

# Characterization of starch degradation during simple heating for bioethanol production from the avocado seed

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**Abstract.** We have successfully identified starch degradation with the presence of glucose from the avocado seed powder by simple heating method. This work was done by varying the composition of the avocado seed powder to obtain the effect of weight fraction increase in improving the concentration of glucose. From the Luff Schoorl test method, we found that the optimal glucose concentrations reached up to 14.86% that was produced from 80 g of the avocado seed powder. Furthermore, SEM showed the crystalline structure of glucose content and swelling of granules after heat treatment. FTIR spectra also indicated the presence of glucose at  $1079.6\text{ cm}^{-1}$  (C-O-H bending) and  $1412.38\text{ cm}^{-1}$  ( $\text{CH}_2$  bending modes).

## 1. Introduction

Bioethanol is one of the most promising alternatives to fossil fuels, which can be produced from various renewable sources rich in carbohydrates. The raw material of bioethanol makers were divided into three groups: sucrose, starchy, cellulosic fibrous (lignocelluloses) materials [1]. Meanwhile, the starch content of the avocado seed reached 85.3% can be used as raw material for bioethanol [2]. Since 2015 Indonesia has availability of the avocado seed as bioethanol source reached 76,516.40 tones with 6,208.60 tones in Central Java. In addition to being economical value, processing of the avocado seed as bioethanol will also reduce 15,303.28 tonnes of the organic waste in national region.

However, conversion of starch into glucose varies considerably depending on the process of starch degradation [3]. In this process, starch granules become hydrated, swell, and release glucose as fermentable sugar [4, 5]. In general, the degradation of complex organic compounds in starch can be done either by hydrolysis acidic or enzymatic methods [6]. During hydrolysis of starch, the amylose and amylopectin molecules undergo biochemical conversion to produce soluble sugar. Although acid hydrolysis is an effective way of generating fermentable sugars from starch, the necessity for acid recovery after hydrolysis has made it unattractive. Moreover, acid hydrolysis is often associated with the production of undesirable by-products that are toxic to microbial cells and inhibit the growth of yeast, thus affecting the overall yield of ethanol fermentation [7].

Furthermore, the pH of the solution is an important factor having significant effect on the overall process of bioethanol production [8]. Most recently, we revealed that simple heating can be used to release of glucose from starch granules without affecting pH solution [9]. Also, Wang *et al.* revealed under hydrothermal treatment, starch granules swell and lose their crystallinity and molecular organization in a process known as gelatinization [10]. Based on these backgrounds, in this work we developed and characterized starch degradation during simple heating for bioethanol production from avocado seed. We prepared the process with weight fraction variation of avocado seed powder and



evaluated correlations their physicochemical and morphological properties to provide a useful information about impact of subsequent reaserch treatment parameters.

## 2. Experimental method

### 2.1. Preparation of raw material

The avocado seed waste collected from local fruits juice stalls around campus area UNNES Sekaran, Gunungpati. First, the seed was washed with plain water and dried under the sun for a week to remove excess moisture. Then, the coat of the seed was peeled to remove the outer layer. After that, the peeled seed was shredded by vegetable grater, followed by drying using microwive at 100 °C for 30 minutes and grinding until it become granulated-powdery the avocado seed.

### 2.2. Starch degradation by simple heating

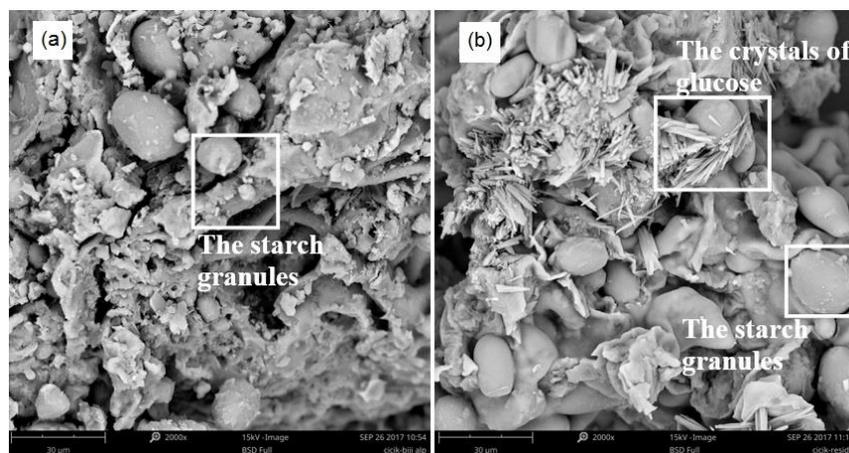
In this work, starch degradation was done by varying weight compotition of the avocado seed powder in range 20-100 g. The powder was mixed with 200 mL of water and heated using magnetic stirrer at 60 °C for 60 minutes. Then, mash of the avocad seed powder was cooled down to room temperature and filtrated to remove the slag.

### 2.3. Analysis and characterization of starch degradation

The filtrated solution was analyzed using Luff Schoorl method to obtain the effect of weight fraction addition in improving the concentration of glucose. In other hand, characterization of physicochemical and morphological properties before and after treatment by simple heating was analyzed using the Fourier-Transform Infrared Spectroscopy (FTIR) and the Scanning Electron Microscopy (SEM).

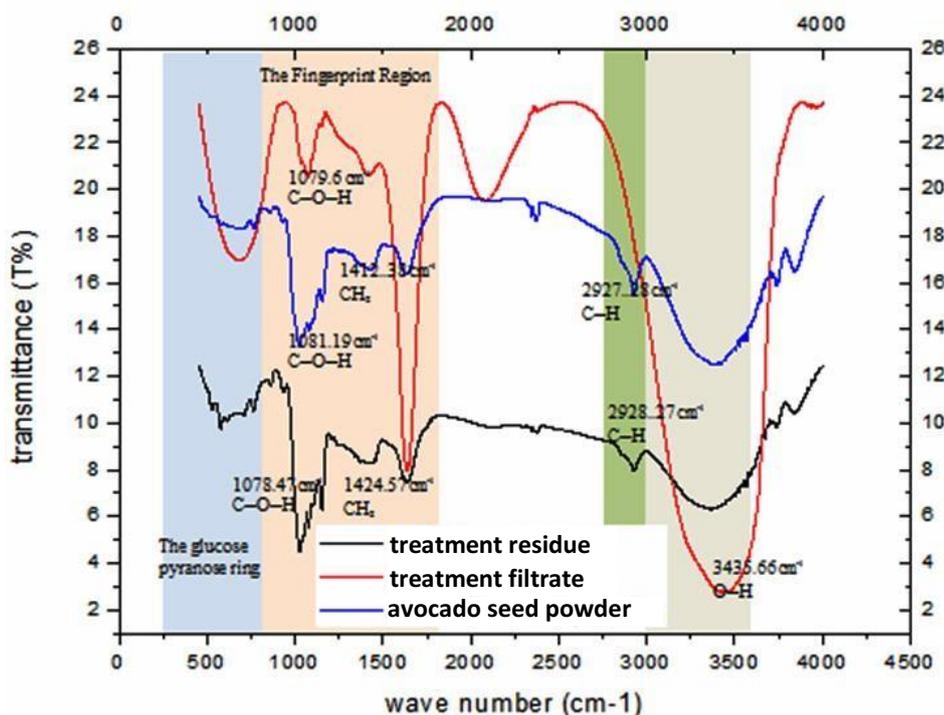
## 3. Results and discussion

SEM characterization result are shown in Figure 1 that explained the morphological effect of simple heating treatment. Figure 1 (a) shows the morphological image of the avocado seed powder before simple heating and it is obtained that the surface is composed by small granules. Otherwise, the avocado seed powder after treatment have the bigger granules (Figure 1 (b)). Both of the granules are displayed in spherical shape with smooth surface [11]. However, this change indicate that the starch granules are swell due to the water adsorption. Previous studies have shown that starch release glucose while the water adsorbed by starch [12]. It is confirmed in Figure 1 (b), where the glucose of starch perform as the reducing sugar in crystals shape [13]. An efficient starch degradation system to release the glucose also analyzed by FTIR.



**Figure 1.** SEM micrographs of (a) the avocado seed powder and (b) the simple heating residue.

Figure 2 shows spectral data of the avocado seed powder, residue, and filtrate after simple heating process by FTIR. Both spectral data of the avocado seed powder and treatment residue have the nearly regions in different transmittance. This is due to the content of the avocado seed powder reduced after hydrolysis but it still similar contents on the functional, chemical, and structural properties of the starch and other. In this work, investigation of starch was performed by four main regions that can be interpretation and characterization of the key bands. This regions were as follows : below 800  $\text{cm}^{-1}$ , 800-1500  $\text{cm}^{-1}$  (the fingerprint region), 2800-3000  $\text{cm}^{-1}$  (C—H stretch region), and the region between 3000-3600  $\text{cm}^{-1}$  (O—H stretch region).



**Figure 2.** FTIR spectra of hydrolysis residue, hidrolisis filtrate, and avocado seed powder.

The complex vibrational modes at below 800  $\text{cm}^{-1}$  region are the skeletal mode vibrations of the glucose pyranose ring [14]. This region of the avocado seed powder was displayed at 684.93  $\text{cm}^{-1}$ , while the treatment residue was showed at 528.04  $\text{cm}^{-1}$ , 576.33  $\text{cm}^{-1}$ , and 762.97  $\text{cm}^{-1}$ . This change shows that there is chemical modification or rupture of chemical bands in compounds such as the pyran ring or guaiacyl ring [15].

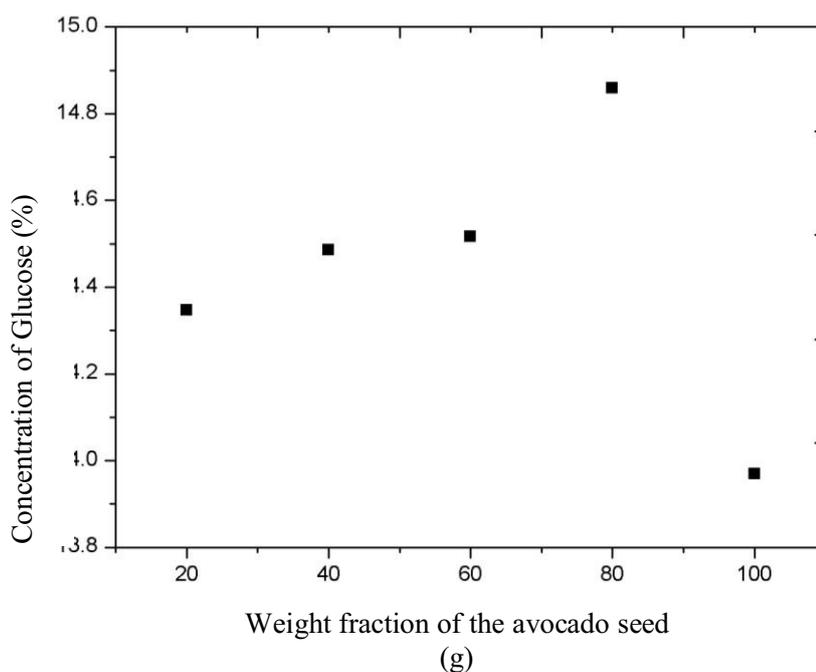
The region between 800-1500  $\text{cm}^{-1}$  provides highly overlapping having complex spectra with difficult assignment. In this regions, the spectra of the avocado seed powder has the FTIR absorption band at 1026.71  $\text{cm}^{-1}$  is attributed to the C(1)—H banding modes, and in other band at 1081.19  $\text{cm}^{-1}$  is identified C—O—H bending. The emerging of 1155.94  $\text{cm}^{-1}$  is due to coupling modes of C—O and C—C stretching, and the infrared band at 1427.83  $\text{cm}^{-1}$  originated from  $\text{CH}_2$  bending modes.

A band at 936.97  $\text{cm}^{-1}$  reported by spectral of treatment residue that indicate the C—O—C vibration of the 3,6 anhydrogalactose bridge and the band also obtained to the glycosidic linkages in starches [16]. However, this band just occurs in spectra of treatment residue because there is deformation in chemical bands after simple heating [17]. In other hand, the spectra of treatment residue between 800-1500  $\text{cm}^{-1}$  has the same of band assignment with the spectra of the avocado seed powder in different absorption band. The C(1)—H banding mode was observed at the band of 1026.97  $\text{cm}^{-1}$  and the other band assignment could be observed at 1078.47  $\text{cm}^{-1}$ , 1153.29  $\text{cm}^{-1}$ , 1424,57  $\text{cm}^{-1}$ .

The water adsorbs of the avocado seed powder and the treatment residue in the amorphous region of starch is identified as a broad infrared band with peak of  $1632.46\text{ cm}^{-1}$  and  $1637.47\text{ cm}^{-1}$ . Both peaks indicate the water molecules vibration adsorbed in the non crystalline region of starch [14]. The region between  $2800\text{--}3000\text{ cm}^{-1}$  in the avocado seed powder and the treatment residue was obtained at  $2927.28\text{ cm}^{-1}$  and  $2928.27\text{ cm}^{-1}$ . This region shows C—H stretching mode observed the variations of amylose and amylopectin in the starch by the change of transmittance [18]. The O—H stretching mode of starch in the avocado seed powder and the treatment residue was obtained at  $3394.41\text{ cm}^{-1}$ ,  $3567.76\text{ cm}^{-1}$ ,  $3365.38\text{ cm}^{-1}$ ,  $3525.8\text{ cm}^{-1}$ , and  $3567.89\text{ cm}^{-1}$ .

An interaction between water and starch after simple heating was performed by the spectra of the treatment filtrate at the Figure 2. This is due to several peaks having the similar assignment with the spectra of the avocado seed powder and the treatment residue. This assignment shows the glucose can be bonded with molecule of water.

Furthermore, the characterization of the glucose in aqueous solution of treatment filtrate was attributed at  $685.14\text{ cm}^{-1}$  (the glucose pyranose ring),  $1079.6\text{ cm}^{-1}$  (C—O—H bending) and  $1412.38\text{ cm}^{-1}$  ( $\text{CH}_2$  bending modes). This spectra is dominated by water component, there are the O—H stretching mode and water adsorbed at  $3435.66\text{ cm}^{-1}$  and  $1634\text{ cm}^{-1}$ . The peak at  $2078.9\text{ cm}^{-1}$  was obtained a combination of hindered rotation and O-H bending (water) that can explained about the vibration water molecules in aqueous solution [19].



**Figure 3.** Concentration of glucose as weight fraction of the avocado seed.

The starch degradation of the avocado seed powder with the water are shown in Figure 3. At 20–80% weight fraction of the avocado seed powder, it was found that the glucose concentration increased with increasing weight fraction. This is due to the increasing of the starch that can react with hydrogen bond in water [20]. After 80%, the glucose concentration remarkably reduces with increasing of weight fraction, since the glucose from the granule starch is not released into the water during simple heating [21]. The maximum amount of glucose concentration was obtained 14.86% of fermentable sugars. Furthermore, William *et al.* have studied that the starch of plant was a semicrystalline polymer and composed with 20–25% amylose [22]. However, glucose as amylose by the result of simple heating is the only one that can convert to become ethanol because it has, a mostly linear chain, typically consists

of up to 3000 glucose molecules interconnected primarily by  $\alpha$ -1,4 glycosidic linkages contain a few branched networks [14]. Additionally, the glucose yield concentration are correlated during fermentation. This due to the fact that the glucose such as nutrients of yeast to convert into ethanol [23].

#### 4. Conclusion

Starch degradation by simple heating was a suitable process to produce the glucose for bioethanol production from the avocado seed powder. The measurement with FTIR showed that the glucose yield in aqueous solution at  $685.14\text{ cm}^{-1}$  (the glucose pyranose ring),  $1079.6\text{ cm}^{-1}$  (C—O—H bending) and  $1412.38\text{ cm}^{-1}$  ( $\text{CH}_2$  bending modes). Further, SEM images show there are changes of granules starch size due to the simple heating process. The optimal condition of the fermentable sugar was produced by 80g of weight fraction, which a concentration at 14.86% using Luff School method.

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