

Utilization of Coffee Skin Fiber as Potential Sources of Reducing Sugar by Means of Enzymatic Hydrolysis

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Abstract

Coffee skin and pulp compromised almost 50–60% of coffee bean, in which most of them will be turned to waste during coffee processing. Until now, the utilization of coffee skin is still limited to low-value products such as compost, fertilizer and feed. Further utilization of coffee skin has been reported by means of chemical or enzymatic degradation of its structure, whether intended to improve the digestibility of feed, improving the nutritional properties or as potential media of fermentation. This research aimed to study the potential of coffee skin fiber to be used as the source of reducing sugar using acid cellulase enzyme and determine the optimum process of hydrolysis of the coffee skin fiber. Delignification using base was carried out prior to enzymatic hydrolysis. Small scale enzymatic hydrolysis was carried out utilizing Sqzyme CSP, an acid cellulase enzyme in various units (200, 400, and 800 units) for 12 and 24 hours. The evaluation of the hydrolysis performance was carried out by total and reducing sugar analysis followed by the quantification using liquid chromatography-mass spectrometer method. The total sugar content was ranged from 0.149 mg/mL to 0.584 mg/mL whereas the reducing sugar content was ranged from 0.013 mg/mL to 0.114 mg/mL with the highest production of sugar was obtained using 800 unit of enzyme and incubated for 24 hours. The glucose content was 36,1 ppm or compromised around 31.57% of total reducing sugar produced in the best treatment. The study shows that the enzyme used in this experiment could be used in first stage of coffee skin fiber hydrolysis process to obtain abundant substrate for potential subsequent process.

Keywords: Belignification, cellulase, lignin, glucose, oligosaccharides

INTRODUCTION

Coffee skin accounted for almost two-fifth part of coffee bean and combined to the pulp, it is accounted for almost 60% of coffee bean (Gathuo *et al.*, 1991). Musatto *et al.* (2011) and Nabais *et al.* (2008) stated that during coffee beans processing, large amount of by-products are generated, in which Berlitz *et al.* (2009) and Esquivel & Jimenez (2012) mentioned that approximately

50% of the coffee fruit turn to waste during the production of green coffee beans. The coffee husk itself mostly constituted by fiber, protein, sugar, pectin and tannin (Wilson, 1985). Until now, there are still limited number of effort to utilize coffee skin/husk, mostly are under-utilized as fertilizer, feed and compost (Richana, 2002). Murthy & Naidu (2012) previously mentioned that coffee husk can be utilized in mould, yeast and enzyme production as substrate due to its high amount

of fermentable sugars. Arrizon *et al.* (2012) further utilize the fermentable sugar obtained from the hydrolysis to produce bioethanol. Similar studies had also been reported by Sampaio *et al.* (2013), and Gouvea *et al.* (2009).

High lignin content has been noticed as important aspect in coffee husk enzymatic hydrolysis. Taherzadeh *et al.* (2007) previously mentioned that lignin-coated cellulose is harder to breakdown and take longer time to be hydrolyzed. Delignification process is crucial in hydrolyzing plant tissues due to the ability of lignin to reduce the accessibility of cellulose to enzymes (Wyman *et al.*, 2005). The removal of lignin can be carried out using dilute acid pre-treatment at higher temperatures which led to shrinking and agglomeration of the lignin (Ooshima *et al.*, 1990). Further, Michalowicz *et al.* (1991) and Selig *et al.* (2007) also mentioned that the dilute acid pre-treatment could result in lignin melting and its relocation that led to cellulase ability to access the cellulose. Delignification can also be carried out using base such as NaOH and Ca(OH)₂ which will attack and breakdown the lignin structure, crystalline and amorphous part of lignocellulose (Gunam *et al.*, 2011)

Acid cellulase enzyme has widely applied in hydrolysis of cellulose. It has been used in the hydrolysis of palm oil empty branches (Caecilia, 2015) and corncob (Ouyang *et al.*, 2009). Further, the increasing concern in enzymatic hydrolysis is occurred due to the cheaper price of the enzyme as its increasing share of world's industrial enzyme market (Zhang & Zhang, 2013). However, the cost of cellulose hydrolysis is still considered high due to its inefficiency to degrade pretreated lignocellulosic material. Thus, the combination of process is needed to achieve optimum hydrolysis process.

Three main types of cellulase enzymes are often to be used to achieve complete cellulose hydrolysis, namely endocellulase

(endoglucanases EC 3.2.1.4), exocellulase (exoglucanase EC 3.2.1.91) and β -glucosidase (cellobiase EC 3.2.1.21). Endoglucanase randomly cut β -1,4-bonds of cellulose chains, generating new ends. Further, it also bind and cleave the cellulose chain to generate glucose, soluble cellodextrins or insoluble cellulose fragment. Some of endoglucanases have an ability to generate other product such as cellobiose or longer cyclodextrins (Cohen *et al.*, 2005; Li & Wilson, 2008; Mejia-Castillo *et al.*, 2008; Parsiegla *et al.*, 2008). This research aimed to study the potential of coffee skin fiber to be used as the source of reducing sugar using acid cellulase enzyme and determine the optimum process of hydrolysis of the coffee husk.

MATERIALS AND METHODS

Fresh coffee skin was obtained from coffee farmer in Sumberwringin, Bondowoso District. Coffee skin was collected during depulping process of Arabica coffee cherry, and was then immediately frozen (-20°C) until used. Hydrolysis agent of the acid cellulase enzyme (Sqzyme CSP) was purchased from Suntaq International Ltd. with the enzyme activity was 10.000 units/g.

The experiment was carried out utilizing factorial complete randomized design with two factors such as duration of hydrolysis (time, hours) and enzyme units (units), in which duration of hydrolysis was divided into 2 levels (12 and 24 hours) and 3 levels of the enzyme units (200, 400, and 800 units).

Preparation of coffee skin fiber (CSF) was carried out to obtain pure delignified fiber of the coffee skin. The preparation was started with delignification process to obtain lignin-free CSF which was carried out based on alkaline hydrolysis method. Crushed coffee skin was mixed with Sodium hydroxide (NaOH) 0.1 M with the ratio of 1:20 (b/v). The mixture was then heated in boiling water

for 3 minutes. After cooled at room temperature, the mixture was then filtered and washed using distilled water as much as 3000 mL. The precipitate obtained was then referred as delignified CSF (DCSF).

Small scale enzymatic hydrolysis was carried out utilizing Sqzyme CSP, an acid cellulase enzyme. As much as 0.5 g of DCSF was diluted in distilled water and adjusted to pH 5.5 using citrate buffer. The mixture was then pre-incubated in using shaker water bath at 55°C for 3–5 minutes and then added with enzyme with certain units (200, 400, and 800 units). The hydrolysis process was carried out at 55°C for 24 and 48 hours based on the experimental design. After certain time of hydrolysis process was fulfilled, the process was then terminated by inactivated the enzyme at 70°C for 15 minutes. The hydrolysate solution was then separated with its precipitate by means of centrifugation at 3500 RPM for 20 minutes. The solution obtained was then referred as sugar hydrolysate solution (SHS). The SHS was then subjected into total sugar, reducing sugar and polymerization degree analysis.

Total sugar analysis was carried out based on the method of Dubois (1956) by means of strong acid hydrolysis. Twenty milliliter of SHS was added with 0.5 mL phenol reagent (5%) and then vortexed. As much as 2.5 sulfuric acid (H_2SO_4) 98% was then added quickly and vortexed, then cooled at room temperature for 2 minutes. The solution was then subjected to absorbance measurement spectrophotometrically at wavelength 490 nm (Perkin Elmer Lambda 35 UV/Vis Spectrophotometer). The content of sugar was measured by comparing the absorbance of the samples with the absorbance of standard glucose solution and referred as mg sugar/mL SHS.

Reducing sugar was analysed based on Dinitrosalicylic acid (DNS) reaction. DNS

reagent was made by diluting 10g NaOH, 0.5 g Na_2SO_4 (sodium sulfate), and $KNaC_4H_4O_6 \cdot 4H_2O$ (kalium sodium tartrate) in 1 L distilled water. The solution was then added with 10 g of DNS until perfectly dissolved. As much as 20 ml of SHS was mixed with 1 mL of DNS and then heated at 100°C for 15 minutes. The heated solution was then cooled at room temperature for 5 minutes and then measured its absorbance at 540 nm (Perkin Elmer Lambda 35 UV/Vis Spectrophotometer). The content of reducing sugar was measured by comparing the absorbance of the samples with the absorbance of standard glucose solution and referred as mg reducing sugar/mL SHS.

Identification of glucose occurrence in SHS was carried out utilizing Liquid Chromatography-Mass Spectrometer. Shimadzu LC-MS 2020 (Shimadzu, Japan) equipped with Waters C 18 column (Waters, USA) was set at column temperature of 40°C and flow rate 1 mL/min. The detection was done using mass spectrometry, whereas the quantification was done using glucose standard.

Data analysis was performed using General Linear Model (GLM) followed by Duncan MRT (multiple range test) post-hoc analysis performed on the statistical package for social science (SPSS) software version 17.0 (IBM Corporation, Armonk, New York, USA). The statistical analyses were performed at 5% significance level. Descriptive analysis using surface and contour plot was done using Sigmaplot ver 12.3 (Systat Software, Inc).

RESULTS AND DISCUSSION

Total sugar content of coffee skin fiber's hydrolysate solution is presented in Figure 1. The total sugar content was ranged from 0.149 mg/mL to 0.584 mg/mL with the highest production of sugar was obtained using 800

unit of enzyme and incubated for 24 hours. Analysis of variance on the total sugar content of the SHS resulted in the conclusion that the effect of time of hydrolysis was insignificant ($p \geq 0.05$), thus the total sugar produced was not significantly different between 12 and 24 hours using the same unit of enzyme. However, the unit of enzyme used was a significant factor to the production of sugar from CSF. It was also evaluated that the increase in enzyme unit used was linear to the total sugar obtained, in which two-fold increase of enzyme unit produced in almost two-fold increase of total sugar produced. It was in agreement with the result of Ouyang *et al.* (2009) which mentioned that the yield of corn cob hydrolysate was increasing as the increase of cellulase enzyme utilized.

High content of sugar in hydrolysate solution indicated that there were carbohydrates released into the solution which confirmed that there were a hydrolysis of cellulose occurred. According to Gunarto (2009), acid cellulase enzyme hydrolyze the β -1,4 glycosidic bond in cellulose resulting in oligosaccharide or glucose. However, it is also stated that the result obtained from hydrolysis directly correlated with the type of enzymes used, whether it was endocellulase, exocellulases or cellobiases. The enzyme used in this experiment was an acid endocellulase enzyme (EC 3.2.1.4) which able to break the non-covalent interactions present in the crystalline structure of cellulose. This specific cleave in internal bonds of cellulose will create new chain ends, in which it could be monomer or oligomer (Enari &

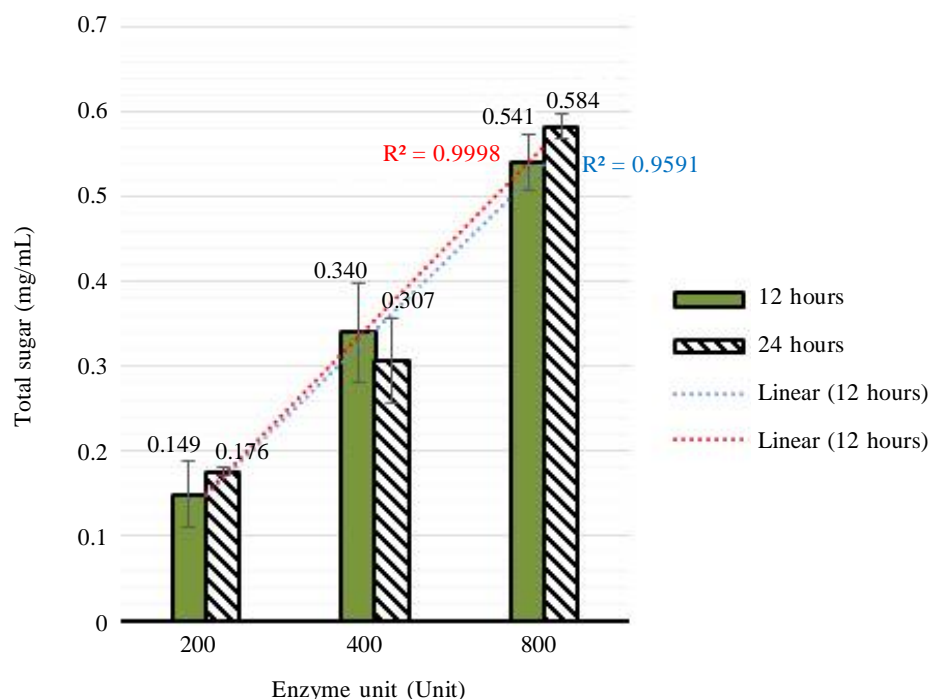


Figure 1. Total sugar content of coffee skin fiber's hydrolysate solution

Fogarty, 1983). Since the hydrolysis of cellulose by enzyme significantly related to its active site requirement, the yield obtained is currently lower to that of acid hydrolysis which could hydrolyze randomly and extensively at any hydrolyzable bonds in term of total soluble sugar obtained as mentioned previously by Wardhana (2014).

On the other hand, the reducing sugar obtained from the hydrolysis process was presented in Figure 2. The reducing sugar content was ranged from 0.013 mg/mL to 0.114 mg/mL following the same linear trend to that of total sugar content. However, there was a significant effect of the interaction time and enzyme unit on the concentration of reducing sugar obtained. It was evaluated that the treatment with longest hydrolysis time using highest unit of enzymes could produce highest reducing sugars concentration (0.114

mg/ml). It was in agreement with the result of Ouyang *et al.* (2009) which mentioned that extensive interaction between enzyme and substrate during long period of incubation time often resulted in higher content of reducing sugar. The result of Ouyang *et al.* (2009) also suggested that the optimum time of hydrolysis varied from one substrate to another and have to be adjusted based on the sample used. However, the result obtained in this experiment was less compared to the report of Caecelia (2015) who utilized empty palm oil brunches which could produce reducing sugar up to 10.31 g/L of hydrolysate. Since dry coffee husk contain high lignin content (23.7-72 % db) in par to empty palm oil brunches (24.15%) (Bondesson, 2015; Bekalo and Reinhardt, 2009; Shenoy *et al.*, 2011), the delignification process needed to be adjusted in order to achieve optimum

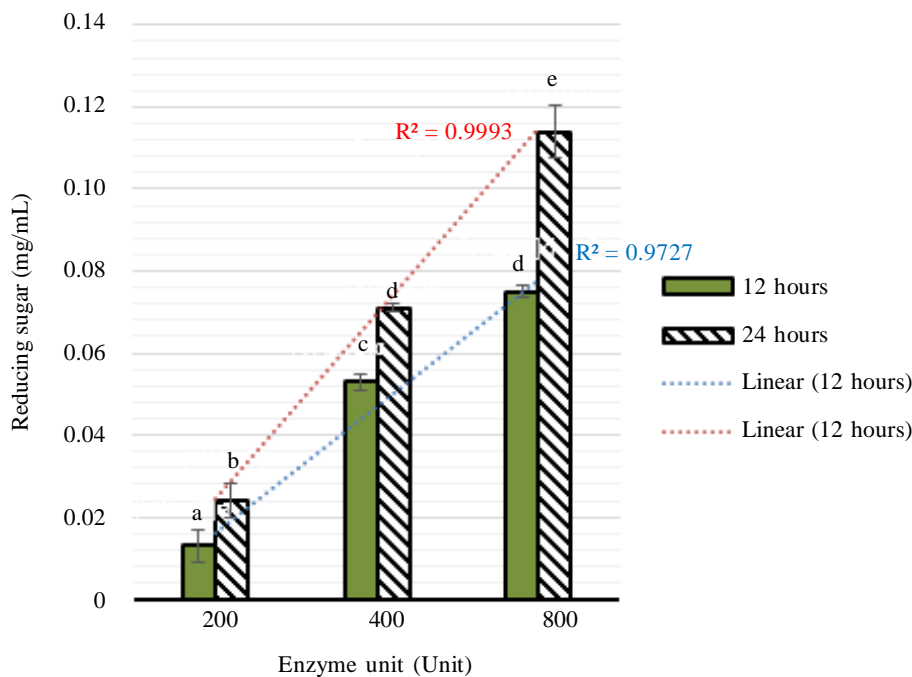


Figure 2. Total reducing sugar content of coffee skin fiber’s sugar hydrolysate solution

degradation of lignin in coffee husk prior to the hydrolysis process.

The production of the monomer of the reducing sugar is often used to measure the performance of the carbohydrate hydrolysis (Bansal, 2009). In this case, the enzymatic degradation of cellulose will endly produce the glucose monomer. Our analysis using liquid chromatography-mass spectrometer on the SHS obtained using 800 unit of enzyme and 24 hours incubation showed that the glucose content was 36.1 ppm or around 31.57% of total reducing sugar produced in the treatment. As mentioned by Lopez *et al.* (2004), the hydrolysis of lignocellulose usually produce glucose, mannose and xilose or the mixture of phenolic compound. However, the cellulose itself which is a bonded chain of glucose, will mainly hydrolized into glucose when it is extensively hydrolyzed and will only produce oligosaccharides when it partly

hydrolyzed (Sukumaran *et al.*, 2005). Thus, with only 31.57% of glucose produced among all the reducing sugar evaluated, it was expected that the oligoscharides reducing sugar was a majority reducing sugars obtained which could be also including dissacharide such as cellobiose. It was concluded that the hydrolysis occured during the experiment was mainly first stage of cellulose hydrolysis, which was the act of endocellulase.

The performance of the hydrolysis could be improved by means of utilizing various type of cellulases such as exocellulase to degrade the product of endocellulase and the use of cellobiase to extensively degrade dissacharides (Bansal *et al.*, 2009). Further, since reducing sugar only compromised around one-fifth of total sugar obtained during hydrolysis, the majority sugar liberated during hydrolysis was non-reducing sugar. Such a non-reducing polysccharide and oli-

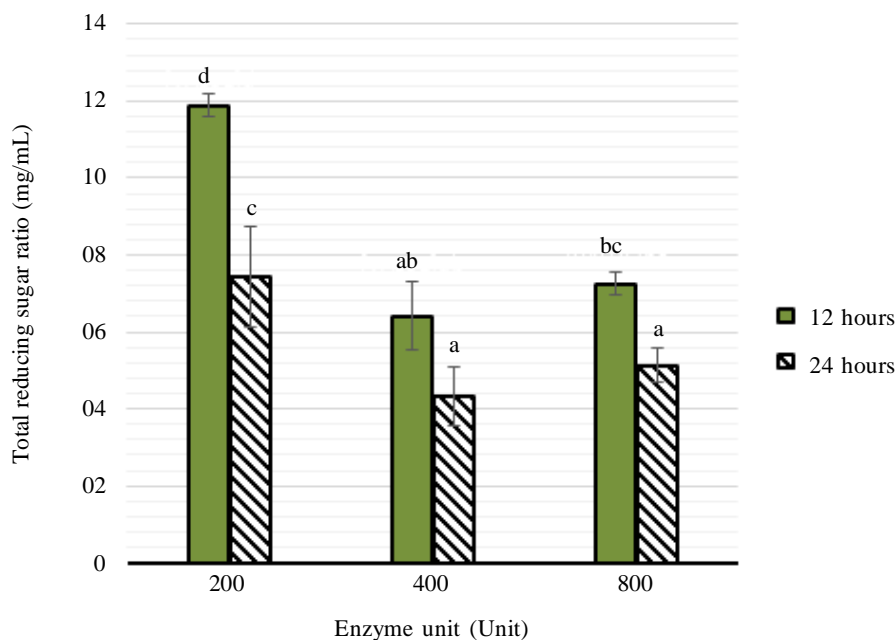


Figure 3. Ratio of total and reducing sugar content of coffee skin fiber's sugar hydrolysate solution

gosaccharide will further need to be degraded to be able to utilized as reducing or fermentable sugar. Thus it is possible to produce higher concentration of glucose by means of utilizing multiple enzymes as reported previously by Ouyang *et al.* (2009) which mentioned that multi-stages hydrolysis could improve the yield of glucose from 43% to 90%.

Ratio of total and reducing sugar as presented in Figure 3 showed that the ratio ranged from 4.34 to 11.89, in which the treatment using 400 and 800 unit of enzymes for 24 hours resulted in lowest ratio, means that it produce higher proportion of reducing sugar compared to non-reducing sugar than other treatments. However, it was also noticed that eventhough the ratio was similar, the yield of total sugar and reducing sugar of the treatment using 800 unit of enzyme were far surpassed that of the 400 (Figure 1 and Figure 2). This phenomenon indicated that the lowest ratio obtained in this experiment might be the lowest possible number that the enzyme could achieved. However, this value is still higher compared to other research as reported by Ruriani *et al.* (2012) which mentioned that the ratio only 8–12 in corncob enzymatic hydrolysis and in par to that of Faridah *et al.* (2013) who reported that the ratio could be as low as 4 to 12 on acid hydrolysis of the arrowroot starch. Girisuta (2007), mentioned that the crystalline structure in coffee husk preventing the hydrolysis of cellulose. However, it is also stated that the longer time of hydrolysis should be carried out to obtain optimum hydrolysis.

CONCLUSIONS

This experiment has successfully reveal the potency of coffee husk to be utilized by means of enzymatic hydrolysis. High proportion of glucose and reducing sugar produced open the possibility of coffee skin fiber to be utilized as fermenteation media

due to its high content of fermentable sugar. However, further research should be done in optimizing the delignification process of coffee husk since current studied still not able to optimize the yield. The use of multiple enzymes can also be carried out to obtain best possible yield of fermentable sugars. It is also concluded that the enzyme used in this experiment could be used in first stage of coffee skin fiber hydrolysis process to obtain abundant substrat for subsequent process.

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