การผลิตไบโอเอธานอลจากเปลือกมันสำปะหลังโดยการทำให้เป็นน้ำตาลควบคู่กับการหมักโดยอะไมโลไลติคยีสต์

Bioethanol Production from Cassava Peels by Simultaneous Saccharification and Fermentation by Amylolytic Yeast

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บทคัดย่อ

การศึกษาการผลิตเอธานอลจากเปลือกมันสำปะหลังที่ผ่านการปรับสภาพวัตถุดิบด้วยกรดซัลฟิวริกเจือจาง โซเดียมไฮดรอกไซด์ และน้ำกลั่น โดยกระบวนการทำให้เป็นน้ำตาลควบคู่กับการหมักแบบการใช้เชื้อเดี่ยว *Saccharomyces diastaticus* 2047, *S. cerevisiae* 7532 และเชื้อผสมระหว่าง *S. diastaticus* 2047 กับ *Candida tropicalis* 5045 ซึ่งเชื้อแต่ละสายพันธุ์สามารถผลิตเอธานอลได้ จากผลการทดลองพบว่า การใช้เชื้อ *S. diastaticus* 2047 ในการหมักวัตถุดิบที่ผ่านการปรับสภาพด้วยความร้อนในน้ำกลั่นที่อุณหภูมิ 135 องศาเซลเซียส ภายใต้ความดัน 15 ปอนด์ต่อตารางนิ้ว สามารถผลิตเอธานอลได้ปริมาณใกล้เคียงกับการปรับสภาพวัตถุดิบด้วยกรดในสภาวะเดียวกัน การใช้เชื้อ *S. diastaticus* 2047 ให้ปริมาณเอธานอลที่สูงกว่าการหมักแบบเชื้อเดี่ยวสายพันธุ์อื่น ส่วนการผลิตเอธานอลโดยเชื้อผสม พบว่า การปรับสภาพวัตถุดิบด้วยกรดซัลฟิวริกเจือจางนั้น จะทำให้ได้ปริมาณเอธานอลที่สูงกว่าเมื่อเปรียบเทียบกับการผลิตเอธานอลโดยเชื้อ *S. diastaticus* 2047

คำสำคัญ : เปลือกมันสำปะหลัง เอธานอล อะไมโลไลติคยีสต์

ABSTRACT

Feasibility study on ethanol production from cassava peels which pretreated with diluted sulfuric acid, diluted sodium hydroxide and distilled water by simultaneous saccharification and fermentation (SSF) with mono-culture of *Saccharomyces diastaticus* 2047 and *S. cerevisiae* 7532 and co-culture of *S. diastaticus* 2047 and *Candida tropicalis* 5045. The results indicated that each strain of mono-culture was able to produce ethanol with high yield. *S. diastaticus* 2047 could fermented the cassava peels pretreated with distilled water at 135°C under pressure of 15 lb/inch^2 to produce ethanol yield as high as the cassava peels pretreated with diluted sulfuric acid under the same condition. The cassava peels pretreated with diluted sulfuric acid and fermented by co-culture of *S. diastaticus* 2047 and *C. tropicalis* 5045 produce ethanol higher than that of by *S. diastaticus* 2047 alone.

Keywords : cassava peels, ethanol, amylolytic yeast

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INTRODUCTION

Bioethanol is being considered as a potential liquid fuel due to the limited amount of natural resources. Cellulose biomass is also being investigated as a potential substrate for bioethanol production (Masami *et al*., 2007). Especially bioethanol produced from non-food lignocellulosic waste products as wood chips and straw or non-food crops as willow could be an environmentally-friendly alternative (Wyman and Goodman, 1993). Cassava peels are the main by-product from processing tuberous roots of cassava for human consumption. The mature root possesses three distinct regions: a central vascular core, the cortex (flesh), and the phelloderm (peels). The peels is 1-4 mm thick and may account for 10-12% of the total dry matter of the root (Nartey, 1979). The analyses of the chemical composition of cassava peels indicate the following chemical composition: dry matter, 86.5-94.5%; organic matter, 81.9-93.9%; crude protein, 4.1-6.5%; neutral detergent fiber, 34.4% and lignin, 8.4%. Cassava peels have been evaluated as a feedstuff for animals (Adegbola and Asaolu, 1986; Obioha and Anikwe, 1982; Osei *et al*., 1990). The carbohydrates, cellulose and hemicellulose are intimately associated with lignin in the plant cell wall. The pentoses might be readily available, but are often found in polymeric chains as xylan, arabinogalactans, arabinans, and mannans. Also, the individual sugars might be methylated or acetylated (Biely, 1985) which can affect the availability. The hexoses can be fermented to ethanol by yeast, whereas the pentoses, can be fermented to ethanol, acetate, lactate, CO_2 and H_2 through the pentose-phosphate pathway with fructose-6-phosphate, glyceraldehyde-3-phosphate and pyruvate as intermediates (Larsen *et al*., 1997). Simultaneous saccharification and fermentation (SSF), which has been studied to reduce the time and steps of bioprocess for the production of ethanol from starch and cellulosic biomass. In SSF process, saccharification process of starch to glucose using enzymes. The glucose is catabolized to ethanol by a fermentative microorganism which performed simultaneously. The objective of this studies was to produce ethanol from cassava peels pretreated by dilute-acid, dilute-base or distilled water and hydrolysis with conversion to ethanol by mono-culture culture and co-culture of amylolytic yeast strain of *S. diastaticus* and *C. tropicalis* to enhance the ethanol production.

MATERIALS AND METHODS

Mono-culture amylolytic yeast strains of *S. diastaticus* 2047 and standard yeast strain of *S. cerevisiae* 7532 were used in this study. Co-culture was mixed between *S. diastaticus* 2047 and *C. tropicalis* 5045. Those were grown on Sabouraud medium at 30°C. Cassava peels from the factory of cassava starch production were milled to flour in the size of 63-425 micrometer by disc mill and dried overnight at 60°C in hot-air oven. The moisture content was 10.5%. 1.5% (w/v) cassava peels in 0.1 M sulfuric acid or 0.025% sodium hydroxide or distilled water was pretreated for 30 min at 135°C under pressure of 15 lb/inch^2. The pretreated cassava peels suspension were neutralized to pH 5.5 for

fermentation process. The enzymes solution (filter-sterilized cellulase 20 FPU per gram substrate, 10 mIU of xylanase and 10 mIU of pectinase solubilized in citrate-phosphate buffer pH 5.0) was used for hydrolysis of pretreated cassava peels. The pretreated sample was supplemented with additional nutrients to give a base medium composition of 1 g/l yeast extract, 1 g/l $MgSO_4 \cdot 7H_2O$, 2 g/l $(NH_4)_2SO_4$, 0.5 g/l KH_2PO_4. The enzymatic hydrolysis was run at the same time as fermentations that were carried out in 125 ml Erlenmeyer flasks containing 50 ml of this medium. The culture was incubated in a rotary shaker with 50 rpm for 48 hr at 30°C. The amount of released glucose was measured using glucose oxidase/peroxidase assay. The amount of reducing sugar produced was determined using the dinitrosalicylic acid (DNS) method. Ethanol concentration was measured by gas chromatography (GC-17A, Shimadzu) using a Chromosorb 101 column and a flame ionization detector.

RESULTS AND DISCUSSION

Saccharification of cassava peels

Cassava peels biomass were used as feed stock for production of fermentable sugars. The cassava peels were pretreated either with distilled water pH 5.5, 0.025% sodium hydroxide or with 0.1 M sulfuric acid as described above, and were used as raw materials for the saccharification experiments. Table 1 shows the yield of reducing sugars from the cassava peels after 24 h of incubation. The method of pretreatment had a pronounced effect on the yield of reducing sugars. Highest yield of reducing sugar (0.72 g/g dry cassava peels) was obtained from diluted acid treated. The value for reducing sugars was calculated from a spectrophotometric determination of sugars and these values may not be directly comparable.

Cassava peels contain sugars in the forms of polysaccharides such as starch and holocellulose. They should be converted to glucose or disaccharides (maltose or cellobiose) for yeast to utilize them efficiently. We used amylolytic yeast and enzyme mixture to saccharify the polysaccharides. The enzymes were added at suspension of the cassava peels powder after pretreatment. The total soluble sugar concentrations increased to 8.45, 9.67, and 10.78 g/l by the additions of the enzyme mixture to the cassava peels pretreated by distilled water, 0.025% NaOH and 0.1 M sulfuric acid, respectively. The enzyme mixture produced soluble sugars and worked more effectively than pretreatment alone. The maximum yield of the sugar concentrations resulted pretreatment by 0.1 M sulfuric acid and hydrolysis by enzyme mixture. Thus, we have used this condition to saccharify the cassava peels in this study. The concentration of total soluble sugars was lower than that of total polysaccharides in the cassava peel. A part of cellulose with a high crystallinity and hemicellulose would remain after the enzyme hydrolysis (Kim *et al.*, 1995). Most of the soluble sugars were glucose. The reaction mixture might contain xylose, maltose, cellobiose or unknown sugars after the enzymatic saccharification.

Table 1 Yield of reducing sugar from enzymatic hydrolysis of pretreated cassava peels.

Pretreatment	Initial sugars (g/l)	Sugars after enzymatic hydrolysis (g/l)	Sugar yield (g/g)
distilled water	1.06	8.45	0.56
0.025% NaOH	4.04	9.67	0.64
0.1 M sulfuric acid	5.17	10.78	0.72
0.1 M sulfuric acid [a]	5.23	4.86	0.32

[a] without enzymatic hydrolysis

Ethanol production by mono-culture and co-culture are summarized in Table 2. The ethanol production by SSF with *S. diastaticus* 2047 was higher than that of *S. cerevisiae* 7532. Fermentation cassava peels pretreated with 0.1 M sulfuric acid, produce maximum ethanol yield of 0.418 g/g dry cassava peels (Fig. 1 A3, Table 2) and substrate pretreated with distilled water (Fig. 1 A1) produced higher ethanol yield than that of with 0.025% sodium hydroxide (Fig. 1 A2). The lowest ethanol yield (0.177 g/g dry cassava peels) was produced by *S. cerevisiae* 7532 obtained from cassava peels pretreated with distilled water (Fig. 1 B1). Ethanol yield by co-culture indicated that cassava peels pretreated with 0.1 M sulfuric acid (0.441 g/g dry cassava peels) produced higher than that of cassava peels pretreated with distilled water (Fig. 2 C1, C2) and it represented for the highest ethanol yield production. So, *S. diastaticus* 2047 and *C. tropicalis* 5045 were appropriate for SSF with co- culture to enhancing the productivity of ethanol. From this study, it was shown that an increase of released fermentable sugars by enzyme increased the ethanol production through the yields of ethanol slightly affected.

Utilizution of cassava peel by mono-culture and co-culture

Results of cassava peel utilization for ethanol production in mono-culture are given in Fig. 1. Ethanol production in mono-culture was comparatively higher with the strain of *S. diastaticus* 2047 (22.4 g/100 g), than with the standard strain 7352 (19.2 g/100 g). Less remaining reducing sugars and glucose were also observed at the end of the fermentation with *S. diastaticus* 2047. Among the different chemical pretreatments of 0.1 M sulfuric acid was found to be optimum for both the strains of *Saccharomyces*, producing maximum ethanol in 18 and 36 h for *S. diastaticus* 2047 and standard strain 7352, respectively. So, the cassava peel substrate used was more suitable to the local strain of *S. diastaticus* 2047 than the standard strain. An ethanol yield of 103.7 % of the theoretical maximum was obtained with the *S. diastaticus* 2047 strain, whereas it was 67.3% with the standard strain, with cassava peel substrate in mono-culture. The results of cassava peel utilization for ethanol production in mono-culture are shown

in Table 2. Cassava peel pretreated with 0.1 M sulfuric acid with co-culture of *S. diastaticus* 2047 and *C. tropicalis* 5045, produced more ethanol than the cassava peel pretreated with water which neither mono-culture of *S. diastaticus* 2047 and *C. tropicalis* 5045. Pretreatment by diluted acid produced more ethanol than water pretreatment due to diluted acid damage the substrate structure for convenient to hydrolysed by enzymes more than water pretreatment. Consequently, fewer reducing sugars were observed with *S. diastaticus* 2047 than with the standard strain 7352. A 1.5 % concentration of the substrate was found to be optimum for both *S. cerevisiue* strains, producing maximum ethanol in 16 h. The *S. diastaticus* 2047 was more suitable for cassava peel utilization than was the standard strain of 7352. The diluted acid pretreated cassava peel gave more ethanol than did the water pretreated cassava peel, with both strains of *S. diastaticus* 2047 in co-culture with *C. tropicalis* and comparatively more left over sugars were accumulated after the fermentation with diluted acid pretreated cassava peel substrate. This might have been due to the damage cassava peel substrate susceptible to enzymatic hydrolysis and suitable for yeast growth in the diluted acid pretreated cassava peel substrate.

Table 2 Comparison of ethanol production in culture with mono-culture culture of *S. diastaticus* 2047 and co-cultures *S. diastaticus* 2047 and *C. tropicalis* 5045 at different cassava peels pretreatment

Process	Ethanol yield (g/g cassava peels) pretreated by		
	distilled water	0.025% NaOH	0.1 M sulfuric acid
SSF with mono-culture culture			
S. diastaticus 2047	0.374 ± 0.011	0.365 ± 0.023	0.418 ± 0.015
S. cerevisiae 7532	0.177 ± 0.019	0.178 ± 0.019	0.280 ± 0.008
SSF with co-culture *S. diastaticus* 2047 and *C. tropicalis* 5045	0.364 ± 0.004	-	0.441 ± 0.015

It was reported in a previous study by Yeon Woo Ryn *et al*. (1994) that 64.3 g/l of ethanol was produced, utilizing 94% of 150 g/l soluble starch, with a mixed culture of mutant M-6 *Schwannimnyces* castelli and *S. cerevisiae*. The starch content in the damaged grains used was lower by 30% and 40% when compared with fresh grains of sorghum (Rehm and Reed, 1996) and rice (Gopalan *et al*., 1996), respectively (Table 1). But as these cassava peels are cheaper than fresh grains, it would still be cheaper to utilize them for ethanol production in co-culture. However, efforts are on to improve the strain

of *S. diastaticus* 2047 to utilize more than 1.5% substrate concentration and to reduce the duration of fermentation. These results indicate that simultaneous saccharification and fermentation of cassava peel starch to ethanol can be conducted efficiently by using a co-culture of amylolytic yeast *S. diastaticus* 2047 and a non-amylolytic sugar fermenter, *C. tropicalis* 5045.

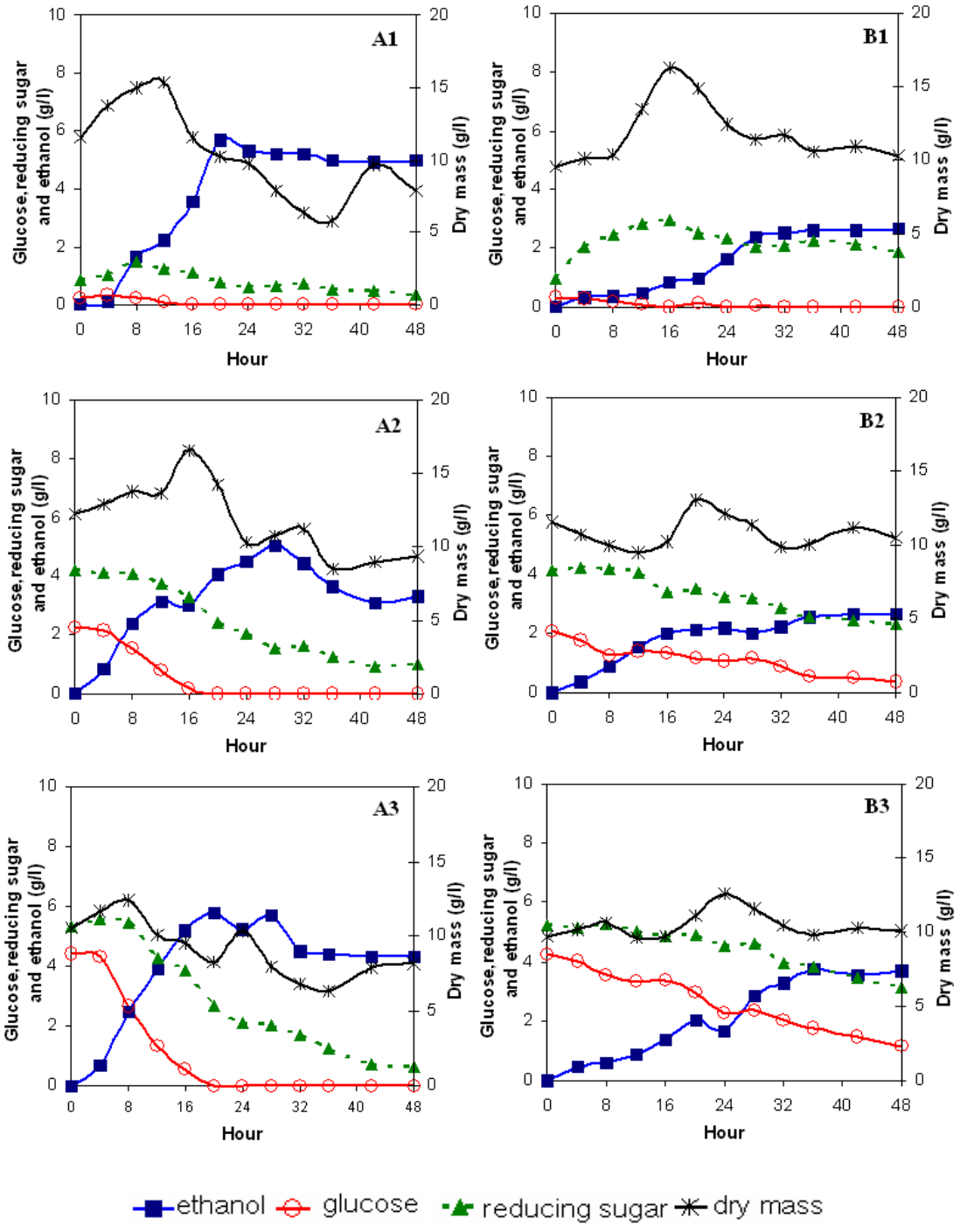

Fig. 1 Simultaneous saccharification and fermentation of cassava peels for ethanol production by *S. diastaticus* 2047 (A) and *S. cerevisiae* 7532 (B) with distilled water (1) 0.025% sodium hydroxide (2) and 0.1 M sulfuric acid (3).

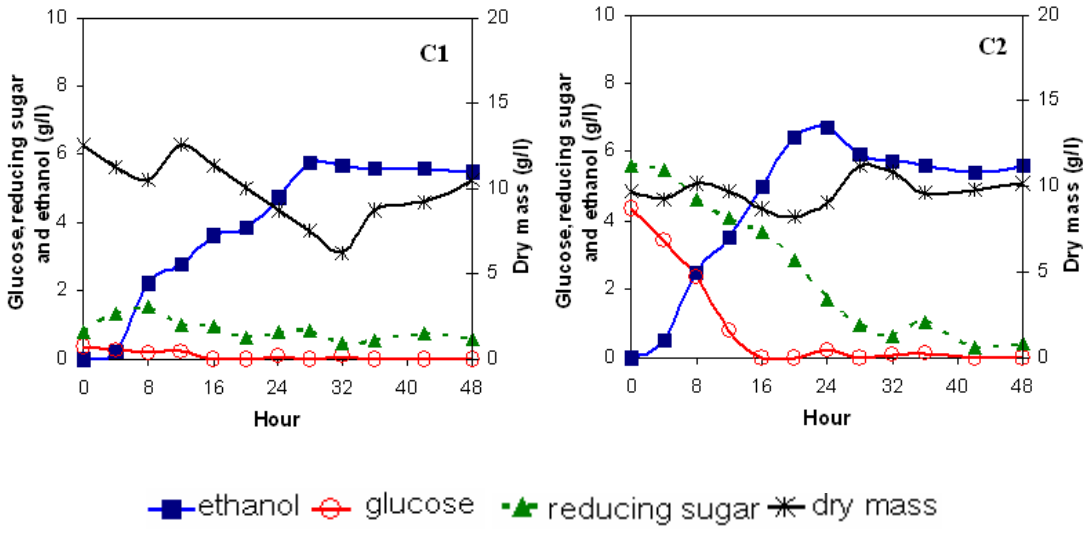

Fig. 2 Simultaneous saccharification and fermentation of cassava peels for ethanol production by simultaneous co-cultured of *S. diastaticus* 2047 and *C. tropicalis* 5045 (C) with distilled water (1) and 0.1 M sulfuric acid (2).

In this study, the performance of ethanol production from cassava peels using SSF process were carried out either with mono-culture of *S. diastaticus* or co-cultures with *C. tropicalis*. The ethanol concentration and productivity by mono-culture of *S. diastaticus* 2047 were approximately 2 folds higher than that of mono-culture of *S. cerevisiae* 7532. Co-culture of *S. diastaticus* 2047 and *C. tropicalis* 5045 could produce the highest ethanol yield from the cassava peels pretreated with diluted sulfuric acid. Pretreatment with diluted sulfuric acid had received some part of sugar prior enzymatic hydrolysate reaction. In this study, the maximum ethanol yield was 0.441 g/g dry cassava peels. This study showed that the cassava peel could be used in the fermentation with high ethanol yield, so it is possibly to be used as an alternative substrate of yeast fermentation for ethanol production. These co-cultures may have several industrial advantages, which can utilize glucose/xylose to ethanol. Effective ethanol production demands selection of suitable fermenting strains from among such diverse microorganisms depending on the biomass feedstock chemical composition.

REFERENCES

Adegbola, A.A., and V.O. Asaolu. 1986. Preparation of cassava peels for use in small ruminant production in Western Nigeria. *In*: Toward Optimum Feeding of Agricultural By-products to Livestock in Africa. Preston, T.R., Nuwanyakpa, M.Y. (Eds.), pp. 109-115. Proc. of Workshop held at the University of Alexandria, Egypt. Oct. 1985. ILCA, Addis Ababa, Ethiopia.

Biely, P. 1985. Microbial xylanolytic systems. Trends in Biotechnology. 3: 286-290.

Gropalan, C., B.V. Ramasastri and S.C. Balasubramanian. 1996. Nutritive Value of Indian Foods. National Institute of Nutrition (NIN), p. 47.

Kim, D.W., Y.K. Jeong, Y.H. Jang, J.K. Lee, K.S.Kim, H. Ryu. 1995. Kinetic mechanism of cellulose hydrolysis by endoglucanase I and exoglucanase II purified from *Trichoderma viride*. Bulletin Korean Chemical Society. 16: 742–747.

Larsen, L., P. Nielsen, and B.K. Ahring. 1997. *Thermoanaerobacter mathranii* sp. nov., an ethanol producing extremely thermophilic bacterium from a hot-spring in Iceland. Archives Microbiology. 168: 114-119.

Masami, O., I. Usui, and N. Urano. 2007. Ethanol production from the water hyacinth *Eichhornia crassipes* by yeast isolated from various hydrospheres. African Journal of Microbiology Research 2: 110-113.

Nartey, F. 1979. Studies on cassava cynogenesis, and biosynthesis of cynogenic glucoside in cassava (*Manihot* spp.), *In*: Nestel, B., MacIntyre, R. (Eds.), Chronic cassava toxicity, IDRC 010e: 73-87.

Obioha, F.C. and P.C.N. Anikwe. 1982. Utilization of ensiled and sun-dried cassava peels by growing swine. Nutr. Rep. Int. 26: 962-972.

Osei, S.A., M. Asiamah and C.C. Atuahene. 1990. Effects of fermented cassava peel meal on the performance of layers. Anim. Feed sci. Technol. 24: 295-301.

Rehm, H.J., Reed, G. 1996. A Multi-volume Comprehensive Treatise Biotechnology. Products of Primary Metabolism. VCU Verlags gesellschaft Mhh, Germany, vol. 6. p. 64.

Yeon, W.R., S.H. Ko, S.Y. Byun and C. Kim. 1994. Direct alcohol fermentation of starch by a derepressed mutant of *Schwanniomyces castelli*. Biotechnology Letter 16: 107-112.

Wyman, C.E. and B.J. Goodman. 1993. Biotechnology for production of fuels, chemicals, and materials from biomass. Applied Biochemistry and Biotechnology. 39/40: 41-59.