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The synergism of hot water pretreatment and enzymatic hydrolysis in depolymerization of lignocellulosic content of palm kernel cake

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ABSTRACT

Palm kernel cake (PKC), mainly composed of mannan, lignin and protein, is abundant renewable resource with commercial value. To develop clean and efficient way for PKC refinery, the method based on the synergism of hot water pretreatment (HWP), steam pretreatment (SP) and enzymatic hydrolysis were developed. HWP of 180 °C, 20 min and SP of 121 °C, 20 min showed similar performance for sugar release from PKC. The main saccharides produced from PKC by HWP and SP were mannose and manno-oligosaccharides, while no furfural formed. The surface structure analyzed by SEM showed that HWP enhanced the microporosity of PKC, and the accessibility of which was increased thereafter. When HWP pretreated PKC was further hydrolyzed with enzyme cocktail (cellulase, xylanase, endo-mannanase), 45% of PKC was solubilized compared with the control. The manno-oligosaccharides produced by HWP and SP were converted to mannose and mannobiose by endo-mannanase. The results suggested that both HWP and SP promote enzymatic hydrolysis of PKC by releasing oligosaccharides and enhancing microporosity, and the synergism of which was effective for PKC decomposition.

1. Introduction

Palm kernel cake (PKC) is co-product after the extraction of oil from palm kernel. The major component of PKC was mannan, cellulose, lignin and protein [1,2]. The character of PKC makes it has various applications, such as feed for ruminants, non-ruminants and poultry, enzyme and bioethanol production [1,3,4]. While the regrid structure of PKC for degradation has limited converting lignocellulose content to valuable products. Pretreatment was therefore adopted to overcome the recalcitrancy of PKC in the refinery process [5].

Biological, chemical and physical methods have been applied to convert PKC into useful products. But some methods, such as acid hydrolysis and steam explosion, were not environmentally friendly, and the product was not suitable for feed [6]. Solid state fermentation (SSF) of fungi such as *Aspergillus* with PKC as substrate was used for enzymes production [6,7]. Chemical and physical pretreatments were studied for process of PKC in recent years. Piyarat Weerachanchai et al. conducted a slow pyrolysis of PKC in a fix-bed reactor, and obtained maximum liquid yield of 54.3%, and the heating value of obtained PKC oil reached 40 MJ/kg [8]. Chee et al. extracted protein from PKC by using trypsin assisted alkaline condition [9]. Suet-Pin Fan et al. studied the microwave-assisted dilute sulfuric acid hydrolysis of deproteinated palm kernel cake (DPKC) [10].

Yan et al. applied hot water pretreatment (HWP) and liquid oxidation(30% H_2O_2) to remove hemicellulose and lignin from PKC, and recover cellulose [11]. PKC can also be hydrolyzed to monosaccharide, and then fermented to ethanol [12]. Henning Jørgensen et al. adopted enzyme cocktail of endo-mannanase, β-mannosidase and cellulase to hydrolyze PKC (35–50% dry matter), and then fermented to final ethanol concentration of 70 g/kg with *Saccharomyces cerevisiae* [1].

Although the pretreatment and processing of PKC have gained increasing study, the economic and efficient way for industrial application was still not developed. HWP was considered to be comparatively clean and efficient method for biomass processing [1,11]. In present work, the processing of PKC with HWP and enzymatic hydrolysis with enzyme cocktail was studied for potential industrial application. The condition of HWP and the combination of which with enzymatic hydrolysis for PKC processing were optimized. The effect of HWP on surface physical structure of PKC and the produced saccharides in different process was also studied.

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Table 1
Chemical composition of PKC with different pretreatments.

Treatment	Cellulose (%)	Cellulose loss (%)	Hemicellulose (%)	Hemicellulose loss (%)	Lignin (%)	Lignin loss (%)	Protein (%)	Protein loss (%)
control	7.46 ± 0.27	NA	45.23 ± 1.41	NA	20.57 ± 2.13	NA	14.81 ± 0.33	NA
E100	4.59 ± 0.52	52.76 ± 2.23	20.84 ± 1.91	64.62 ± 1.07	28.29 ± 1.17	−5.85 ± 4.37	16.92 ± 1.33	12.04 ± 7.61
T190	4.61 ± 0.54	52.06 ± 6.43	24.38 ± 2.6	58.16 ± 4.95	24.47 ± 1.76	7.74 ± 6.63	13.63 ± 0.66	28.5 ± 6.45
T190E	4.87 ± 0.34	64.57 ± 1.2	24.51 ± 1.81	70.61 ± 1.03	37.46 ± 0.22	1.15 ± 0.58	13.16 ± 1.33	51.8 ± 3.84

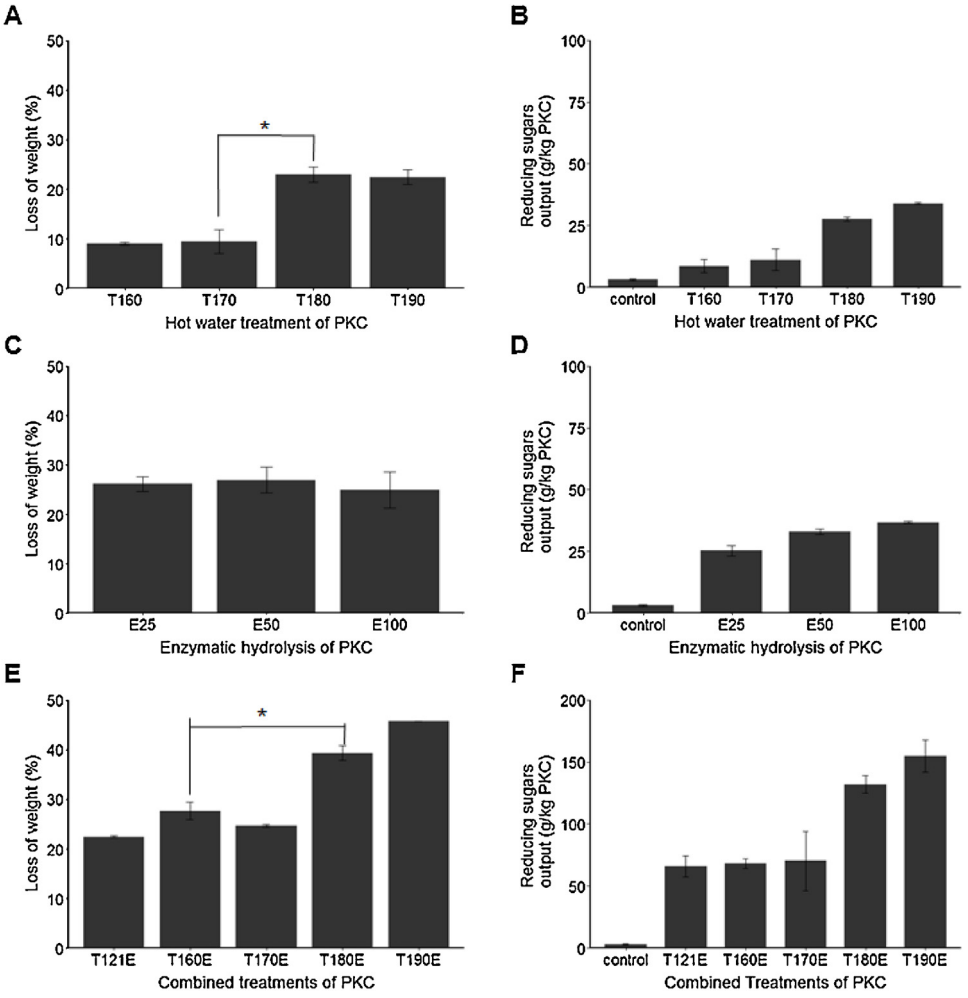

Fig. 1. Processing of PKC with hot water pretreatment and enzymatic hydrolysis. (A) Loss of weight from PKC pretreated with hot water at different temperature. The temperature and incubation period of pretreatments: 160 °C, 10 min (T160); 170 °C, 15 min (T170); 180 °C, 20 min (T180); 190 °C, 30 min (T190). (B) Reducing sugar produced from PKC with hot water t at different temperature. (C) Loss of weight from PKC hydrolyzed with different enzyme cocktail. The cellulase and xylanase were same in each group at 15 U/g and 20 U/g respectively, the endo-mannanase was 25 U/g (E25), 50 U/g (E50), 100 U/g (E100). (D) Reducing sugar produced from PKC hydrolyzed with different enzyme cocktail. (E) Loss of weight from PKC treated with the combination of hot water and enzymatic hydrolysis. The hot water pretreatments were T160, T170, T180, T190, the enzymatic hydrolysis was conducted at E100 condition. T121E means PKC treated with the combination of hot water pretreatment (121 °C, 20 min) and enzymatic hydrolysis (E100). (F) Reducing sugar produced from PKC treated with the combination of hot water and enzymatic hydrolysis.

2. Materials and methods

2.1. Material and enzymes

Palm kernel cake (PKC), with dry matter content of 95%, was purchased from Yantai Internation trade Co., Ltd, China. The composition of PKC was listed in Table 1. Endo-mannanase was purchased from Sunson Industry Group Co., Ltd., Ningxia, China. Xylanase and cellulase were obtained from Yangshao Biochemical Engineering Co., Ltd., Sanmenxia, China. The activity of endo-mannanase, xylanase and cellulase was determined by the 3,5-dinitrosalisylic acid method with respective konjac mannan (Klamar®), beechwood xylan (Sigma Aldrich®) and cellulose microcrystallline (Ourchem®) as substrate. One unit (IU) of enzyme activity was defined as the amount of enzyme produced 1 μmol of reducing sugar in 1 min.

2.2. Processing of PKC with hot water and enzymatic hydrolysis

Pretreatment of PKC with hot water: HWP was performed in a micro-pressure reactor, which was filled with PKC (10 g) and distilled water (70 mL). The mixture of PKC was pretreated at different conditions (160 °C, 10 min; 170 °C, 15 min; 180 °C, 20 min; 190 °C, 30 min), then terminated by quenching in water bath. In addition, PKC was pretreated at 121 °C for 20 min in sterilizing pot. The treated PKC was collected by filtration and then dried at 60 °C, and the liquid fraction was saved at −20 °C for sugar analysis. The con-