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Solvent extraction of antioxidants from steam exploded sugarcane bagasse and enzymatic convertibility of the solid fraction

Jingbo Li, Jianghai Lin, Wenjuan Xiao, Yingxue Gong, Mingming Wang, Pengfei Zhou, Zehuan Liu *

Research Center for Molecular Biology, Institutes of Life and Health Engineering, College of Life Science and Technology, Jinan University, Guangzhou 510632, PR China

HIGHLIGHTS

- Antioxidants were solvent-extracted from steam exploded sugarcane bagasse (SESB).
- Solid, phenolic and total sugar yields of different extractions were different.
- The extracts showed potent antioxidant activities.
- Enzymatic convertibility of solid fraction after extraction was enhanced.

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ABSTRACT

Solvent extraction of steam exploded lignocellulosic biomass may be a potential way to obtain antioxidative extracts and to enhance the enzymatic convertibility of the solid residue. Boiling solvent extraction (BSE) showed higher solid and phenolic yields than room temperature extraction. Solubilities of phenolics and sugars were higher in anhydrous ethanol (AE) and deionized water (DW) than in ethyl acetate under each individual extraction condition. The antioxidant activities of the AE and DW extract obtained under BSE were better than those of 10 mM vitamin C. Conversion of the solid fractions into reducing sugar using Celluclast 1.5 L and Novozym 188 after AE and DW extraction was 95.13% and 92.97%, respectively, higher than that obtained with SESB (88.95%).

1. Introduction

Sugarcane bagasse (SCB) is a waste from sugar production. SCB has been a feedstock to produce bio-alcohol (Dekker and Wallis, 1983) after conversion of the cellulose and hemicellulose into fermentable sugars by acidic or enzymatic catalysis. Certain pretreatments to reduce crystallinity and degree of polymerization or remove part of the lignin and hemicellulose need to be employed in order to enhance enzymatic hydrolysis (Sun and Cheng, 2002).

Steam explosion is the most commonly used method to pretreat lignocellulose (Sun and Cheng, 2002) and the typical effects of such pretreatment are substantial breakdown of the lignocellulosic structure, degradation of hemicellulose and delignification (Castro et al., 2008); however, some degradation products such as furfural, 5-hydroxymethylfurfural, acetate acid and lignin-derived phenolics inhibit microbial conversion of steam exploded lignocellulose (Cruz et al., 2001). These inhibitors can be removed either by physico-chemical procedures such as adsorption, neutralization and overliming (Cruz et al., 1999) or by solvent extraction (Conde et al., 2011; Cruz et al., 1999, 2001). Solvent-detoxified media display good fermentation performance. Low boiling point solvents and lignin-derived antioxidants are easily recovered, and these antioxidants might be used as a cheap, renewable source of food additives.

Phenolic compounds, since they have antimicrobial activity (Garrote et al., 2004), are undesirable in microbial fermentation media (Cruz et al., 2001). Additionally, cellulase can be inhibited by phenolics (Ximenes et al., 2010). Therefore, simultaneous antioxidant extraction and detoxification of steam exploded or autohydrolyzed lignocellulosic materials is a reasonable way to solve the antimicrobial problem prior to further conversion of pretreated lignocellulose into alcohol. Although several authors have studied extraction of antioxidants from steam exploded or hydrolyzed lignocellulosic materials (Castro et al., 2008; Conde et al., 2009; Cruz et al., 2001; Hongzhang and Liying, 2007), the effects of different solvents on the extraction and the subsequent enzymatic convertibility of the solid fractions after solvent extraction have not been explored.

* Corresponding author. Tel.: +86 20 85222863 800; fax: +86 20 85222863 888.
E-mail address: zhliu@jnu.edu.cn (Z. Liu).

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The objective of the present study was to investigate the solid, phenolic and sugar yields from SESB when using anhydrous ethanol, ethyl acetate and deionized water under different extraction conditions. The antioxidant activities of the obtained extracts were evaluated. The impact of the solvent extraction on the enzymatic convertibility of the solid fraction after extraction was also studied.

2. Methods

2.1. Preparation of SESB

SCB was kindly provided by the Qianwu sugar refinery, Zhuhai, Guangdong, China. After air-drying, it was stored at room temperature in woven bags. Steam explosion was performed by using equipment designed by the Gentle Science & Technology Co. Ltd., Henan, China. The parameters of the steam explosion treatment: 220 °C, liquid/solid ratio (LSR) of 1:1 (w/w), residence time of 5 min. The product (SESB) was stored at 4 °C until use.

2.2. Solvent extract preparation

SESB was oven-dried at 85 °C for 12 h, milled to pass 0.15 mm screen, and homogenized. Anhydrous ethanol (AE, Damao, Tianjin, China, H_2O content $\leqslant$0.3%), ethyl acetate (EA, Damao, Tianjin, China) and deionized water (DW) were used as solvents. LSR of 20:1 (v/w) was employed in all extractions. Room temperature extractions (RTE) were conducted in a rotary shaker for 1 and 24 h and labeled as RTE1 and RTE24, respectively. Boiling solvent extracts (BSE) were prepared at the boiling point of the solvent in a Soxhlet extractor for 20, 40 and 60 min and labeled BSE20, BSE40 and BSE60, respectively. Extracts were collected by filtering through filter paper (Shuangquan 102, Xinhua Paper Industry Co., Ltd., Hangzhou, China) and the solid fractions were oven-dried at 85 °C overnight and stored at room temperature.

2.3. Analytical methods

Total phenolic content was determined according to the method described by Kim et al. (2006). A known volume of a sample was mixed with 0.5 mL of Folin–Ciocalteau reagent (Guangzhou Qiyun Biological Technology Co., Ltd.) and 0.75 mL of 20% (w/v) sodium carbonate (Na_2CO_3) solution. The mixture was allowed to stand in a water bath at 40 °C for 40 min and the absorbance was measured at 765 nm by a UV/Vis spectrophotometer (UNICO, UV-2000, Shanghai, China). A mixture of distilled water and reagents was used as blank control. The total phenolic content was expressed as gallic acid equivalent (GAE, Tianjin Guangfu Fine Chemical Research Institute, Tianjin).

Reducing sugar was quantified according to the dinitrosalicylic acid (DNS) method (Miller, 1959) and expressed as glucose equivalent.

Total sugar was determined with the phenol/sulfuric acid method (DuBois et al., 1956) and expressed as xylose equivalent (XE).

An analysis of the reducing sugar content in the solid fraction was determined according to the Laboratory Analytical Procedures from the National Renewable Energy Laboratory (Sluiter et al., 2008). After a two-step analytical acid hydrolysis procedure, the released reducing sugar was determined using the DNS method.

Thin-layer chromatography (TLC) was performed on a silica gel plate using the mixture of n-butanol, pyridine and water (6:4:1, v/v/v) as the developing solvent. The sugars were visualized by spraying the plates with a mixture of 1 mL phosphoric acid and 10 mL stock solution (2 g diphenylamine, 2 mL aniline, 100 mL acetone) and oven-drying at 85 °C.

Fourier transform infrared spectroscopy fingerprints (FTIR) was conducted with a BRUKER EQUINOX 55 Fourier transform infrared spectrometer, in transmission mode using a KBr wafer with a resolution of 4 cm^{-1}.

2.4. Antioxidant and free radical scavenging activity

2.4.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH (Tokyo Kasei Kogyo Co., Ltd.) radical scavenging activity was determined according to Li et al. (2012). Vitamin C was used as positive control. Samples with different volumes were tested after dilution to 2 mL.

2.4.2. Reducing power

The reducing power of extracts was determined according to Yen and Chen (1995). Vitamin C was used as positive control. Samples with different volumes were tested after dilution to 1 mL.

2.4.3. Hydrogen peroxide inhibition ability

The ability of different extracts to scavenge hydrogen peroxide was determined following the method of Yen and Chen (1995) with some modifications. Hydrogen peroxide (2 mL, 30%) was dissolved in 250 mL of phosphate-buffered saline (PBS, pH 7.4). The hydrogen peroxide concentration was determined spectrophotometrically at 230 nm. Extracts with different volumes were added to 0.6 mL of hydrogen peroxide solution and distilled water was added to a total volume of 4.6 mL. Absorbance of different samples at 230 nm was recorded after 10 min against distilled water. The hydrogen peroxide inhibition ability (HPIA) was calculated according to the following equation.

$$\text{HPIA}\ (\%) = \frac{A_c - (A_{sh} - A_s)}{A_c} \times 100\%$$

where A_c is the absorbance at 230 nm of the mixture of distilled water and hydrogen peroxide solution, A_{sh} is the absorbance of the mixture of samples and hydrogen peroxide solution and A_s is the absorbance of the mixture of samples and distilled water.

2.4.4. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity (HRSA) was determined according to Smirnoff and Cumbes (1989), with modifications. Extracts with different volumes were placed into the test tubes and distilled water was added to a total volume of 200 μL. Subsequently, 0.5 mL of 0.3% H_2O_2, 0.5 mL of 2 mM $FeCl_2$ and 0.5 mL of 9 mM salicylic acid–ethanol solution were added consecutively and finally 3 mL of distilled water was poured into each tube. The mixtures were incubated in a water bath at 37 °C for 30 min. Absorbance at 510 nm was recorded against distilled water using a UV/Vis spectrophotometer. Vitamin C was used as positive control. The hydroxyl radical scavenging activity (HRSA) was calculated by the following equation:

$$\text{HRSA}\ (\%) = \frac{A_0 - (A_x - A_{x0})}{A_0} \times 100\%$$

where HRSA is the hydroxyl radical scavenging activity of the extract (%); A_0 is the absorbance of distilled water, $FeCl_2$, H_2O_2 and salicylic acid–ethanol solution; A_x is the absorbance of sample solution, $FeCl_2$, and salicylic acid–ethanol solution; A_{x0} is the absorbance of sample solution, $FeCl_2$, H_2O_2 and distilled water.

2.5. Enzymatic convertibility

Enzymatic convertibilities of solid fractions from solvent extracted SESB were determined at 2% (w/v) of substrate in 50 mL 0.1 M citric acid/citric sodium buffer (pH 4.8), containing 80 μg/