The Effect of Acid Hydrolysis on Degradation of Lignoselulosa Bond in Cassava Peel as Fish Feed

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ABSTRACT

The experiment was done at Microbiology Laboratory Faculty Fishery and Marine Science Padjadjaran University. The objective of this experiment was to know the degradation of lignocelulose bound in cassava peel through the acid hydrolysis. The research is done by experimental methods, using Completely Random Design ; consisting of five treatments and three replications. Parameters measured were the ratio of type and amount of dilution, the reducing sugar concentration and DE (Dextrose equivalent), lignin, cellulose, and hemicelullosa content in hydrolysis product.Results of this experiment showed that : a) Type of dilution was water, with ratio 1 substrate : 5 water, b) The concentration acid 0.5% gave the highest reducing sugar concentration of hydrolysis product 2642 ppm and DE 4.8% , c) The lowest lignin, cellulose and hemicelullose content were gave from treatment with 0.75% acid treatment.

Keywords : acid hydrolysis, lignocelluloses bound, cassava peel waste, fish feed.

INTRODUCTION

Various agricultural effluents have fish feed benefits, one of which is cassava peel. The cassava peel has sufficiently large starch content, making it possible to become a source of carbohydrate in fish fodder; the coarse fiber content is, however, still high, requiring further processing for better nutritional value.

Proximate analysis (2010) indicated sufficiently high coarse fiber content in cassava peel effluent, whereas lignin, cellulosa and hemicellulosa values were respectively 7.88%, 6.06% and 2.48%. Cellulosa is a frame of plant network which protects starch, whereas hemicellulosa are fibrils which covers the plant frame. Further, lignin, cellulosa and hemicellulosa.form a very strong lignocellulosa complex bond, making it indigestable in the fish digestive tract.tau The lignocellulosa bond does not dissolve in water and needs a temperature of more than 100^2 to change its composition and tautness (Fengel, 1995).

Acid hydrolysis using sulphuric acid (H_2SO_4) is one of the processes applicable to process agricultural effluent-based fodder ingredients, where the lignocellulosa biomass displayed wth acid at a certain temperature and pressure for a certain period of time will result in tautness in the lignocellulosa bond, facilitating the working of the enzyme used in the hydrolysisprocess enzymatically (Wyman et al., 2005). In addition, chemical hydrolysis will produce sugar monomer from cellulosa polymer and hemicellulosa. at the end of the reaction.

However, the sulphuric acid dosage used in the treatment of acid hydrolysis varied in every substrate, necessitating a research to be conducted to find the proper dosage of use of acid for the processing of cassava peel effluent to have better nutritonal composition as fish feed ingredient.

RESEARCH METHOD

This research used the descriptive and experimental method. The descriptive method was used in amount optimization and type of solvent, whereas the experimental method was used for the determination of sulphuric acid dosage used in the hydrolysis process.

The experiment was conducted using the Complete Random Design which consisted of 5 (five) treatments, respectively with 3 (three) replications. The treatment consisted of:

A : Addition of 0.10% sulphuric acid
B : Addition of 0.25 % sulphuric acid
C : Addition of 0.50% sulphuric acid
D : Addition of 0.75% sulphuric acid
E : Addition of 1.00% sulphuric acid

The data obtained from this experiment were analyzed using Anava, then the inter-treatment effect was tested using the Duncan Test at 95%.

This research consisted of various research stages which comprised:

1) Determination of type and amount of solvent; 10 grams of cassava starch were put into an Erlemeyer tube of 125 ml; then the following amounts of aquadest solvent were added: 30

ml, 40 ml, 50 ml, 40 ml, 50 ml, 60 ml, and 70 ml. The procedure was then repeated using acetate buffer solvent.

2) Dilute acid hydrolysis; 10 grams of cassava starch were put into an Erlemeyer tube of 125 ml; then the following amounts of aquadest solvent were added: 30 ml, 40 ml, 50 ml, 40 ml, 50 ml, 60 ml, and 70 ml. The procedure was then repeated using acetate buffer solvent.

The parameters to be observed in determining type and amount of solvent were: gelatine consistency, whereas in acid hydrolysis the reduction agent sugar contents used the DNS method and contents of lignin, cellulosa, and hemicellulosa hydrolysis products.

RESULTS AND DISCUSSION

1. Optimization of Solvent Type and Volume

The hydrolysis process in starch-based substrate needed stages of gelatinazation. Cassava peel flour is a starch-rich ingredient source; starch content in cassava peel came up to 30.8721% (b/b) (Andriani, 2009). Starch does not dissolve in water; however, if starch suspension is heated, gelatinazaton comes about, in which starch granules will absorb water and expand, making it incapable of returning to its original condition (Winarno, 2004). Hydrolysis of starch, which was previously not gelatinized, will produce a lower reduction agent sugar compared to hydrolysis of starch which was previously gelatinized (Slominska et al., 2003).

Some of the factors affecting the gelatinazation process were temperature, solvent type and volume. The starch gelatinization temperature ranged from 60^0 to 72^0 (Meyer, 1973 in Djafar et al., 2000). The results of optimization of type and amount of solvent in this research are presented in Table 1.

Table 1. Results of Determination of Amount and Type of Solvent for the Hydrolysis Process

Amount of Solvent (ml)	**Type of Solvent**	
	Acetate Buffer	Aquadest
30	Dry/Caramel	Dry/Caramel
40	Dry/Caramel	Dry/Caramel
50	Thick gelatine	Thick gelatine
60	Thick gelatine	Thick gelatine
70	Liquid	Liquid

Optimization results showed that the best type of solvent used was aquadest with a weight ratio of substrate weight to solvent of 1 : 5. This conformed to the research conducted by Wirakartakusumah (1984) which stated that the best startch suspension was the ratio of starch to water of 1:5.

The type and amount of solvent would affect the hydrolysis process, because the gelatine produced at a balanced water to starch ratio would have an ideal consistency and viscosity in the hydrolysis process. Birch and Parker (1979) stated that good gelatine would facilitate enzyme to break the starch polysacharide chain, because the enzyme used in starch could not attack the starch granules in intact condition. According to Reed (1975), after expanding the starch granules became more susceptible to chemicals, mechanical force and enzyme work.

5.2. Reducing Agent Sugar Content as a Result of Hydrolysis Process

The Hydrolysis process using sulphuric acid would result in the forming of sugar which for the greater part took the form of the result of simplification of the complex bond found in cassava peel substrate. Research results indicated that at various concentrations of sulphuric acid used, the highest reducing agent sugar concentration was obtained at a sulphuric acid concentration of 0.50%, that is, 2642 ppm (Table 2).

Table 2. Reducing Agent Sugar Content at Every Treatment

H_2SO_4 Concentration (%)	Absorbant		Sugar Content (ppm)
	Abs	Abs	
0.01	0	0.0208	1607.5
0.25	0.042	0.0208	1757.5
0.50	0.028	0.0208	2642
0.75	0.032	0.0208	2012
0.1	0.02	0.0208	1312

The reducing agent sugar concentration tended to increase in line with increasing concentration of sulphuric acid added. This was due to the fact that the acid broke up starch molecules randomly, and the break-up produced simpler molecules consecutively until the forming of glucose. The acid first cut off the amorph part in the starch molecule, because it tended to be more labile with respect to strong acid.

When cassava peel four is hydrolyzedwith acid catalyst there will be severing of C-O-C bond which further will result in glucose and some of tis polymers. When the process is continued, it will increase the sugar proportion with low molecular weight. The polymers were then hydrolized until they become glucosa (Junk and Pancoast, 1977, in Judoamidjojo, 1922).

The efficiency of the hydrolysis process could be measured by the Dextrose Equivalent (DE) value, in which the DE value was a percentage of the ratio of reduction agent sugar as a result of hydrolysis obtained against the total amount of carbohydrate contained in cassava peel flour (Winarno, 2004).

Research results indicated that hydrolysis of cassava peel flour using acid at 0.50% concentration produced the highest DE at 4.80% (Table 3). This DE value indicated that not all of the carbohydrate in cassava peel flour could hydrolyzed by the acid.

Table 3. The DE value at Every Treatment of Acid Concentration

H_2SO_4 Concentration (%)	Sugar Content (ppm)	Total Sugar (ppm)	DE (%)
0.10	1607.5	54840.580	2.93
0.25	1757.5	54840.580	3.20
0.50	2642	54840.580	4.80
0.75	2012	54840.580	3.66
1.00	1312	54840.580	2.39

This conformed to the statement by Judoamidjojo, et al. (1992), that starch conversion using acid would only produce a maximum DE of 55%. He also stated that a conversion with a DE of more than 55% would result in the sugar molecules'recombining and resulting in a color forming material, also called the Browning effect (Judoamidjojo, 1992) and a bitter-tasting component (Winarno, 1983). Further, Howling (1979) in Judoamidjojo et al. (1992) stated that the starch hydrolysis process using acid had only a DE value of 30 - 35%.

The hydrolysis process will be more efficient when conducted by using a combined method of acid hydrolysis and enzyme hydrolysis treatment. In this process, the starch was first hydrolyzed with acid, then hydrolysis was continued with using amylolitic enzyme until it DE reached 61 - 65%. This hydrolysis process produced a product which did not a bitter taste and which did not crystalize quickly (Tjokroadikoesoemo, 1986).

5.3. Composition of Lignin, Cellulosa, and Hemicellulosa

Hydrolysis is one of the processes that can be conducted to decompose starch and coarse fiber which formed the lignohemicellulosa complex bond to become a simpler compound, like sugar.

Research results indicated that hydrolysis using acid at different concentrations was capable of decomposing the lignohemicellulosa complex bond, bringing about proportional decrease in lignin, cellulosa, and hemicellulosa inside. Content of lignin, cellulosa, and hemicellulosa increasingly decreased with the increase in acid concentration. This was suspected to be due to cellulosa dissolution by acid used in the hydrolysis process. Winarno

(1997) stated that cellulosa had a high degree of polymerization and easily dissolved in water but dissolved with difficulty in alkali (base).

On the basis of results of statistical testing conducted on lignin content (%) produced from H_2SO_4 hydrolysis results at various concentrations, the inter-treatment variety (H_2SO_4 concentration) was known to be very significant. Testing was therefore continued with the Duncan Multiple Range test at 5% significant level to find which treatment gave rise to the said variety (Table 4)

Table 4. Effect of H2SO4 concentration on Lignin Content (%) in Cassava Peel Flour

Treatment H_2SO_4 (%) Concentration	Average Treatment Lignin (%)	Average Treatment Cellulosa (%)	Average Treatment Hemicellulosa (%)
Without Treatment	7.8800 a	2.4800 a	6.0600 a
0.10	4.8500 b	2.3700 a	3.5400 c
0.25	4.4200 c	2.0000 a	3.7400 b
0.50	4.2400 d	2.1400 a	3.4800 c
0.75	3.7500 e	2.8700 a	3.1500 d
1.00	3.7000 e	3.2200 a	1.6000 e

Explanation: Average treatment marked with the same letter does not significantly vary according to the Duncan Multiple Distance Test at 5% significant level.

On the basis of results of statistical analysis presented in Table 4, administration of H_2SO_4 concentration with different concentrations produced amounts of lignin and hemicellulosa which were degraded differed very significantly, but at 0.75% and 1.00% H2SO4 concentration the degraded amount of lignin did not differ very significantly. On the other hand, difference in H_2SO_4 concentration did not give a significant effect on cellulosa degradation in cassava peel flour on the basis of a 5% significant level.

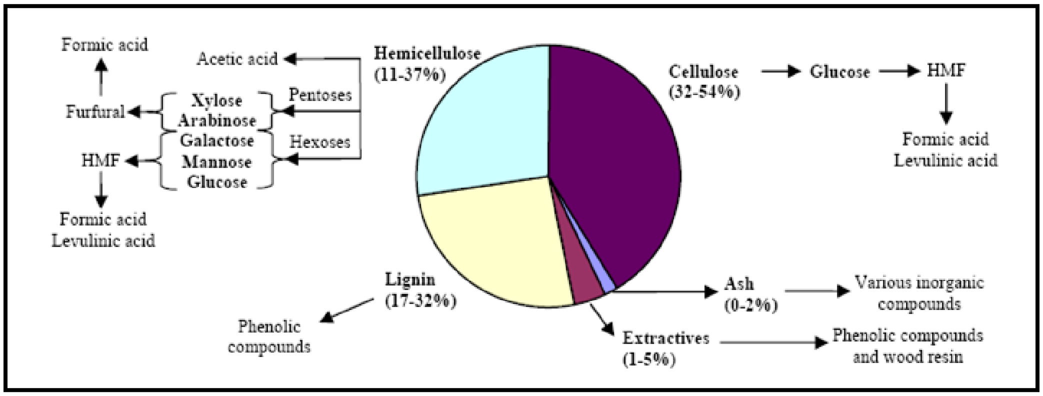

Fig. 1. Parts of Lignocellulosa and Product of Hydrolysis Result
(Taherzadeh & Karimi, 2007)

The release of lignocellulosa bond in this research was thought to be due to the fact that basically chemical hydrolysis involved lignocellulosa biomass displayed with acid at a certain temperature and pressure for a certain period of time would result in sugar monomer from cellulosa and hemicellulosa polymer. Composition of lignocellulosa and components of the product of hydrolysis results are presented in Fig. 1.

Dilute acid hydrolysis was conducted at high temperature and easily dissolvedin acidbut dissolved with difficulty in alkali (base). Dilute acid hydrolysis is also known as two-stage acid hydrolysis. The first stage was hydrolysis with dilute acid, then the hydrolysate produced was fermented to produce ethanol. The first stage was carried out in a "softer" condition and would hydrolyze hemicellulosa, for instance, 0.7% sulphuric acid at 190^0C. The second stage was carried out at a higher temperature, but at lower acid concentration to hydrolyze cellulosa, like that at 2150C at 0.4% concentration of sulphuric acid (Hamelinck et al., 2005 in Isroi, 2008).

CONCLUSION AND SUGGESTION

Conclusion

Acid hydrolysis using H_2SO_4 was capable of degrading lignocellulosa bond in cassava peel effluent substrate. The highest content in lignin, cellulosa, and hemicellulosa was found in hydrolysis using 0.75% concentration, the values of which were respectively 3.75, 2.87 and 3.15%. Acid hydrolysis at 0.50% concentration produced the highest DE of 4.80%.

Suggestion

Decline in coarse fiber content (lignin, cellulosa, and hemicellulosa) by way of acid hydrolysis did not indicate maximal result; it is therefore suggested to continue hydrolysis using enzyme to obtain more optimal results.

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BIBLIOGRAPHY

Andriani, Y. 2009. *Reduksi Asam Sianida (HCN) Secar Fisik Dan Kimiawi Sebagai Pra-Perlakuan Pengolahan Kulit Singkong Sebagai Pakan Ikan (Physical and Chemical Reduction of Cyanide Acid (HCN) as Pre-Treatment of Cassava Peel Processing as Fish Fodder). Scientific paper Laboratorium Budidaya Ikan (Fishery Culture Laboratory).*FPIK Universitas Padjadjaran.Not yet published. Bandung.

Birch, G. G. dan K. J. Parker. 1979. *Sugar: Science and Technology*. London: Applied Science Publishers Ltd.

Djaafar, Titiek. F. Mudjisihono, and S. Rahayu 2000 *Sago Processing Technique,* Yogyakarta Publishers Kanisius

Fengel, D. 1995 *Wood: Chemistry, Ultrastructure, Reactions* Publishers: Gadjah Mada University Press. Yogyakarta

Isroi, 2008 Lignocellulosa Characteristics Obtained May 13, 2011 From http://isroi.wordpress.com/2008/11/23.karakteristik-lignoselulosa/.

Judoamidjojo, M., A. A. Darwis, and E.G. Said. 1992 *Fermentation Technology* Publsihers: Rajawali Jakarta.

Reed, G. 1975 *Enzymes in Food Processing*. New York: Academic Press.

Slominska, L., M. Klisowska dan A. Grzeskowiak. 2003 *Degradation of Starch Granules by Amylases*.Electronic Journal of Polish Agricultural Universities.Series Food Science and Technology. Vol: 6 Issue 2.

Taherzadeh, MJ. and K. Karimi. 2007 Acid Base Hydrolysis Processes For Ethanol From Lignocellulose Materials ; A Review. *Bio Resources, 2 (3) :*472-499.

Tjokroadikoesoemo, P.S. 1986 *HFS dan Industri Ubi Kayu Lainnya (HFS dan other Cassava Industries)*. Publishers: Gramedia Jakarta.

Winarno, F.G. 1997 *Kimia Pangan dan Gizi (Foodstuff Chemistry and Nutrition)*. Publishers: PT Gramedia Pustaka Utama. Jakarta.

Wyman, C.E. *1994 Etanol from lignocellulosic biomass : Technology, economycs, and opportunitie*s .*Bioresources Technology* : 50.3-15.