Effect of Microwave Treatment on Acid and Enzymes Susceptibilities of Sago Pith

T.C. Sunartia, M. Dwikoå, V. Derosya, A. Meryandini

Department of Agroindustrial Technology, Bogor Agricultural University, Kampus IPB Darmaga, Bogor 16002, Indonesia
Department of Biology, Bogor Agricultural University, Kampus IPB Darmaga, Bogor 16002, Indonesia

Abstract

Sago palm is considered to be an ideal crop for starch-based sweetener and alcohol fuel feedstock since there is negligible competition between food and fuel in Indonesia and land resources were under-utilized. Starch accumulates in the pith of the sago trunk. Composition of dried sago pith is mainly starch and fiber components. Direct conversion of sago pith into fermentable sugar will reserve the water and save the energy for starch extraction and drying. This research investigated the effects of sago pith pretreatment as heating in water and in dilute sulfuric acid using microwave heating and autoclaving. Liquefaction of starch was conducted using α-amylase on 95 °C, and enzyme consortium between dextrozyme (amyloglucosidase and pullulanase), cellulase dan xylanase on 50 °C for saccharification process. Changes on structures were monitored by microscopy examination, and filtrate characteristics. Conversion process which included the pretreatment was carried out by microwave heating in water or in dilute acid, starch, and fiber hydrolysis into fermentable sugar. The results shows that direct heating of sago pith in water by microwave treatment can swell and gelatinize the starch, make the fiber more amorphous and more susceptible for enzyme reaction. Heating in dilute acid directly converted the starch and fiber into depolymerized products and gave more simple sugars (DP of 1.11-2.56) compared to autoclaved heating of sago pith (DP of 2.00-2.11). Microwaved-treatment produced lower DP of sugars during liquefaction (DP of 3.8-13.3) compared to autoclaved heating (DP of 7.3), but expressed some higher DP of sugar during saccharification (DP 3.1-3.8) compared to autoclaved heating (DP of 3.0).

Keywords: Sago pith; acid hydrolysis; enzymatic hydrolysis; microwave treatment; fermentable sugars.

1. Introduction

Currently bioethanol produced from glucose obtained from starchy materials. Sago starch, is derived from the pith of numerous kinds of palm trees, namely Metroxylon sp. Yatsugi [1] reported that the
highest starch yield in these samples was almost 40% of sago pith. Starch accumulates in the pith of the sago trunk. Fujii et al. [2] have reported that composition of dried sago pith was mainly starch (81.51-84.72%), and fiber (3.20-4.20%) components.

Microwave irradiation is an alternative method for hydrolysis of starch. The use of microwave irradiation to generate heat is very promising to be applied in the conversion of biomass to simple sugars. There are some reports regarding the use of microwave heating in the degradation of starch from different kinds of starchy materials, such as wheat, rice, potato, and corn, in water or dilute acid solutions. Microwave heating could convert starch directly to glucose in relatively short time. Compare with conventional heating, the reaction rate of starch hydrolysis to glucose was accelerated 100 times under microwave irradiation as reported by Kunlan et al. [3]. There might be some secondary decomposition materials produced after microwave heating of starch suspension, especially when the medium used is acid or the temperature used is quite high with a quite long heating time.

One of important steps for the utilization of cellulose contained in lignocellulosic materials is pretreatment. This step is still considered to be the rate limiting step in an economically feasible process for enzymatic hydrolysis of cellulose. The aims of pretreatment are mainly to degrade or reduce lignin content, to reduce cellulose crystallinity, and to increase porosity and surface area of the materials. Microwave irradiation is one of pretreatment methods for lignocelluloses. Temperature and time of heating treatment affects weight loss and the loss of chemical component from the lignocelluloses. Heating time and energy consumption in microwave heating operation are low. Previously Matsumoto et al. [4] and Khan et al. [5] have reported that microwave treatment can be used for saccharification of starch in water and Warrand & Jannsen [6] and Yu et al. [7] in acid hydrolysis can produce oligosaccharides. Sunarti et al. [8] have reported that microwave-assisted heating in dilute acid directly converted the starch and fiber into depolymerized products and gave more simple sugars compared to autoclaving treatment. Even heating in dilute acid produced high soluble total sugars with low degree of polymerization, the process also released hydroxymethylfurfural as undesired by-products and high amount salt produced from neutralization of acid, which inhibit the Saccharomyces ellipsoides during ethanol fermentation.

Some previous research has been conducted to convert starch and lignocellulosic materials to glucose syrup and continue to ethanol. The conversion includes two processes: hydrolysis of starch, cellulose and hemicellulose to fermentable sugar, and fermentation of the sugars to ethanol by yeast or bacteria. Sakano et al. [9] has reported the susceptibility of sago starch to enzymes; and it shows that upon the addition of amylolytic and debraching enzymes, the enzymatic susceptibility of gelatinized sago starch was similar to that of other gelatinized starches. The hydrolysis of cellulose and hemicellulose are usually catalyzed by cellulase and xylanase enzymes. These enzymes condition have similarity for commercial amyloglucosidase. Therefore we proposed a new development system conducting by simultant consortium enzymes. This research (1) developed the saccharification process of starch and fiber directly from sago pith by using enzymatic process (2) investigated the effect of microwave treatment to enzymatic susceptibility of sago pith, especially during amylase liquefaction and saccharification by using consortium of amyloglucosidase, xylanase, and cellulase compared to acid hydrolysis.

2. Experiment

2.1. Materials

Sago pith from Metroxylon sp. was provided from local sago starch industry in Cimalpar, Bogor, Indonesia. Native sago pith was rasped and dried in sun drying and follow by 50 °C oven drier, and then pulverized and passed 35 mesh screens. The enzymes were commercial food grade enzymes for
α-amylase (thermamyl), dextrozyme (pullulanase & amyloglucosidase), cellulase and xylanase. Microwave oven Sharp type R-348 C output 1000 W High power and 32 L capacity was used for pretreatment procedure.

2.2. Pretreatment of sago pith

Sago pith mainly consists of starch and limited amount of fiber. Pre-treatment was investigated as microwave heating in water, and conventional heating by autoclaving as comparison. Sago pith slurries (10%) were poured into glass jar and lid, then heating on microwave oven on 30-100% of power level (output 1000 Watt) for certain time. Heating duration (in seconds’ unit) was decided for every power level depends on degree of gelatinization and water availability of heated slurry.

2.3. Enzymatic saccharification of sago pith

Enzymatic saccharification was conducted by regulating the enzyme application. Enzyme activity for each enzyme and consortium enzymes were analyzed as amylase, CMC-ase, and xylanase. The research investigated the saccharification of pre-treated sago pith using simultant reaction of consortium enzyme. Liquifying enzyme, α-amylase (1.75 U/g) worked on temperature 90 °C, saccharifying enzyme (dextrozyme, 0.3 U/g) worked together with cellulolytic (1 U/g) and xylanolytic (1 U/g) enzymes on 50 °C. Saccharifying products were examined as total sugar as described by Dubois et al. [10] total reducing sugar by Miller [11] and degree of polymerization of hydrolyzate. The fiber residue was examined and the changes on fiber structures were analyzed using polarized microscopic examination.

3. Results and Discussion

Raw sago pith obtained from local small-scale industry in Cimahpar village, Bogor, had high moisture content as 17.9%, therefore the pith should be oven dried, and during the process, dark color of sago pith came from browning reaction of phenolic compounds. Previously Sunarti et al. [8] has reported the proxymate components of sago pith. Carbohydrate is the major component in sago pith, especially starch (73%) and crude fiber (7.84%). Similar research Fujii et al. [2] also reported high content of starch (81-85%) on the pith. Sunarti et al. [8] described crude fiber as second component in pith also contained high amount of cellulose (10.36%), and small quantity of lignin, therefore delignification of sago pith was not necessary to be conducted.

3.1. Pretreatment of sago pith

Starch and fiber are main components in sago pith, which are insoluble materials in cold water. Heating pretreatment, such as autoclaving and microwave treatments are needed to increase the solubility and to break down the crystallinity of starch and fiber. The suspended sago pith slurry converted into almost homogenous gel form as products of starch swelling and fiber structure degradation into more amorphous form. Gelatinized starch and amorphous fiber are more sensitive to the physical treatment, chemicals and enzymatic susceptibility to produce simple sugars.

Microwave (MW) treatment influenced to the degradation of some polysaccharides into monomer and oligomers as reported by Khan et al. [5], and Warrand & Jannsen [6]. Microwave energy can penetrate into materials heated, so that it directly and uniformly heating the materials. Energy irradiated by MW is absorbed by polysaccharide material and changes into heat, and high amount energy produced quick heat penetration, and cleavages the glycosidic polysaccharide linkages. Acid catalysts also gave more
advantages since it can convert starch, and also fiber as cellulose and hemicellulose in the short time. Changes of starch and fiber structure during microwave treatment in water and dilute acid are shown in Figure 1. On Figure 1a untreated sago pith showed native granule of starch, and compacted form of fiber, but Figure 1b showed that microwave heating caused the granule swelling and gelatinized it. Figure 1c heating in dilute acid caused gelatinized and hydrolysis directly into depolymerized products. Most of the native starch granules disappeared, and converted into gel forms products. Blue color of fiber fraction also indicated not completely degradation of crystalline parts. It shows how effective the microwave heating in dilute acid compared to heating in water.

![Figure 1](image.png)

Fig. 1. Microscopic structures of (a) native sago pith, microwave heating of sago pith in (b) water and (c) dilute acid. Magnification 200×

Soluble total sugar is the important parameter to be determined since it can reflect the efficacy of microwave treatment of sago pith in water and dilute acid. Total sugar is determined the amount of soluble reducing sugar, and its oligomers. Previously Sunarti et al. [8] reported the amount of total sugar ranged from 32 g/L to 285 g/L from microwave treatment in dilute acid. The highest amount of soluble sugar came from 0.3 M of acid concentration, heating on 70% power level for 3 min. DP (degree of polymerization) expressed the number of monomer unit in the molecule. The process in dilute acid produced more simple sugar compared to heating in water. The DP of products is ranged from DP 1.11-2.56. Attack actions of acid dilute hydrolysis are quick and random, but can react not only to the starch nor hemicellulose. Even this process produced very high soluble sugar, but the undesired product HMF (hydroxy methyl furfural) also accumulated in high amount as 0.01 g/L (10 ppm). Longer time for heating caused evaporated of filtrate volume, and this shown the concentrated of soluble fraction, as express in the previous pretreatment.

3.2. Enzymatic saccharification of sago pith

Microwave treatment to the starch granules in the water caused the granule swelling and gelatinized it, and then the enzymes easily attacked the reaction site and hydrolysis it. Remained water in gel products after MW treatment correlated to the hydrolytic requirement of free water availability to induce the enzymatic reaction. Liquefaction process initiated the hydrolytic process of sago pith. Gelatinized sago pith produced from previous heating process showed different degree of gelatinization. Products from liquefaction process really depend on the effect of heating process both from microwave heating and autoclaving. Table 1 shows different soluble fraction indicated with high total sugar content, and degree of depolymerization is shown in number of DP (degree of polymerization). Alpha amylase cleaved the α-1,4 glycosidic bonds in amylose and amylopectin in starch to produce α-limit dextrin and
maltooligosaccharides. This enzyme is gelatinized starch degrading enzyme, works randomly as endo attack enzyme. So the liquefying process needs short time to liquefy the starch.

Table 1. Effect of microwave treatment to total sugar (TS) and degree of polymerization (DP)

| Treatment | Liquefaction | Saccharification |
|-----------|--------------|------------------|
|           | TS (g/L)     | DP              | TS (g/L) | DP              |
| Autoclaving | 34.2<sup>a</sup> | 7.3            | 134.18<sup>b</sup> | 3.0            |
| Microwave* |              |                 |          |                |
| 30% - 320 s | 36.2<sup>a</sup> | 5.4            | 149.7<sup>b</sup> | 3.2            |
| 30% - 270 s | 67.3<sup>b</sup> | 10.2           | 143.0<sup>a</sup> | 3.7            |
| 30% - 120 s | 68.7<sup>a</sup> | 10.5           | 144.5<sup>a</sup> | 3.7            |
| 50% - 180 s | 36.2<sup>ab</sup> | 5.6            | 149.5<sup>a</sup> | 3.8            |
| 50% - 120 s | 38.4<sup>ab</sup> | 6.2            | 138.5<sup>ab</sup> | 3.3            |
| 50% - 60 s  | 86.1<sup>b</sup> | 13.3           | 131.9<sup>a</sup> | 3.8            |
| 70% - 60 s  | 42.5<sup>b</sup> | 7.7            | 140.4<sup>ab</sup> | 3.8            |
| 70% - 55 s  | 21.5<sup>b</sup> | 3.8            | 174.6<sup>b</sup> | 3.2            |
| 70% - 50 s  | 26<sup>b</sup>  | 4.5            | 137.3<sup>b</sup> | 2.8            |
| 100% - 50 s | 72.1<sup>ab</sup> | 11.6           | 146.2<sup>bc</sup> | 4.4            |
| 100% - 40 s | 40.9<sup>ab</sup> | 5.8            | 145.5<sup>b</sup> | 3.9            |
| 100% - 30 s | 67.2<sup>a</sup>  | 11.0           | 134.2<sup>ab</sup> | 3.1            |

*Microwave treatment (power level – duration)

High power level of microwave treatment also influenced to structural starch changes, since it produced high DP products compared to autoclaving treatment. Direct high heating especially in irradiation, influenced to the momentum movement which produced high energy. Gelatinization of starch in water needs time to penetrate, and then influenced to the liquefying products with DP 3.8-13.3. High degree of gelatinization will produce more simple sugars.

Saccharification process means the process to produce simple sugars, as sequence process after liquefaction process. Dextrozyme is mixed enzyme between amylloglucosidase and pullulanase. Amyloglucosidase is an exo-enzyme, attacks limit dextrin and maltooligosaccharide from the reducing site, and slowly release monomers especially glucose. Amyloglucosidase mainly cleaved the α-1,4 glycosidic bonds and slowly attack the α-1,6 glycosidic bonds. This made a debranching enzyme pullulanase to improve the degradation of α-1,6 glycosidic bonds and produce more linear maltooligosaccharides.

Sago pith consist mainly starch component, but since 35 mesh-screened native pith is used in this research. Most of starch granules entrapped in fiber matrix. Introduction of cellulolytic and xylanolytic enzymes improved the granule release into the system, by softened the cell-wall components of the pith. Increasing the simple sugar components also followed by the accumulation of celooligosaccharides and xylooligosaccharides as the products of cellulase and xylanase reaction to the fiber component. Even cellulose and xylanase are complex enzymes consist of endo-cellulase, exo-cellulase, and β-cellobiase for cellulase, and endo-xylanase, exo-xylanase, and β-xylosidase, the process needs more longer time to more high efficient process. Enzyme consortium works on temperature of 50 °C for 48 h. More amorphous form formed from heating and liquefying process, made easily the enzymes to degrade the dextrin to simple sugars. The end products of saccharifying process are described on Table 1.
Saccharification process is a simultant process with liquefying process, since no inactivation process conducted in this step. Continuous simultant liquefying enzyme shows its activity since it increase the soluble total sugar happened in the saccharification process. Cellulase and xylanase also play an important role to release the starch, as resistant starch type 1, from cell-wall, and then degrade by α-amylase. More simple sugars are produced from this step as DP 2.8-4.4, since Some residue (21-36%) also remain after saccharification Development saccharification process is need to improve the process efficiency, since more simple sugars DP~1 are desired for ethanol substrate. Effects of this step process are monitored in microscope examination of the residue on Figure 2. High amount of residues are related to the amount of remain unreacted starch and fiber, and indicated insufficient process of sago pith saccharification. Weigh loss of samples not only caused by starch hydrolysis. Solubilization of hemicellulose also increases with the increment of temperature process as reported by Magara et al. [12].

![Figure 2. Microscopic structure of sago pith residue after enzymatic saccharification. (a) autoclaving (b) microwave treatment. Magnification 100×](image)

Figure 2 shows the remaining fiber component in the residue. There is no starch granule in slurry, but most of fiber is still in solid form. Some entrapped starch granules in cell-wall might be accumulated in fiber. Dominated of blue crystalline fraction reflected from all treatment indicated that autoclaved heating in water gelatinized the starch, but not degraded the fiber, Figure 2a, while microwave treatment also made fiber more amorphous form, Figure 2b. Blue crystalline parts dominated the residue, but some of the fiber also reflected yellow part in the residue.

4. Conclusion

Sago pith is an abundant carbohydrate sources, which mainly consists of starch and fiber. Direct heating of sago pith in water by microwave treatment can swell and gelatinize the starch, make the fiber more amorphous, and more susceptible for enzyme reaction. Heating in dilute acid directly converted the starch and fiber into depolymerized products and gave more simple sugars compared to heating of sago pith in water.

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