.8 | 1.5 | 1.5 |
| Galactose (Gal) | 1.8 | 1.1 | 2.3 |
| Ara/Xyl[b] | 0.025 | 0.022 | 0.031 |
| GlcA/Xyl[c] | 0.021 | | 0.033 |

[a] Yield was calculated as the percentage of the weight of the dry dewaxed sample; [b] Ara/Xyl, arabinose to xylose ratio; [c] GlcA/Xyl, glucuronic acid to xylose ratio. Measurements were carried out in duplicate and the relative standard deviation was less than 5%. n.d.: not detected

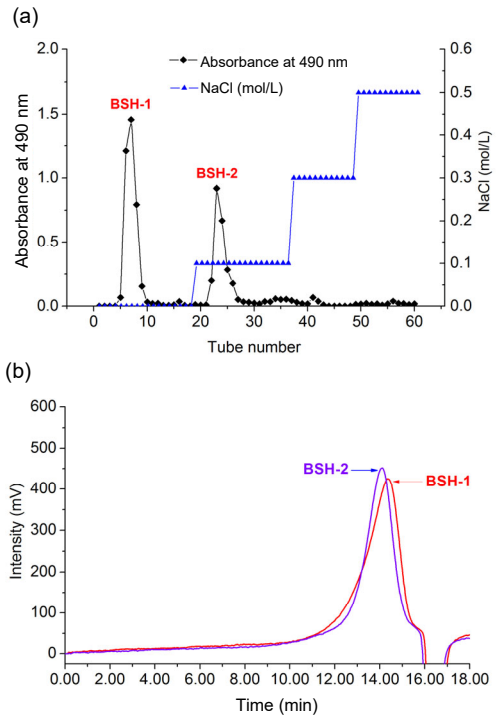


**Fig. 2 Elution profile of BSH on a DEAE-sepharose Fast Flow column (a) and gel permeation chromatography profiles of BSH-1 and BSH-2 (b)**

The free volume outside of the particles ($V_o$) was 5 ml and the total volume ($V_t$) of the gel column was 20 ml. The peak values of the molecular weights ($M_p$) of BSH-1 and BSH-2 were about 6300 and 7600 g/mol, respectively. The weight-averaged molecular weights ($M_w$) of BSH-1 and BSH-2 were about 12 800 and 11 300 g/mol, respectively. The number-averaged molecular weights ($M_n$) of BSH-1 and BSH-2 were about 8000 and 8600 g/mol, respectively

## 3.3 Structural characterization of hemicellulosic fractions

### 3.3.1 Molecular weight

The GPC profiles of BSH-1 and BSH-2 both presented as a single and symmetrical narrow peak (Fig. 2b), indicating that they were relatively homogeneous polymers. Their $M_w$ values were estimated to be about 12 800 and 11 300 g/mol, respectively.

### 3.3.2 Chemical composition

The chemical analyses showed that the total sugar contents of BSH-1 and BSH-2 were 95.3% and 92.5%, respectively. Both BSH-1 and BSH-2 contained small amounts of Klason lignin (2.4% and 3.6%, respectively) and protein (0.3% and 0.5%, respectively) (Table 1). The above data suggested that the hemicelluloses obtained from steam-exploded bamboo shavings had a satisfactory degree of purity. After acid hydrolysis of the hemicelluloses, the monomeric sugars released were reacted with PMP and then monitored by UPLC. Xylose was the major sugar constituent in BSH-1 and BSH-2, comprising 89.9%–92.0% of the total sugar composition, whereas arabinose was present in only small amounts (2.0%–2.8%) (Table 1). Minor quantities (1.1%–2.3%) of mannose and galactose were also identified in BSH-1 and BSH-2. BSH-1 contained 3.4% glucose, which may have arisen from degraded low-molecular-weight cellulose or (1→3)(1→4)-β-D-glucan (Peng *et al.*, 2011b). BSH-2 contained 3.0% glucuronic acid, which may have originated from 4-*O*-methyl-α-D-glucuronic acid (4-*O*-Me-α-D-Glc*p*A) as side chains (Peng *et al.*, 2011b). In general, the arabinose to xylose (Ara/Xyl) ratio is considered to be indicative of the degree of linearity or branching of hemicelluloses. A high Ara/Xyl ratio indicates a short-chain polymer with more branching while a low Ara/Xyl ratio suggests a linear hemicellulosic polymer with less branching (Peng *et al.*, 2011a). In the present study, the Ara/Xyl ratios of BSH-1 and BSH-2 were 0.022 and 0.031, respectively (Table 1), much lower than those of alkali-extractable or alkali/ethanol-extractable hemicelluloses from steam-exploded bamboo (*P. pubescens*) stems (Sun *et al.*, 2014). This result indicates that water-extractable hemicelluloses from steam-exploded bamboo (*P. pubescens*) shavings have a relatively low degree of arabinose branching and appear to be more linear than alkali-extractable or alkali/ethanol-extractable hemicelluloses.

### 3.3.3 FT-IR analysis

Fig. 3 shows the FT-IR spectra of BSH-1 and BSH-2, which appeared to be typical of polysaccharide structures. The broad intense peak around 3420 $cm^{-1}$ indicated the stretching vibration of O–H. The signals at 2928 and 1379 $cm^{-1}$ come from the stretching and bending vibrations of C–H. The peak at 1646 $cm^{-1}$ was due to the presence of bound water in the hemicelluloses. The bands at 1735 and 1250 $cm^{-1}$ were attributed to the C=O and C–O linkages in the acetyl group of xylan (Peng *et al.*, 2011b), implying that both BSH-1 and BSH-2 were acetylated. The absorption at 1735 $cm^{-1}$ in BSH-2 was from its uronic ester groups (Sukhbaatar *et al.*, 2014). The weak

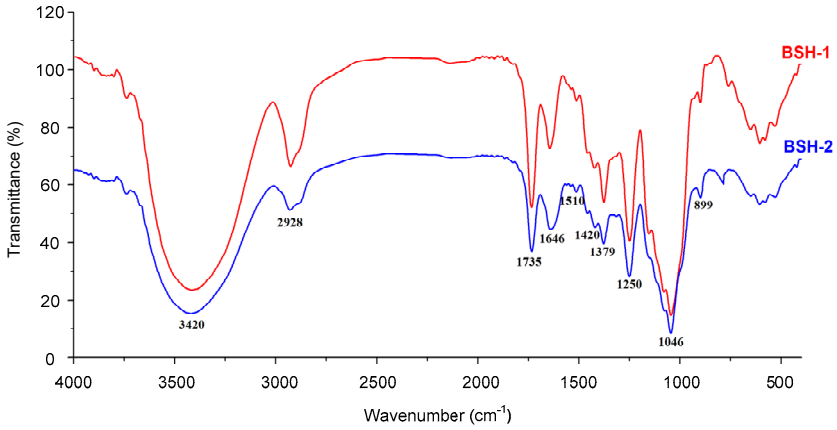


**Fig. 3 FT-IR spectra of BSH-1 and BSH-2**

absorbance at 1510 $cm^{-1}$ suggested that both BSH-1 and BSH-2 were slightly contaminated with minor amounts of bound lignin. The typical signal of xylopyranosyl (Xyl*p*) units appeared at 1046 $cm^{-1}$ (C–O, C–C stretching or C–OH bending) (Sun *et al.*, 2014). Finally, the important band at 899 $cm^{-1}$ indicated the presence of dominant β-glycosidic linkages between the sugar units in the hemicelluloses (Geng *et al.*, 2003).

### 3.3.4 NMR analysis

NMR spectroscopy has been viewed as a powerful non-destructive tool to investigate the fine structure of polysaccharides. Fig. 4 gives the $^{1}H$ and $^{13}C$ NMR spectra of BSH-1 and BSH-2. Their signal assignments were made according to previous studies (Hoffmann *et al.*, 1992; Teleman *et al.*, 2000; Peng *et al.*, 2011a; 2011b; Sun *et al.*, 2014).

As shown in Fig. 4a, the relative structural complexity of BSH-1 was exhibited by (i) major signals at $\delta$ 4.42 (H-1), 4.05 (H-5eq), 3.72 (H-4), 3.50 (H-3), 3.41 (H-5ax), and 3.24 ppm (H-2), corresponding to nonsubstituted (1→4)-β-D-Xyl*p* residues (Teleman *et al.*, 2000; Peng *et al.*, 2011a); (ii) a small signal at $\delta$ 5.32 ppm, indicating the arabinofuranosyl (Ara*f*) units monosubstituted at *O*-3 of Xyl*p* residues (Hoffmann *et al.*, 1992); and (iii) strong signals at $\delta$ 2.05 and 2.09 ppm, corresponding to the hydrogen in methyl groups of 2-*O*-acetylated Xyl*p* residues and 3-*O*-acetylated Xyl*p* residues, respectively (Teleman *et al.*, 2000). Six signals at $\delta$ 4.36 (H-1), 4.00 (H-5eq), 3.69 (H-4), 3.47 (H-3), 3.32 (H-5ax), and 3.14 ppm (H-2) were observed. We deduced that these signals came from another group of nonsubstituted (1→4)-β-D-Xyl*p* residues. The result was similar to that of Teleman *et al.* (2000) who found several groups of signals from nonsubstituted (1→4)-β-D-Xyl*p* residues. The $^{1}H$ NMR spectrum of BSH-2 is shown in Fig. 4b. Compared with BSH-1, additional peaks were observed in the spectrum of BSH-2. For instance, the sharp singlet at $\delta$ 3.35 ppm and the signal at $\delta$ 5.19 ppm were characteristics of $OCH_3$-4 and H-1 of 4-*O*-Me-α-D-Glc*p*A, respectively (Teleman *et al.*, 2000).

The $^{13}C$ NMR spectrum of BSH-1 (Fig. 4c) shows five main signals at $\delta$ 102.66, 77.33, 74.63, 73.65, and 63.94 ppm, corresponding to the C-1, C-4, C-3, C-2, and C-5 of the (1→4)-β-D-Xyl*p* units, respectively (Peng *et al.*, 2011b; Sun *et al.*, 2014). Moreover, four signals at $\delta$ 103.63, 77.13, 74.54, and 73.73 ppm were observed. Likewise, they can be attributed to C-1, C-4, C-3, and C-2 of another group of nonsubstituted (1→4)-β-D-Xyl*p* residues (Teleman *et al.*, 2000). The strong signals at $\delta$ 21.37/173.96 ppm and 21.60/174.49 ppm were indicative of methyl and carbonyl in acetyl groups for 2-*O*-acetylated Xyl*p* residues and 3-*O*-acetylated Xyl*p* residues, respectively (Teleman *et al.*, 2000). The small signal at $\delta$ 61.44 ppm was attributed to C-5 of Ara*f* units (Peng *et al.*, 2011a). In the $^{13}C$ NMR spectrum of BSH-2 (Fig. 4d), the signals at $\delta$ 176.95, 98.74, 73.24, 83.16, and 61.00 ppm were characteristics, respectively, of COOH, C-1, C-3, C-4, and $OCH_3$-4 of 4-*O*-Me-α-D-Glc*p*A residues (Peng *et al.*, 2011a; 2011b; Sun *et al.*, 2014). For a better understanding, we have summarized the $^{1}H$ and $^{13}C$ NMR chemical shifts of BSH-1 and BSH-2 in Tables S1 and S2.

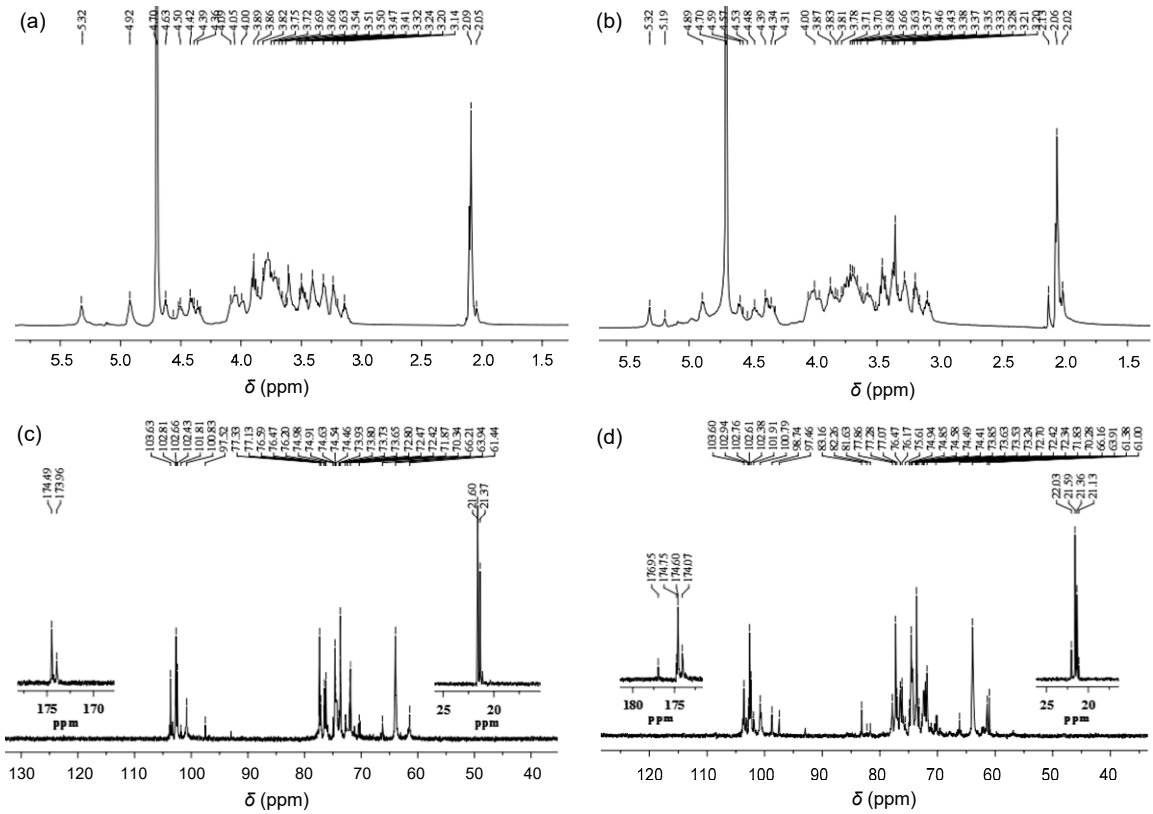


**Fig. 4 $^{1}$H NMR and $^{13}$C NMR spectra of BSH-1 and BSH-2**

(a) $^{1}$H NMR spectrum of BSH-1; (b) $^{1}$H NMR spectrum of BSH-2; (c) $^{13}$C NMR spectrum of BSH-1; (d) $^{13}$C NMR spectrum of BSH-2

Wen *et al.* (2011) reported that the 2D-HSQC spectrum can clearly show direct correlations between the hydrogen and carbon atoms of the glycosyl residues and allows accurate quantitative analysis. To understand more about the structures of BSH-1 and BSH-2, we applied the 2D-HSQC technique in this study. The marked $^{1}$H/$^{13}$C cross-peaks in the HSQC spectrum of BSH-1 confirmed the structural elements of the (1→4)-β-D-Xyl*p* backbone, 2-*O*-acetylated Xyl*p* units, 3-*O*-acetylated Xyl*p* units, reducing end residues of Xyl*p* units (exhibiting β conformation), and Ara*f* units (Fig. 5a). The HSQC spectrum of BSH-2 (Fig. 5b) additionally gives the cross-peaks of the structural elements of 4-*O*-Me-α-D-Glc*p*A units and 2,3-di-*O*-acetylated Xyl*p* units. For both BSH-1 and BSH-2, several different chemical environments could be observed for their Xyl*p* residues. The anomeric proton resonances originating from these groups could be divided into the following eight types: (i) reducing end residues exhibiting β conformation; (ii) unsubstituted internal residues; (iii) terminal (non-reducing end) residues; (iv) 2-*O*-acetylated internal residues; (v) 3-*O*-acetylated internal residues; (vi) 2,3-di-*O*-acetylated internal residues; (vii) 4-*O*-Me-Glc*p*A 2-*O*-substituted internal residues; and (viii) Ara*f* 3-*O*-substituted internal residues. By integrating the areas of anomeric hydrogen/carbon correlation peaks in HSQC spectra, we obtained the rough molar ratios of different glycosyl residues in BSH-1 and BSH-2 (Table 2). Note that the relative molar ratio of 4-*O*-Me-α-D-GlcA to Xyl in BSH-2 (0.064) obtained from the integral in HSQC was higher than that from sugar analysis (0.033) (Table 1). This discrepancy might be explained by the fact that aldouronic acids are difficult to hydrolyze under acidic conditions (Wen *et al.*, 2011).

Taking the results of various analytical techniques (chemical analysis, FT-IR, and NMR) into account, we deduce that BSH-1 was *O*-acetylated-arabinoxylan, consisting of a linear (1→4)-β-D-Xyl*p* backbone decorated with branches at *O*-2 of acetyl groups (8.4%) or at *O*-3 of α-L-Ara*f* (2.2%) and acetyl groups (22.8%); while BSH-2 was *O*-acetylated-(4-*O*-methyl-glucurono)-arabinoxylan, consisting of

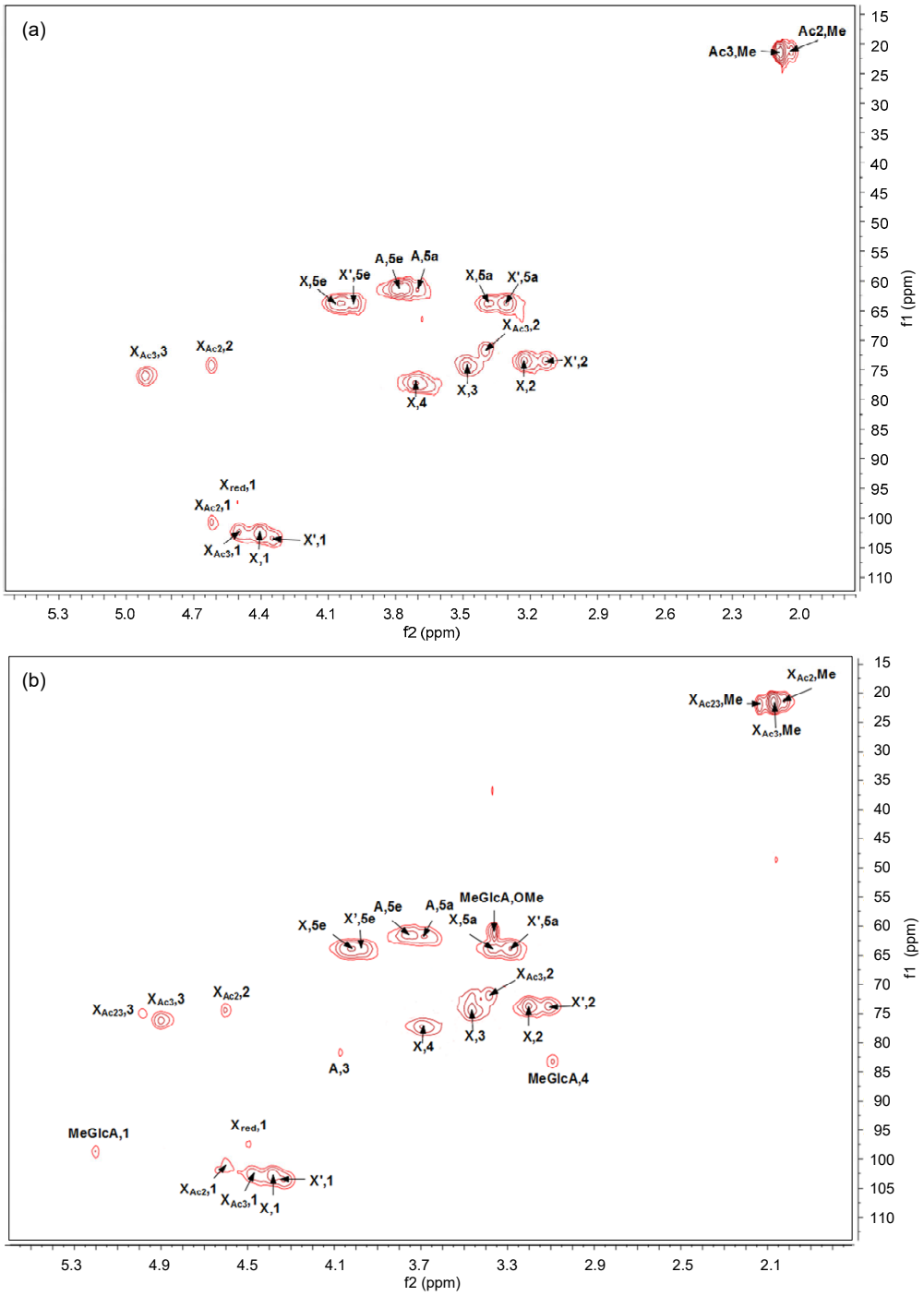


**Fig. 5 HSQC spectra of BSH-1 (a) and BSH-2 (b)**

The following designations are used: X (X')=$X_{int}$, Xyl internal; $X_{red}$, Xyl with reducing end; A, Ara*f*; MeGlcA, 4-*O*-Me-D-Glc*p*A; $X_{Ac2}$, 2-*O*-acetylated Xyl; $X_{Ac3}$, 3-*O*-acetylated Xyl; $X_{Ac23}$, 2,3-di-*O*-acetylated Xyl; a, axial; e, equatorial

a linear (1→4)-β-D-Xyl*p* backbone decorated with branches at *O*-2 of acetyl groups (7.0%) and 4-*O*-Me-α-D-Glc*p*A (6.4%) or at *O*-3 of α-L-Ara*f* (3.1%) and acetyl groups (18.1%). Also, about 1.4% of its (1→4)-β-D-Xyl*p* units were di-acetylated (at *O*-2 and *O*-3).

### 3.4 Immunomodulatory activity in vitro

#### 3.4.1 Effects of BSH, BSH-1, and BSH-2 on splenocyte proliferation

Splenocyte proliferation is a key event in the activation cascade of both cellular and humoral

**Table 2 Integrated values of anomeric hydrogen/carbon correlation peaks of glycosyl residues in HSQC spectra of BSH-1 and BSH-2**

| Glycosyl residue* | BSH-1 | BSH-2 |
|---|---|---|
| $X_{int}$ (X and X') | 1.000 | 1.000 |
| $X_{red}$ | 0.039 | 0.040 |
| $X_{term}$[a] | 0.039 | 0.040 |
| $X_{MeGlcA2}$[b] | n.d. | 0.109 |
| $X_{A3}$[c] | 0.036 | 0.052 |
| $X_{Ac2}$ | 0.136 | 0.119 |
| $X_{Ac3}$ | 0.369 | 0.306 |
| $X_{Ac23}$[d] | n.d. | 0.024 |
| $X_{total}$ | 1.619 | 1.690 |
| $X_{MeGlcA2}/X_{total}$ | | 0.064 |
| $X_{A3}/X_{total}$[e] | 0.022 | 0.031 |
| $X_{Ac2}/X_{total}$ | 0.084 | 0.070 |
| $X_{Ac3}/X_{total}$ | 0.228 | 0.181 |
| $X_{Ac23}/X_{total}$ | | 0.014 |

* The following designations are used: $X_{int}$, Xyl internal; $X_{red}$, Xyl with reducing end; $X_{term}$, Xyl with terminal end; $X_{MeGlcA2}$, MeGlcA 2-*O*-linked Xyl; $X_{A3}$, Ara 3-*O*-linked Xyl; $X_{Ac2}$, 2-*O*-acetylated Xyl; $X_{Ac3}$, 3-*O*-acetylated Xyl; and $X_{Ac23}$, 2,3-di-*O*-acetylated Xyl. MeGlcA, 4-*O*-methyl-glucuronic acid. [a] Equal to $X_{red}$; [b] Refers to the H1/C1 signals of MeGlcA, as the H1/C1 peak of $X_{MeGlcA2}$ overlapped that of $X_{int}$; [c] Calculated from $X_{A3}/X_{total}$; [d] Refers to the H3/C3 peak of $X_{Ac23}$, as the H1/C1 peak of $X_{Ac23}$ overlapped that of $X_{int}$; [e] Calculated from Ara/Xyl in Table 1, as the H1/C1 peak of Ara was not detected; n.d., not detected

immune responses. T- and B-lymphocytes are mainly responsible for cellular and humoral immunity, respectively. The ability to stimulate the proliferative response of lymphocytes (expressed as the SI) is one of the important indicators used to evaluate the immune function of drugs (Ke *et al.*, 2013). Generally, the SI of the control (medium alone) was close to 1. A higher value would indicate a stimulatory effect on splenocyte proliferation. While investigating the possible synergistic effect between the activity of BSH-1 and BSH-2, we also took BSH into account. Fig. 6a shows that BSH at 100–1000 μg/ml and BSH-2 at 10–1000 μg/ml were able to induce splenocyte proliferation, while BSH-1 did not exert any effect. Thus, BSH-2 performed better than BSH-1 in inducing splenocyte proliferation. Considering the structural differences between BSH-1 and BSH-2, we speculate that the uronic acid in BSH-2 might contribute to its stronger activity in stimulating lymphocytes. The result was similar to that of Yi *et al.* (2012), who reported that the acidic polysaccharides were more sensitive in stimulating lymphocyte proliferation. However, other studies have reported that neither the uronic acid content nor the distribution pattern of the uronic acid side chains was a determinant of the immunostimulatory activity of 4-*O*-methyl-glucuronoxylans (Ebringerová *et al.*, 2002). Therefore, more studies should be done to explain the above structure/function relationships.

3.4.2 Effects of BSH, BSH-1, and BSH-2 on phagocytic activity of macrophage RAW264.7

Macrophages provide the first line of defense against microbial invaders and malignancies by secreting inflammatory mediators, such as cytokines, chemokines, and NO (Ma *et al*., 2014). Activation of macrophages is a pivotal event in innate and adaptive immunity for initiation and propagation of defense against pathogens or cancer cells (Ma *et al.*, 2014). Some studies report the activation effects of polysaccharides on macrophages, as polysaccharides can recognize the various receptors on macrophage membranes (Ma *et al.*, 2014; Zheng *et al.*, 2014). In this study, we assessed the effects of BSH, BSH-1, and BSH-2 on the phagocytic activity of murine macrophage cell line RAW264.7 by neutral red uptake assay. All three hemicelluloses enhanced the phagocytosis of RAW264.7 in a prominent dose-related response (Fig. 6b). The enhancements of BSH at 10–1000 μg/ml, BSH-1 at 10–1000 μg/ml, and BSH-2 at 100–1000 μg/ml were significant compared with the blank control group. Among the three hemicelluloses, BSH-1 showed the best activity in enhancing the phagocytosis of macrophage, followed by BSH and BSH-2. BSH-1 at 10–100 μg/ml showed an enhancing effect corresponding to 5 μg/ml of the positive control (LPS). The above data suggest that the naturally acetylated hemicelluloses isolated from bamboo shavings can effectively promote the phagocytic function of macrophages in vitro.

3.4.3 Effects of BSH, BSH-1, and BSH-2 on NO release from macrophage RAW264.7

NO, an intracellular messenger, is one of the major effector molecules of macrophages for killing tumor cells and pathogenic microorganisms (Boscá *et al.*, 2005). BSH, BSH-1, and BSH-2 markedly induced NO production from macrophage RAW264.7 at 10–1000 μg/ml (Fig. 6c). BSH-1 displayed the strongest activity, followed by BSH and BSH-2. The lowest effective concentration of BSH, BSH-1, and BSH-2 in inducing NO release from macrophage RAW264.7 was 10 μg/ml, which was lower than that reported for polysaccharides from *Schisandra chinensis* (Turcz.) Bail (Zhao *et al.*, 2013) or *Inonotus*

*obliquus* (Fan *et al.*, 2012). The results suggest that the hemicellulosic polysaccharides isolated from steam-exploded bamboo shavings have a good capacity for stimulating macrophages.

Taken together, BSH-1 clearly showed a stronger activity in stimulating macrophages than BSH-2. In consideration of their structural differences, we speculate that the higher content of acetyl groups in BSH-1 may have contributed to its better performance in stimulating macrophages. This finding is in line with that of Chen *et al.* (2014). Therefore, the acetyl groups attached to the polysaccharide might play an important role in its activation of macrophages.

## 4 Conclusions

In summary, the present study obtained three hemicelluloses (BSH, BSH-1, and BSH-2) from steam-exploded bamboo shavings. BSH-1 and BSH-2 were purified fractions of BSH. Chemical and structural analyses revealed that BSH-1 was *O*-acetylated-arabinoxylan ($M_w$: 12 800 g/mol) and BSH-2 was *O*-acetylated-(4-*O*-methyl-glucurono)-arabinoxylan ($M_w$: 11 300 g/mol). BSH-1 had more acetyl groups than BSH-2. Investigation of immunomodulatory activity in vitro showed that BSH (at 100–1000 μg/ml) and BSH-2 (at 10–1000 μg/ml) significantly enhanced mouse splenocyte proliferation whereas BSH-1 had no effect; BSH (at 10–1000 μg/ml), BSH-1 (at 10–1000 μg/ml), and BSH-2 (at 100–1000 or 10–1000 μg/ml) markedly increased the phagocytosis activity and NO release of murine macrophage RAW264.7. BSH-1 had stronger activity than BSH-2 in stimulating macrophages, while BSH-2 showed better activity than BSH-1 in inducing splenocyte proliferation. Our results demonstrated that the water-extractable hemicelluloses from steam-exploded bamboo shavings were naturally acetylated and immuno-active in vitro. We also performed additional animal experiments and found that BSH had good immunostimulatory activity in vivo (data unpublished). In view of the abundance of bamboo and its by-products in China, the identification of immuno-active hemicelluloses from bamboo shavings may not only provide a new method allowing dietary control of immune-related diseases, but also promote the development of bamboo shavings as highly valued by-products.

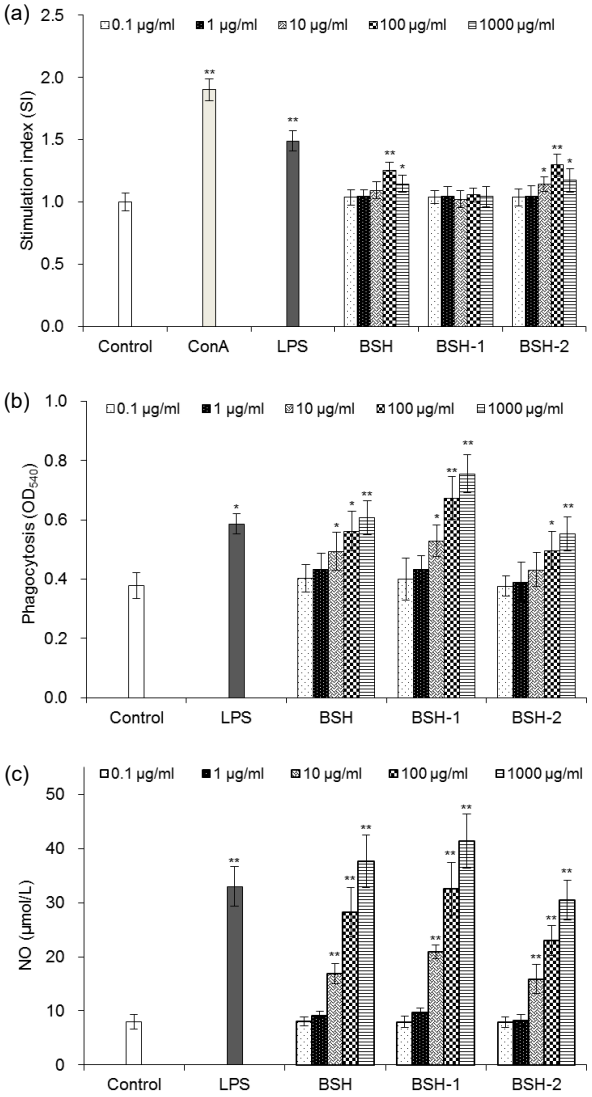


**Fig. 6 Immunomodulatory activities in vitro of BSH, BSH-1, and BSH-2**

(a) Effects of BSH, BSH-1, and BSH-2 on mouse splenocyte proliferation. Splenocytes ($2.5\times10^6$ cells/well) were incubated with ConA (2.5 μg/ml), LPS (7.5 μg/ml), and different concentrations (0–1000 μg/ml) of BSH, BSH-1, and BSH-2 for 68 h. (b) Effects of BSH, BSH-1, and BSH-2 on phagocytosis of murine macrophage RAW264.7 by neutral red uptake assay. RAW264.7 cells ($1\times10^5$ cells/well) were incubated with LPS (5 μg/ml) and different concentrations (0–1000 μg/ml) of BSH, BSH-1, and BSH-2 for 48 h. Absorbance was determined at 540 nm. (c) Effects of BSH, BSH-1, and BSH-2 on NO production of murine macrophage RAW264.7. RAW264.7 cells ($1\times10^5$ cells/well) were incubated with LPS (5 μg/ml) and different concentrations (0–1000 μg/ml) of BSH, BSH-1, and BSH-2 for 48 h. Each value is presented as mean±SD of three separate experiments. $^*$ $P<0.05$, $^{**}$ $P<0.01$ compared with the blank control group. A value of $P<0.05$ denotes the presence of a statistically significant difference according to Dunnett's method

## Compliance with ethics guidelines

Ju-qing HUANG, Rui-ting QI, Mei-rong PANG, Cong LIU, Guang-yu LI, and Ying ZHANG declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

## References


Boscá, L., Zeini, M., Través, P.G., *et al*., 2005. Nitric oxide and cell viability in inflammatory cells: a role for NO in macrophage function and fate. *Toxicology*, **208**(2):249-258. http://dx.doi.org/10.1016/j.tox.2004.11.035

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**(1):248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3

Cao, L., Liu, X., Qian, T., *et al*., 2011. Antitumor and immunomodulatory activity of arabinoxylans: a major constituent of wheat bran. *Int. J. Biol. Macromol.*, **48**(1):160-164. http://dx.doi.org/10.1016/j.ijbiomac.2010.10.014

Chen, Y., Duan, J., Qian, D., *et al*., 2010. Assessment and comparison of immunoregulatory activity of four hydrosoluble fractions of *Angelica sinensis in vitro* on the peritoneal macrophages in ICR mice. *Int. Immunopharmacol.*, **10**(4):422-430. http://dx.doi.org/10.1016/j.intimp.2010.01.004

Chen, Y., Zhang, H., Wang, Y., *et al*., 2014. Acetylation and carboxymethylation of the polysaccharide from *Ganoderma atrum* and their antioxidant and immunomodulating activities. *Food Chem.*, **156**(8):279-288. http://dx.doi.org/10.1016/j.foodchem.2014.01.111

Dai, J., Wu, Y., Chen, S., *et al*., 2010. Sugar compositional determination of polysaccharides from *Dunaliella salina* by modified RP-HPLC method of precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone. *Carbohyd. Polym.*, **82**(3):629-635. http://dx.doi.org/10.1016/j.carbpol.2010.05.029

Dubois, M., Gilles, K.A., Hamilton, J.K., *et al*., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**(3):350-356. http://dx.doi.org/10.1021/ac60111a017

Ebringerova, A., Hromadkova, Z., Heinze, T., 2005. Hemicellulose. *Adv. Polym. Sci*., **186**:1-67. http://dx.doi.org/10.1007/b136816

Ebringerová, A., Kardo Ová, A., Hromádková, Z., *et al*., 2002. Immunomodulatory activity of acidic xylans in relation to their structural and molecular properties. *Int. J. Biol. Macromol.*, **30**(1):1-6. http://dx.doi.org/10.1016/S0141-8130(01)00186-6

Fan, L., Ding, S., Ai, L., *et al*., 2012. Antitumor and immunomodulatory activity of water-soluble polysaccharide from *Inonotus obliquus*. *Carbohyd. Polym.*, **90**(2):870-874. http://dx.doi.org/10.1016/j.carbpol.2012.06.013

Geng, Z.C., Sun, R.C., Sun, X.F., *et al*., 2003. Comparative study of hemicelluloses released during two-stage treatments with acidic organosolv and alkaline peroxide from *Caligonum monogoliacum* and *Tamarix* spp. *Polym. Degrad. Stabil.*, **80**(2):315-325. http://dx.doi.org/10.1016/S0141-3910(03)00015-6

Gírio, F.M., Fonseca, C., Carvalheiro, F., *et al*., 2010. Hemicelluloses for fuel ethanol: a review. *Bioresource Technol.*, **101**(13):4775-4800. http://dx.doi.org/10.1016/j.biortech.2010.01.088

Gong, L., Huang, L., Zhang, Y., 2012. Effect of steam explosion treatment on barley bran phenolic compounds and antioxidant capacity. *J. Agric. Food Chem.*, **60**(29):7177-7184. http://dx.doi.org/10.1021/jf301599a

He, M., Wang, J., Qin, H., *et al*., 2014. Bamboo: a new source of carbohydrate for biorefinery. *Carbohyd. Polym.*, **111**(20):645-654. http://dx.doi.org/10.1016/j.carbpol.2014.05.025

Hoffmann, R.A., Geijtenbeek, T., Kamerling, J.P., *et al*., 1992. $^{1}$H-N.m.r. study of enzymically generated wheat-endosperm arabinoxylan oligosaccharides: structures of hepta- to tetradeca-saccharides containing two or three branched xylose residues. *Carbohyd. Res.*, **223**:19-44. http://dx.doi.org/10.1016/0008-6215(92)80003-J

Ke, C., Qiao, D., Luo, J., *et al*., 2013. Immunostimulatory activity and structure of polysaccharide from *Streptococcus equi* subsp. *zooepidemicus*. *Int. J. Biol. Macromol.*, **57**(6):218-225. http://dx.doi.org/10.1016/j.ijbiomac.2013.03.033

Kenealy, W., Horn, E., Davis, M., *et al*., 2007. Vapor-phase diethyl oxalate pretreatment of wood chips: Part 2. Release of hemicellulosic carbohydrates. *Holzforschung*, **61**(3):230-235. http://dx.doi.org/10.1515/HF.2007.040

Klinke, H.B., Thomsen, A.B., Ahring, B.K., 2004. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Appl. Microbiol. Biotechnol.*, **66**(1):10-26. http://dx.doi.org/10.1007/s00253-004-1642-2

Leopold, B., 1961. Chemical composition and physical properties of wood fibers. I. Preparation of holocellulose fibers from loblolly pinewood. *TAPPI*, **44**(3):230-232.

Ma, Y., Xing, Y., Mi, H., *et al*., 2014. Extraction, preliminary characterization and immunostimulatory activity *in vitro* of a polysaccharide isolated from *Strongylocentrotus nudus* eggs. *Carbohyd. Polym*., **111**(1):576-583. http://dx.doi.org/10.1016/j.carbpol.2014.04.010

Maekawa, E., 1976. Studies on hemicellulose of bamboo. *Wood Res.: Bull. Wood Res. Inst. Kyoto Univ.*, **59**:153-179.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, **65**(1):55-63. http://dx.doi.org/10.1016/0022-1759(83)90303-4

Nagata, J., Higashiuesato, Y., Maeda, G., *et al*., 2001. Effects of water-soluble hemicellulose from soybean hull on serum antibody levels and activation of macrophages in rats. *J. Agric. Food Chem.*, **49**(10):4965-4970. http://dx.doi.org/10.1021/jf0104883

Peng, P., Peng, F., Bian, J., *et al*., 2011a. Isolation and structural characterization of hemicelluloses from the bamboo species *Phyllostachys incarnata* Wen. *Carbohyd. Polym.*, **86**(2):883-890. http://dx.doi.org/10.1016/j.carbpol.2011.05.038

Peng, P., Peng, F., Bian, J., *et al*., 2011b. Studies on the starch and hemicelluloses fractionated by graded ethanol precipitation from bamboo *Phyllostachys bambusoides* f. shouzhu Yi. *J. Agric. Food Chem.*, **59**(6):2680-2688.

http://dx.doi.org/10.1021/jf1045766
Scheller, H.V., Ulvskov, P., 2010. Hemicelluloses. *Annu. Rev. Plant Biol.*, **61**(1):263-289. http://dx.doi.org/10.1146/annurev-arplant-042809-112315
Sella Kapu, N., Trajano, H.L., 2014. Review of hemicellulose hydrolysis in softwoods and bamboo. *Biofuel. Bioprod. Bior.*, **8**(6):857-870. http://dx.doi.org/10.1002/bbb.1517
Song, H., Yang, R., Zhao, W., *et al*., 2014. Innovative assistant extraction of flavonoids from pine (*Larix olgensis* Henry) needles by high density steam flash-explosion. *J. Agric. Food Chem.*, **62**(17):3806-3812. http://dx.doi.org/10.1021/jf405412r
Sukhbaatar, B., Hassan, E.B., Kim, M., *et al*., 2014. Optimization of hot-compressed water pretreatment of bagasse and characterization of extracted hemicelluloses. *Carbohyd. Polym.*, **101**(1):196-202. http://dx.doi.org/10.1016/j.carbpol.2013.09.027
Sun, S., Cao, X., Xu, F., *et al*., 2014. Structure and thermal property of alkaline hemicelluloses from steam exploded *Phyllostachys pubescens*. *Carbohyd. Polym.*, **101**(1): 1191-1197. http://dx.doi.org/10.1016/j.carbpol.2013.09.109
Teleman, A., Lundqvist, J., Tjerneld, F., *et al*., 2000. Characterization of acetylated 4-*O*-methylglucuronoxylan isolated from aspen employing $^{1}H$ and $^{13}C$ NMR spectroscopy. *Carbohyd. Res.*, **329**(4):807-815. http://dx.doi.org/10.1016/S0008-6215(00)00249-4
Wang, Q.H., Shu, Z.P., Xu, B.Q., *et al*., 2014. Structural characterization and antioxidant activities of polysaccharides from *Citrus aurantium* L. *Int. J. Biol. Macromol.*, **67**(6):112-123. http://dx.doi.org/10.1016/j.ijbiomac.2014.03.004
Wen, J., Xiao, L., Sun, Y., *et al*., 2011. Comparative study of alkali-soluble hemicelluloses isolated from bamboo (*Bambusa rigida*). *Carbohyd. Res.*, **346**(1):111-120. http://dx.doi.org/10.1016/j.carres.2010.10.006
Yang, D., Zhong, L., Yuan, T., *et al*., 2013. Studies on the structural characterization of lignin, hemicelluloses and cellulose fractionated by ionic liquid followed by alkaline extraction from bamboo. *Ind. Crop. Prod.*, **43**(5):141-149. http://dx.doi.org/10.1016/j.indcrop.2012.07.024
Yi, Y., Zhang, M., Liao, S., *et al*., 2012. Effects of alkali dissociation on the molecular conformation and immunomodulatory activity of longan pulp polysaccharide (LPI). *Carbohyd. Polym.*, **87**(2):1311-1317. http://dx.doi.org/10.1016/j.carbpol.2011.09.014
Yu, Z., Xu, D., Lu, L., *et al*., 2016. Immunomodulatory effect of a formula developed from American ginseng and Chinese jujube extracts in mice. *J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.)*, **17**(2):147-157. http://dx.doi.org/10.1631/jzus.B1500170
Zhao, T., Mao, G., Mao, R., *et al*., 2013. Antitumor and immunomodulatory activity of a water-soluble low molecular weight polysaccharide from *Schisandra chinensis* (Turcz.) Baill. *Food Chem. Toxicol.*, **55**(1):609-616. http://dx.doi.org/10.1016/j.fct.2013.01.041
Zhao, W., Yang, R., Zhang, Y., *et al*., 2012. Sustainable and practical utilization of feather keratin by an innovative physicochemical pretreatment: high density steam flash-explosion. *Green Chem*., **14**(12):3352-3360. http://dx.doi.org/10.1039/c2gc36243k
Zheng, W., Zhao, T., Feng, W., *et al*., 2014. Purification, characterization and immunomodulating activity of a polysaccharide from flowers of *Abelmoschus esculentus*. *Carbohyd. Polym.*, **106**(1):335-342. http://dx.doi.org/10.1016/j.carbpol.2014.02.079

## List of electronic supplementary materials

Table S1 $^{1}H$ NMR chemical shifts (relative to an internal $H_2O$, $\delta_H$=4.70 ppm) of constituent monosaccharide residues in BSH-1 and BSH-2

Table S2 $^{13}C$ NMR chemical shifts (relative to an internal acetone-$d_6$, $\delta_C$=30.50 ppm) of constituent monosaccharide residues in BSH-1 and BSH-2

## 中文概要

**题　目：竹茹中天然乙酰化半纤维素的分离、化学结构和免疫活性研究**

**目　的：**以竹材加工中产生的大宗副产物（竹茹）为原料，对其中的半纤维素进行分离纯化、结构解析及体外免疫活性的评价，旨在为竹茹半纤维素（BSH）作为免疫功能因子的研发和竹材加工副产物的高值转化提供理论依据和实践指导。

**创新点：**首次采用蒸汽爆破预处理联合热水提取法从竹茹中获得有免疫调节活性的天然乙酰化半纤维素。

**方　法：**采用蒸汽爆破预处理联合热水抽提法从竹茹中提取半纤维素；通过阴离子交换柱层析法对优化条件下制得的 BSH 进行分离；利用化学法和波谱法对分离得到的两个半纤维素组分（BSH-1 和 BSH-2）进行化学结构解析；采用小鼠脾脏淋巴细胞转化增殖试验、小鼠巨噬细胞 RAW264.7 吞噬中性红以及分泌 NO 试验评价 BSH、BSH-1 和 BSH-2 的体外免疫调节活性。

**结　论：**当蒸汽爆破压力为 2.2 MPa 和维压时间为 1 min 时，BSH 的得率达到最大，为(2.05±0.22)%（以脱蜡样品干重计），高于未经处理对照样品 5.7 倍。通过 DEAE-Sepharose Fast Flow 阴离子交换柱对 BSH 进行分离，获得一个中性组分 BSH-1（得率 0.79%，纯度 95.3%）和一个酸性组分 BSH-2（得率 1.06%，纯度 92.5%）。化学结构解析结果表明 BSH-1 为乙酰化的阿拉伯木聚糖（分子量：12 800），而 BSH-2 为乙酰化的 4-甲氧基-葡萄糖醛酸阿拉伯木聚糖（分子量：11 300）。体外免疫试验结果表明 BSH 和 BSH-2 能够显著刺激小鼠脾脏淋巴细胞增殖，而 BSH-1 无此活性；BSH、BSH-1 和 BSH-2 三者均能显著促进巨噬细胞 RAW264.7 吞噬中性红及分泌释放 NO，且呈现明显的剂量依赖性。综上所述，采用蒸汽爆破预处理联合热水提取法从竹茹中获得的半纤维素含天然乙酰基，并具有体外免疫刺激活性。

**关键词：**竹茹；蒸汽爆破预处理；天然乙酰化半纤维素；化学结构；免疫调节活性