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Alternative Source of Beta-Glucan From Oil Palm (*Elaeis Guineensis*) Trunk Fiber

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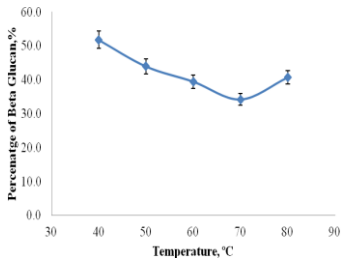
Graphical abstract



Abstract

This study was conducted to develop the method of extracting beta-glucan from the fiber of *Elaeis guineensis* or oil palm trunk (OPT) and to evaluate its potential. From the previous studies, beta-glucan usually extracted from barley, oats and also bacteria. In this research, wet separation method which is also known as aqueous alcohol method was used to extract beta-glucan from the oil palm trunk fiber. This indirect method of producing beta-glucan was found optimized at conditions of 50% of ethanol as solvent, 4 hours pretreatment duration and temperature of 70ºC. Finally beta-glucan was analyzed by enzymatic method and the percentage of beta-glucan extracted in this study was 34.17 percent.

Keywords: Oil palm trunk; beta-glucan; ethanol concentration; pre-treatment duration; enzymatic reaction temperature

Abstrak

Kajian ini dilakukan bagi membangunkan kaedah mengekstrak beta-glukan daripada serat *Elaeis guineensis* atau batang kelapa sawit (OPT) dan untuk mengkaji potensinya. Daripada kajian terdahulu, beta-glukan biasanya dihasilkan daripada barli, gandum dan juga bakteria. Dalam kajian ini, kaedah pemisahan basah iaitu kaedah alkohol berakues digunakan untuk mendapatkan ekstrak beta-glukan daripada serat batang kelapa sawit. Kajian ini mendapati kaedah tidak langsung bagi mengeluarkan beta-glukan pada kondisi optimum 50% pelarut etanol selama 4 jam praolahan pada suhu tindakbalas 70ºC. Pada analisis peringkat akhir, kaedah berenzim digunakan dan didapati sebanyak 34.17 peratus beta-glukan berjaya dihasilkan.

Kata kunci: Serat batang kelapa sawit; beta-glukan; kepekatan etanol; masa pra-rawatan; suhu tindakbalas enzim

1.0 INTRODUCTION

Vasanthan and Temelli [1] highlighted the approval of the health claims by FDA for oats in 1997 and for barley in 2005. It states that the demand for oats and barleys fractions enriched in beta-glucan has been on the rise. Many supplement and food companies are investing and putting more effort into new product development according to the demand of the market because of the health claims. The production of the beta-glucan concentrates has been started by several ingredients companies. The beta-glucan is enriched in different levels depending on which processing technologies are employed. There are some differences in the physiochemical properties of those commercial products [1].

Cui [2] mentioned that beta-glucan from cereal grains have been extensively researched over the past several decades based on their postulated health benefits, including attenuating blood glucose level and lowering serum cholesterol level. In addition, even a small amount of a beta-glucan-enriched bedtime snack has been shown to significantly decrease postprandial glucose in children with type 1 diabetes [3]. Beta-glucan is a carbohydrate which consist of glucose units linked through β-1, 3 or β-1, 4 glycosidic linkage (Figure 1). Beta-glucan is one of the most important structural polysaccharides in the cell walls of bacteria, fungi and plants [4]. The existences of beta-glucan normally occur as cellulose.

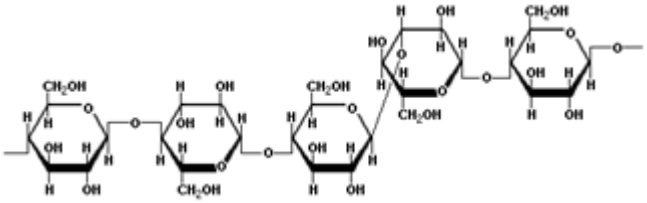


Figure 1 Structure of beta-glucan [4]

Beta-glucan was obtained from various sources and previous studies have been shown that the extraction of beta-glucan were from barleys [1, 5, 6], oats [1, 7] and microorganisms [8, 9]. Several methods have been used producing it which including aqueous alkali, aqueous thermo-mechanical and aqueous alcohol method. Beta-glucan form long cylindrical molecules containing up to about 250,000 glucose units. Beta-glucan occurs in the bran of grains such as barley and oats, and they are recognized as being beneficial for reducing heart disease by lowering cholesterol and reducing the glycemic response. They are used commercially as fat substitutes and additive to modify food texture [4].

In present study, the trunk of oil palm or *Elaeis guineensis* is utilized due to the main components of fiber are cellulose and lignin [10]. Oil palm trunk (OPT) is abundant, easy to obtain locally and it does not require importing from other countries. Thousands of tonnes of oil palm trunk produced annually in Malaysia [11]. In 1997, Malaysia produced about 13.2 million tonnes of oil palm biomass including trunk, fronds, and empty fruit bunches [12]. Palm oil has made impressive and sustained growth in the global market over the past four decades, and it is projected in the period 2016 – 2020, the average annual production of palm oil in Malaysia will reach 15.4 million tonnes [4]. The chemical composition of the oil palm trunk fibre (% dry weight, w/w) is as the following: cellulose 41.2%, hemicelluloses 34.4%, lignin 17.1%, ash 3.4%, extractives 0.5%, and ethanol solubles 2.3% [13].

Thus, the objective of this study was to develop the method of producing beta-glucan from the fiber of oil palm trunk (OPT) and to evaluate its potential.

■2.0 EXPERIMENTAL

2.1 Sample Preparation

Dried OPT fibers were provided by FaiberPalm Sdn. Bhd., Selangor. The fibers were grounded into different particle sizes and kept in air tight container for further processing.

2.2 Pre-treatment of Fiber

OPT fibers were weighted approximately 3g and pretreated with different ethanol concentration and prepared as slurry in 30% of ethanol (approx 96%, Grade AR, MW=46.07g/mol from QReC). Experiment was repeated with 40%, 50 %, 60%, 70%, 80%, 90% and 100% of ethanol (v/v). The optimum concentration of ethanol was used for next test. The optimized pretreatment duration was determined using optimized ethanol concentration in duration of 1 hour, 2 hours, 3 hours, 4 hours and 5 hours. Then, it underwent vacuum filtration method for 15 minutes to remove other components. Filtration was going to remove starch concentrate while the fiber particles that are riched in beta-glucan were retaining on the screen. After that, the beta-glucan concentrate on the screen was re-slurried in the 30% of ethanol. Experiment was repeated using similar concentration of ethanol as the previous step. Next, the mixture was sequentially treated with protease and thermostable alpha-amylase (Sigma-Aldrich) to hydrolyze protein and starch that are bound to the fiber particles. First, they were treated together with the mixture at 40ºC. Experiment was conducted and repeated by treating the thermostable alpha-amylase with 50ºC, 60ºC, 70ºC and 80ºC to determine the optimum processing temperature. The enzyme treating duration is 1 hour and with 50µl for each enzyme. Beta-glucan rich cell wall fiber particulates free from bound starch and protein were then recovered by vacuum filtration. The filtration process took approximately an hour. Fiber concentrates containing beta-glucan could then be obtained through this technique.

2.3 Beta-Glucan Analysis using DNS Method

2.3.1 Treatment with Amyloglucosidase

An extracted sample was mixed with distilled water and then incubated in amyloglucosidase of high purity from *Aspergillus niger* solution (Sigma-Aldrich) for 1 hour. A control was also prepared before extraction was also prepared. Finally, each set of samples was prepared to determine the percentage of beta-glucan. The percentage of beta-glucan was assumed represented by the percentage of glucose [4]. Glucose concentration was determined by dinitrosalicylic acid (DNS) calorimetric method.

2.3.2 Preparation of Dinitrosalicylic Acid (DNS) Calorimetric Reagent

There are two reagents prepared in order to carry out the DNS calorimetric method. The first chemical reagent was 40% of potassium sodium tartarate (J.T.Baker) prepared from 40 g of potassium sodium tartarate mixed with 100ml of distilled water. The second reagent prepared was 1% of dinitrosalicylic acid reagent. Approximately 10g of sodium hydroxide (QReC), 2 g of phenol and 0.5 g of sodium sulfide (Merck), and 10 g of dinitrosalicylic acid (Fluka) were mixed and made up to 1 liter with distilled water.

2.3.3 Dinitrosalicylic Acid (DNS) Calorimetric Analysis

Each sample of 1 ml was transferred into different test tube. Distilled water of 1 ml was added to dilute the sample. Then, 3 ml of the DNS reagent was added into the same test tube. On the other hand, 2 ml of distilled water and 3ml of DNS reagent was mixed and use as blank. The test tubes that contain sample which including the blank were put inside the water bath with the temperature at 80ºC. The test tubes were removed until the mixture changes to reddish brown in colour. The test tubes were cooled to room temperature. 1 ml of the 40% potassium sodium solution was added into each test tube. All the samples were measured by the OD at 540 nm using spectrophotometer.

■3.0 RESULTS AND DISCUSSION

3.1 Analysis of Beta-Glucan using DNS Method

Most of the mixed-linkage of beta-glucan analyzed according to the procedures provided by Megazyme International Ireland

Ltd. with their test kits [6]. In current study, all the beta-glucan extracted were analyzed using the DNS method. The standard curve of DNS was prepared and used to refer the glucose concentration for different absorbance of samples. In the DNS method, about 40% of potassium sodium tartarate solution was added into the sample to stabilize the colour of the mixture. Sample that contains glucose changes to reddish brown after heating. Blank remained in yellow colour when it does not have any glucose contents. The 1% of dinitrosalicylic acid reagent cannot expose to light so this step should be perform immediately after the reagent being taken out and mixed with samples to avoid exposure to light and cause inaccurate absorbance reading.

This method assumed the end product is beta-glucan and it was being hydrolyzed by glucosidase during incubation at 40ºC. According to Cui [2], glucosidase is used to break the oligosaccharides bonds of beta-glucan. The sample which has high absorbance means it has high glucose content which equally high percentage of beta-glucan.

3.2 Effect of Ethanol Concentration

Effect of concentration on solvent is one of the important factors for extraction as it may affect the concentration of extracted compound [14]. In this study, solvent that has been used is ethanol and different concentrations were applied in order to determine the optimum condition for beta-glucan extraction. The results of glucose concentration obtained using different concentration of ethanol was shown in Table 1.

Glucose concentration increased with the increasing concentration of the ethanol. Glucose concentration for each sample is representing the beta-glucan concentration extracted from each sample. Hence, the percentage of beta-glucan extracted also increased with the increasing of ethanol concentration. Redmond and Fielder [15] reported that inaccuracy will occur in utilizing low concentrations of short-chain alcohols for the precipitation of the glucan.

Table 1 Glucose concentration produced from different concentration of ethanol

Ethanol concentration as solvent, %	Glucose concentration, g/L
30	0.287
40	0.690
50	1.159
60	1.333
70	1.416
80	1.486
90	1.551
≈100	1.620

Figure 2 presents the effect of ethanol concentration contributes to the percentage of beta-glucan. It can be observed that increasing the concentration of ethanol increased the percentage of beta-glucan extracted. From Figure 2, approximately 100% use of ethanol could extract the highest amount of 91.8% of beta-glucan. The lowest concentration of 30% of ethanol only extracted 16.2% of beta-glucan. However, clearly it can be seen that the increase of 30% to 50% of ethanol gave drastic increase in the amount of extracted beta-glucan meanwhile at higher percentage concentration of ethanol, more consistent increase was observed in the percentage of glucan extraction. Pretreatment using 50% of ethanol managed to extract 65.65% of beta-glucan. Hence, 50% of ethanol as solvent was further used to investigate the optimum pretreatment duration for 3g of OPT fiber. Although 60% and the above concentration of the ethanol will provide higher beta-glucan, due to the economic potential, 50% of ethanol is considered the best choice among others.

Vasanthan and Temelli [1] reported their findings that 50 percent ethanol concentration as solvent was optimum for beta-glucan extraction from barley and oats grains. On the other hands, Johansson *et al*. [7] stated polysaccharides precipitation must use at least 60 percent of ethanol. Besides, Redmond and Fielder [15] stated that concentrations of greater than 50 percent solvent by volume can cause severe dehydration of the beta-glucan and result in the need for homogenizer to disperse the beta-glucan.

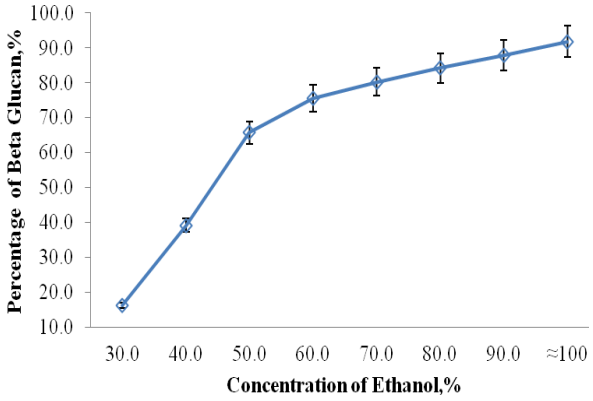


Figure 2 The effect of ethanol concentration as solvent to the percentage of extracted beta-glucan

3.3 Effect of Pretreatment Duration on the OPT Fibers

The purpose of this study is to find an additive to increase the efficiency of the beta-glucan production so the pretreatment duration of the oil palm trunk is also become one of the major factors. The variables for the pretreatment duration were varied for every hours starting from the 1st hour until the percentage of beta-glucan extracted become constant. All of the pretreatment was taking place under room temperature condition. Table 2 shows the pretreatment duration for 3 grams of OPT fiber and the resultant production of glucose concentration from each duration. It can be observed that the glucose concentration increased with the increase of pretreatment duration, however later it became constant after 4 hours pretreatment. Longer pre-treatment duration provided sufficient duration to disrupt the structure of lignin in OPT as plant fiber contains lignin. The glucose content become constant after 4 hours pretreatment which showed it reached the optimum condition.

Table 2 Glucose concentration produced from different pretreatment duration

Pretreatment duration, hours	Glucose Concentration, g/L
1	0.725
2	1.128
3	1.273
4	1.454
5	1.455

Beta-glucan is non-starchy polysaccharides found in walls of endosperm and aleurone cells of barley and oat grains [16]. These polysaccharides are polymers of β-D-glucopyranose [17], where about 30% of glucose residues are C(O)3-linked and 70% are C(O)4-linked. Works by Samyar *et al*. [18] have found that optimum pretreatment for wheat by using alcohol was at 220◦C and 5 minutes but in current study the optimum condition for OPT fiber pretreatment was 4 hours at much lower temperature, 25◦C.

Figure 3 shows the percentage of extracted beta-glucan in relation to the pretreatment duration. It can be observed that a constant value achieved after 4 hours of the pretreatment (which represented as well by the glucose concentration). The highest percentage of extracted beta-glucan after 4 hours pretreatment duration was 82.4% while the lowest achieved at 1 hour pretreatment duration was 41.1%. Besides, a relevant mathematical model showed the relation between beta-glucan extraction and pretreatment duration as shown in Figure 4. It demonstrated the duration of pretreatment has a direct relationship with beta-glucan extracted. After 4 hours pretratment, the structure of OPT was completely disorganized and facilitate the enzymatic reaction.

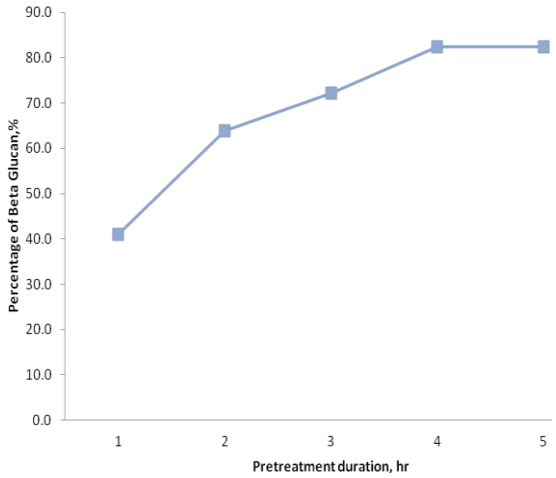


Figure 3 The effect of pretreatment duration by using 50% of ethanol

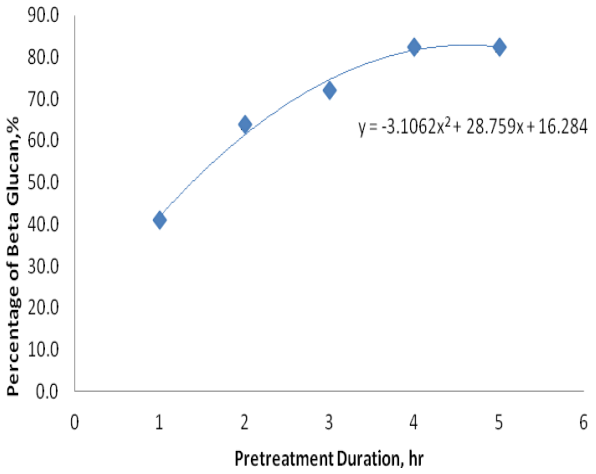


Figure 4 Mathematical model of relation between beta-glucan extraction and pretreatment duration

3.4 Optimum Temperature for α-amylase

Acidic and alkaline extraction methods reduced the viscosity of beta-glucan [19]. According to Asif *et al*. [19], high viscosity of extracted beta-glucan makes it an alternative as thickening agents in different food applications. Beta-glucan is non-starchy polysaccharides [16] so it could not be hydrolyzed by α-amylase. Hence enzymatic extraction method was found to be the most suitable due to highest yield and more starch could be removes during extraction of beta glucan [19].

The influence of operating temperature for alpha amylase on beta-glucan production is very important in order to produce high purity of them. The temperature range for normal alpha amylase is between 25ºC until 37ºC [20]. In current study thermally stable α-amylase was used to recover the starch hydrolysate. The glucose concentration production from different operating temperature for thermal stable α-amylase is presented in Table 3.

The glucose concentration decreased when the temperature was increased from 40ºC until 70ºC and further up to 80ºC. This is due to the enzyme activity was not react at optimum condition and it denatured after 70ºC. Hence, analyzed beta-glucan still contained the residue of starch hydrolysate which not being removed by thermo stable alpha amylase. The enzyme was stable for 1 hour at 60ºC and 70ºC [21-22]. The point at which there was a turn on the percentage of beta-glucan showed the optimum condition for thermal stable alpha amylase. It can be summarized the optimum temperature for alpha amylase in this reaction was at 70ºC.

Table 3 Glucose concentration production from different operating temperature for thermally stable α-amylase

Temperature, °C	Glucose Concentration, g/L
40	0.91
50	0.78
60	0.70
70	0.60
80	0.72

Figure 5 shows the percentage of beta-glucan versus the treatment temperature for thermal stable alpha amylase. Hence, when operated at the optimum condition, the percentage of beta-glucan was 34.17%.

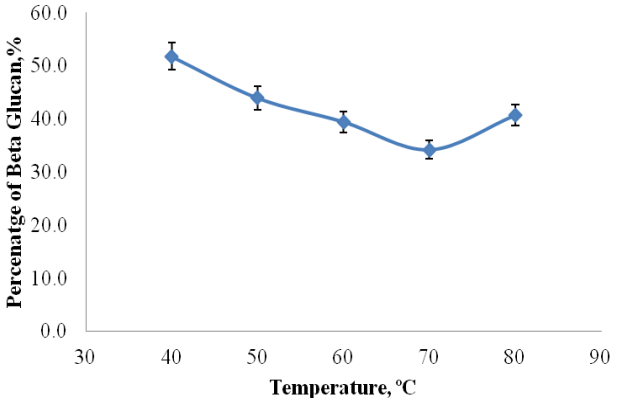


Figure 5 The effect of different temperature for α-amylase treatment

4.0 CONCLUSIONS

In a nut shell, this study showed the pretreatment parameters affect the percentage of beta-glucan extracted. Those parameters were concentration of the ethanol, pretreatment duration and also the operating temperature for the thermal stable alpha amylase. From the overall results, it can be concluded that using 50% of ethanol as solvent for 4 hours pretreatment duration and temperature of 70ºC for the thermal alpha amylase reaction could produce yield of the beta-glucan which is 34.17%.

Acknowledgement

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