SEMIMICRO DETERMINATION OF CELLULOSE CONTENT IN SAGO AND PITH FLOUR

(Penetapan kadar selulosa tepung sagu dan tepung empulur sagu dengan metoda semimikro)

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Ringkasan

Tepung Sagu serta tepung empulur sagu selain mengandung komponen utama pati, juga mengandung komponen lain seperti protein, lemak, sellulosa, flavan, dan polyphenol. Kadar komponen abus (minor) sering menunjang peranan penentu untuk menyimak kualitas pati, sehingga analisis kuantitatifnya perlu dilakukan. Study ini adalah untuk memantau keampuhan metoda Anthrone dalam penetapan kadar selulosa pada tepung sagu dan tepung empulur sagu melalui hidrolisis dengan katalis asam.

Dalam kajian ini, asam aseto nitrat digunakan sebagai bahan pengendap selulosa dan pengusir komponen lain seperti pati, lignin, ksilosan, dan hemiselulosa. Endapan selulosa dihidrolisa dengan 67% H2SO4 agar diperoleh komponen β-D-glukosa. Kadar β-D- glukosa setelah direaksikan dengan senyawa Anthrone diukur dengan alat spektrophotometer pada gelombang 620u, dimana senyawa selulosa murni (Avicell) digunakan sebagai senyawa baku yang dibandingkan dengan larutan blanko. Tepung empulur sagu mengandung kadar selulosa yang jauh lebih tinggi dibanding yang terkandung dalam tepung sagu.

I. INTRODUCTION

It is believed that there are 200 genera which consist of 4.000 species of palms in the world (Anonymous, 1969). Among the palms, mainly tropical plants, there are eight genera which have stems that produce starch. Five of them grow only in Southeast Asia and Oceania (Ruddle *et al.*, 1978; Johnson, 1977). The most important genus, Metroxylon, has been exploited on an industrial scale (Knight, 1969).

The species which are economically the most important in the Metroxylon genus are *M. sago* and *M. rumphii* (Flach, 1983). These sago palms can grow up to 10 m. in height, and about 40 cm. in diameter. The sago tree after 8-12 years growth is felled and is processed to collect the sago flour during flowering (Flach, 1983).

The estimated area which covered by good quality of sago palm trees are about 1.0 million ha in Papua New Guinea, 1.1 million ha In Indonesia, 33,000 ha in Malaysia, and in the Southeast Asia countries about 20,000 ha. Totally there are about 2,200 million ha palm tree stands (Flach, 1983).

Flach (1983) said that approximately 16-20 trunks of sago trees are harvested for every ha of palm trees. Each trunk is capable of producing about 850 kgs of pith or 250 kgs of dry flour.

Therefore, Southeast Asia area is estimated to produce about 9 to 11 million tons of dry sago flour annually. The stem is shredded to obtain the pith and then it is decanted in water to obtain the sago flour. The sago flour is rich in starch content, but poor in fat and protein content, For these reasons, many of the nativ change their staple food from sago flour to rice or corn instead.

Until now, there are only a few articles dealing with sago flour components. Due to its potentially and its highly in starch content, it is suggested that further studies in sago flour determination should be intensified. This study is needed in order to seek other possibilities of the uses in industry which consume a great amount of starch such as paper, textile, and food industry. This experiment is aimed to determine the cellulose content both in the sago and pith flour. The experiment used the anthrone method (Updegraff, 1969).

II. METHODS

A. Samples

Samples of sago and pith flour were obtained from a small sago mill at Bogor, Indonesia. Both were sun

dried before they were sent to Corvallis, The United State of America. The pith was ground into 140-mesh prior to determine the cellulose content.

B. Procedures

1. Moisture Content

Weighted the sample about 300 mg (Ww), then the sample is oven dried for 2 hrs or more until the constant weight (Wo) is obtained. The Moisture Content (MC) can be calculated by the following formula :
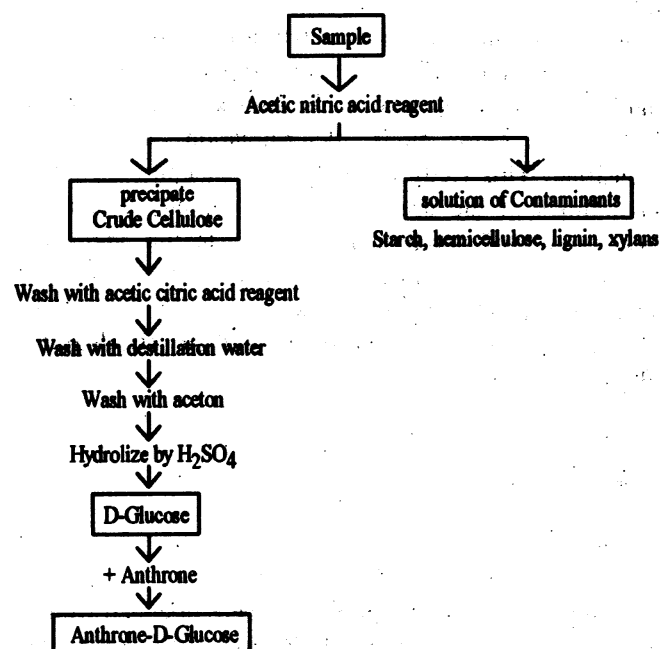
$$MC = \frac{Ww - Wo}{Wo} \times 100\%$$

2. Reagents

- The acetic nitric acid reagent is prepared by mixing 150 ml of 80 % of acetic acid and 15 ml of concentrated nitric acid.
- The 67% H_2SO_4 is prepared by diluting 136 ml of 98% H_2SO_4 in 64 ml distilled water.
- The Anthrone reagent is prepared by dissolving 200 mg of Anthrone in 100 ml concentrated H_2SO_4, prepared fresh daily. Chill about 2 hours in a refrigerator prior to using it.

3. Cellulose Content

This procedure is adopted from Updegraff Technique (Updegraff, 1969)

The procedural steps are as follows :

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        Sample
          ↓
Acetic nitric acid reagent
    ↓              ↓
precipate      solution of Contaminants
Crude Cellulose   Starch, hemicellulose, lignin, xylans
    ↓
Wash with acetic citric acid reagent
    ↓
Wash with destillation water
    ↓
Wash with aceton
    ↓
Hydrolize by H₂SO₄
    ↓
D-Glucose
    ↓
+ Anthrone
    ↓
Anthrone-D-Glucose
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Concentration is detected using a Spectrophotometer at 620μ

The procedure is as follows :

1. Place about 500 mg of sample in a small screw top tube
2. Add 1.0 ml acetic nitric acid reagent, mix well on Vortex mixer. Then add 2.0 ml of the same reagent and remixing
3. Cover the top of the tube loosely and place in a boiling water bath for 30 minutes
4. Centrifuge 5 minutes at 2000 rpm. Decant and discard supernatant . Wash twice with 3.0 ml acetic nitric acid reagent
5. Add 10.0 ml distilled water, centrifuge 5 minutes, decant and discard supernatant. Wash twice with 10 ml distilled water
6. Wash tree times with acetone then discard and air dry the aceton
7. Add 10.0 ml 67% H2SO4 (v/v). Let it stand on water bath at 36°C for 1 hour
8. Place 1.0 ml of the solution into 100 ml volumetric flask, dilute it to 100 ml with distilled water
9. Place 1.0 ml of the solution into a convenient screw top tube. Add 4.0 ml of distilled water
10. Place tube in an ice bath to cool, add 10.0 ml Anthrone reagent. Mix well on a Vortex mixer
11. Cover the top loosely and place the tube in a boiling water bath for 16 minutes. Cool in an ice bath for 3 minutes. Let it stand at room temperature for 10 minutes.
12. Read on a spectrophotometer at a wavelength of 620μ. Against the reagent blank

4. Standard Curve

The stock standard is prepared by dissolving 10.00 mg of pure cellulose (Avicell) in 10.0 ml of 67% H_2SO_4 with gentle heat. Then dilute to 100 ml with distiled water in a 100 ml volumetric flask. Analyze 0 (Blank), 0.4, 0.8, 1.2, and 1.6 ml of the stock standard. To each of these add a proper quantity of distilled water to reach 5.0 ml of the final volume. Each of these are treated as the same treatment as the sample on step #9 of the cellulose content procedure.

5. Sensitivity

The sensitivity of this procedure is 0.0007-0.0009 Å for the change of 0.1 mg of the cellulose content.

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III. RESULT AND DISCUSSION

A. Moisture Content

The moisture content of the sago flour is 13.87 % $\pm$ 0.57 % and the pith flour is 14.16 % $\pm$ 1.34 % respectively. The moisture content is high due to OH groups in the carbohydrate molecules which attract H_2O in the vicinity.

Meanwhile, pith flour is higher in moisture content then sago flour because the pith flour has more amorph region in cellulose and hemicellulose compound. Both components are more hygroscophic than starch.

B. Cellulose Content

The relation between absorbances and concentrations (in mg/ml) on the cellulose standard solution is shown in the following equation :

$Y = 0.0121 + 11.62X$ where X is Absorbance and Y is Concentration (mg/ml)

By reading the absorbance in a spectrophotometer (Spectronic 20) of the treated sample, we can calculate the concentration of the glucose of either the pith or the sago flour.

The cellulose contents in sago and pith flour are 1.24 $\pm$ 0.24% and 3.60 $\pm$ 0.31% respectively. The cellulose cantent in pith is 0.60% higher that what Wina et al. (1986) got (3.00 $\pm$ 0.37%). Even the difference is not too large it is better to be discussed. The difference is probably caused by two factors : the sample and the method. The sample could be differ for various reasons such as the differences in tree site, tree age, and tree variety.

The result of the method depends upon the effectiveness of the some factors such as :
- how to eliminate contaminants
- how to isolate cellulose
- how to hydrolyze cellulose into D-glucose, and
- how to measure D-glucose

In the experiment the acetic nitric acid reagent is used to remove contaminants such as starch, lignin, hemicellulose, xylan, cellulosan, and polyphenol which are in the sago and pith flour. This reagent left cellulose as a precipitate. It is important to add the proper amount of the reagent. Too little reagent will leave some contaminants which will mix with cellulose, and the result is higher than the real cellulose content. However, too much reagent will degrade some cellulose component, and the result will be lower than the real amount. Therefore, the appropriate amount of acetic

nitric acid reagent and the washing is the important things to be held to get the most accurate result.

Acid hydrolysis will liberate a polysaccharide into its monosaccharide such as arabinose, xylose, galactose, mannose, and glucose. There are some types of acids which can be used to hydrolyze polysaccharide such as H_2SO_4 (the most common used), HCL, and Trifluoro-acetic acid (TFA) (Biermann, 1989).

Sulfuric acid at 67% concentration (v/v) will swell cellulose and then dilution to 1 M H_2SO_4 will further hydrolyze cellulose into its mono saccharide (β-D-glucose) as a reaction follows :
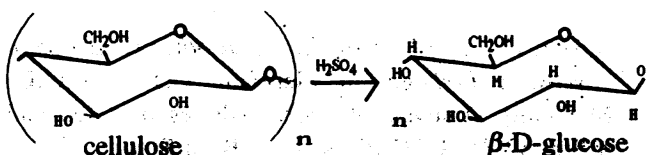
This method is known as Saeman hydrolysis (Saeman et al. 1954) which has many variations. Jeffery et al. (1964) used 77% H_2SO_4 followed by 6.6% H_2SO_4 for 4.5 hours. Laver et al. said that it is better than the Jeffery method. Oades (1967) used 72 % H_2SO_4 and then 0.5 M H_2SO_4 in studying carbohydrates in soils peats. To hydrolyze the plant cell wall, Blakeney et al. (1983) used 72% H_2SO_4 followed by 1.0 M H_2SO_4 for 3 step conditions :

a) at 121°C for 1 hour; b) 100°C for 2 hours; and c) 100°C for 3 hours. The highest recovery of mono-glucose was for condition c).

Beside acid hydrolysis there are enzymatic hydrolysis which use enzyme to liberate monosaccharide from a polysaccharide, Biermann (1989) said that this method able to select the determination of carbohydrate constituent by the selective glycosides.

There are many methods to detect monosaccharide in a solution such as colorimetric using phenol-sulfuric acid (Dubois et al. 1956) or using Anthrone sulfuric acid (Updegraff, 1969) as used in this experiment. Scott and Melvin (1953) said that the blank solution in Anthrone method, is poor reproducibility.

The other methods using chromatography (Gas-, Gas-Liquid-, or High Parformance Liquid Chromatography (Biermann and McGinnis, 1989). Chromatography is able to detect many kinds of monosaccharide simultaneously.

Conclusions

The antrone method is simple to do, but tedious for a lot number of samples.

It cannot differentiate monosaccharides such as arabinose, xylose, galactose, mannose, and glucose, all of these will calculated as glucose. The apparatus much less expensive, but consume more time compare to chromatography method.

The antrone method is sensitive, and necessary to pay special attention to control the condition of standard and blank treatment with Anthrone reagent to give reproducible results.

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