

# Renewable Utilization of Cassava Coat Solid Waste Using Fungal Enzyme Technology

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## ABSTRACT

Utilization of cassava is mostly concerned with the efficiency of producing starch and converting starch to sugars or other products through enzyme hydrolysis or fermentation. Following starch manufacturing, a large amount of solid waste remains, especially the peeled-off coat obtained in the first step. This paper showed that the overall utilization of cassava coat-waste through enzyme technology resulted in the beneficial regaining of sugars and value-added products from the remaining fiber. The enzymes used were glucoamylase and polygalacturonase that were obtained from the fungi *Aspergillus niger* J8 and *Rhizopus stolonifers* 26R, respectively, isolated in Thailand. The two enzymes were tested for their efficiency in digesting uncooked cassava starch. The production of the enzymes could be undertaken using cheap agricultural substrates. The two fungal enzymes showed a synergistic effect on raw starch digestibility of cassava coat-solid waste and of the whole cassava tuber, whereas a combination of the fungal glucoamylase with a commercial pectinase showed no such effect. An optimum ratio of polygalacturonase and glucoamylase at only 3:2 resulted in 115 and 301 mg of reducing sugars being released from 1 g of solid waste in 8 and 72 h, respectively, which was 3.3 times higher than with a commercial pectinase. The remaining non-starch fiber was processed into two value-added biomaterials, a charcoal enzyme-digested cassava fiber (EDCF) and a composite board. The EDCF was a good source of alternative energy and had a calorific value of 3,555 cal/g, which was comparable to charcoal made from corncobs and rice straw. By introducing the renewable utilization of cassava solid waste with enzyme technology produced in Thailand, cassava manufacture may gain more benefit not only from the value-added products, but also from using domestic microbial enzymes and technology which involve low cost investment.

**Keywords:** value-added products, cassava, *Rhizopus stolonifers*, polygalacturonase, *Aspergillus niger*, glucoamylase

## INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the main agricultural crops important for food and feed. Value-added products including hydrogen (Buranakarl *et al.*, 1985; Chitradon *et*

*al.*, 2008), ethanol (Ueda *et al.*, 1981; Lin and Tanaka, 2006), lactic acid (Anuradha *et al.*, 1999; John *et al.*, 2006), citric acid (Pandey *et al.*, 2000), fumaric acid (Carta *et al.*, 1998; Carta *et al.*, 1999) and high fructose syrup are obtained from microbial fermentation and enzyme utilization.

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The process of extracting cassava starch produces tonnes of cassava coat that has been peeled off the tuber. The solid waste still contains 60~70% starch per solid dry weight that is entrapped in complex fibrous structures of cellulose and non-starch polysaccharides, mostly pectin. With such a rich source of organic carbohydrates, renewable utilization of the solid cassava waste is of considerable interest.

From the viewpoint of saving time and energy, hydrolyzing raw starch without pre-gelatinization has shown promise (Ueda *et al.*, 1981; Buranakarl *et al.*, 1985, 1988; Ngmcharoenwong *et al.*, 2004; Mahakhan *et al.*, 2005; Chitradon *et al.*, 2008). Due to composition and structure of the cassava coat, amylolytic enzymes are inefficient in the utilization of the starch that is bound within the complex fiber in the cassava coat and the ground tuber. US Patent Application 20050100996 (2004), showed the success of using a multi-enzyme of  $\alpha$ -amylase with pullulanase and protease in enhancing starch utilization in corn solids (Lantero and Shetty, 2004). There has been considerable research on the utilization of cassava starch and pulp using enzyme technology to obtain fermented products (Buranakarl *et al.*, 1985, 1988; Lotong *et al.*, 1987; Chitradon *et al.*, 1992, 1993, 1996a, 1996b). However, little work has been carried out involving the cassava coat and non-starch carbohydrate fiber, thus resulting in this study to explore further beneficial uses of the biomass residue. The renewable utilization of biomass residue can help solve environmental problems and added value to the waste.

## MATERIALS AND METHODS

### Enzymes

An endopolygalacturonase (PG) of *Rhizopus stolonifera* 26R isolated in Thailand (Chitradon *et al.*, 1992, 1996a, b) was used. A high yield of the enzyme was produced from culture

grown on wheat bran, rice bran and rice husk (6:12:2). The enzyme has main activity in pectin degradation. The crude enzyme is stable to high temperatures up to 50~55 °C with an optimum temperature of 55 °C and it is stable to a wide range of pH from 3.5 to 8.0 with an optimum pH of 5.25.

A raw starch-digestive glucoamylase of *Aspergillus niger* J8 isolated in Thailand (Lotong *et al.*, 1987; Chitradon *et al.*, 1993) was used. A high yield of the enzyme was produced from culture grown on wheat bran and rice husk (18:2). The crude enzyme is stable to high temperatures up to 40-50 °C and a wide range of pH from 4.0 to 10.0. It exhibits maximum activity at 55 °C and pH 5.0.

Pectinex Ultra SP-L, a commercial pectinase from NOVO Industri, Irvine, Denmark was also used.

### Analytical methods

#### Pectinase activity

Pectinase activity was assayed by determination of diminishing substrate viscosity using an Ostwald viscometer, Agarwal Scientific Glass Industries, Nanhai, USA according to Deuel and Stutz (1958) at pH 5.25. One unit of enzyme was defined as the amount of enzyme that could reduce the viscosity of 1% pectin by 50% in 10 min.

#### Raw starch-digestive amylase activity

Raw starch-digestive amylase activity was assayed by determination of reducing sugars liberated from digesting raw starch at pH 5.0 using the modified Somogyi-Nelson method (Nelson, 1944). One unit of the enzyme was defined as the amount of enzyme that liberated 1 mmol of reducing sugars within 1 min. The starch remaining in the solid waste of cassava was determined with the iodide method (Neufeld and Ginsburg, 1966).

#### Protein determination

Protein content was determined followed

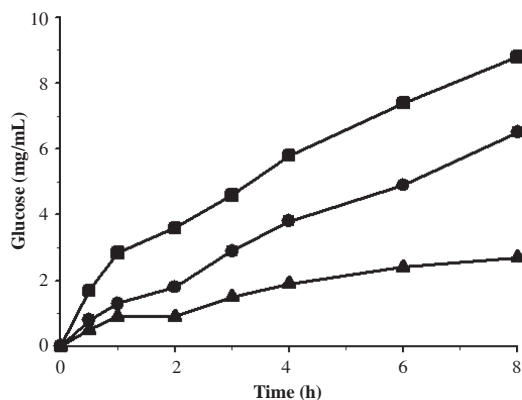
Lowry's method (Lowry *et al.*, 1951) with bovine serum albumin as the standard.

### Enzymatic hydrolysis of ground cassava tuber and peeled-coat of cassava

Uncooked ground tuber or peeled-coat was digested with a single enzyme and the mixed enzymes (pectinase:glucoamylase, 1:1, 2:2 or 3:2) in 8 h. The total units of the mixed enzymes was equal to the total units of single enzyme. The ratio of enzyme unit per gram of uncooked substrate was 1:1. The efficiency of the *Rhizopus stolonifera* 26R PG was compared with commercial pectinase.

### Value-added products of solid fiber that remained after using enzymes hydrolysis

Cassava coat, (10 kg) was digested with the mixed enzymes at different ratios. Solid fiber was separated and further utilized by pressing through machines to make charcoal and composite board. The properties of both value-added products were determined.

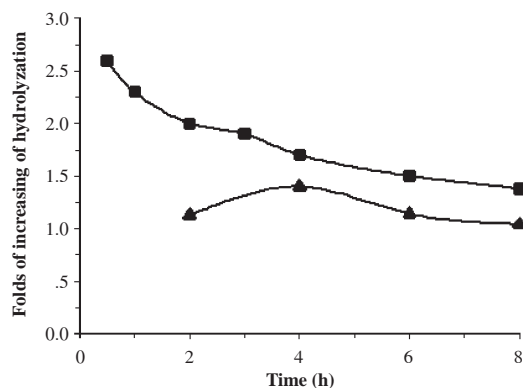


**Figure 1** Glucose liberated from reaction mixture of the enzymes with whole tuber of cassava, using one unit per gram of ground tuber at the same volume at 37 °C. ~▲~, *Rhizopus stolonifera* 26R polygalacturonase; ~●~, *Aspergillus* J8 glucoamylase; ~■~, the mixed enzymes in the ratio of 1:1.

## RESULTS

### Capability of digesting whole cassava tuber using *Rhizopus* PG, *Aspergillus* glucoamylase and the mixed enzymes

Adding *Rhizopus* PG to the glucoamylase helped liberate more reducing sugars (9 mg/mL) than using only *Aspergillus* glucoamylase (6.5 mg/mL) from 1 g tuber in 8 h (Figure 1). PG could enhance the amylolytic activity in the whole tuber by 1.33 times. This showed the synergistic effect of PG on glucoamylase raw starch activity, which have been due to PG polymerizing the pectin and pectic substances that are components of the cassava coat, which then released more starch granules from the tuber and resulted in increased starch digestion. When compared with the commercial pectinase, *Rhizopus* PG was more efficient (Figure 2). The highest rate of the mixed enzymes with *Rhizopus* PG was observed at 0.5 h, while that of the commercial pectinase was detected at 4 h. There was also a greater increase in hydrolyzation.



**Figure 2** Relative comparison of hydrolyzation of raw cassava starch from the whole tuber by the mixed enzymes of pectinase from *Rhizopus niger* 26R and glucoamylase from *Aspergillus niger* J8 (~■~) compared with the mixed enzymes of Pectinex Ultra SP-L and glucoamylase (~▲~).

### Digestion of cassava coat solid waste by the mixed enzymes

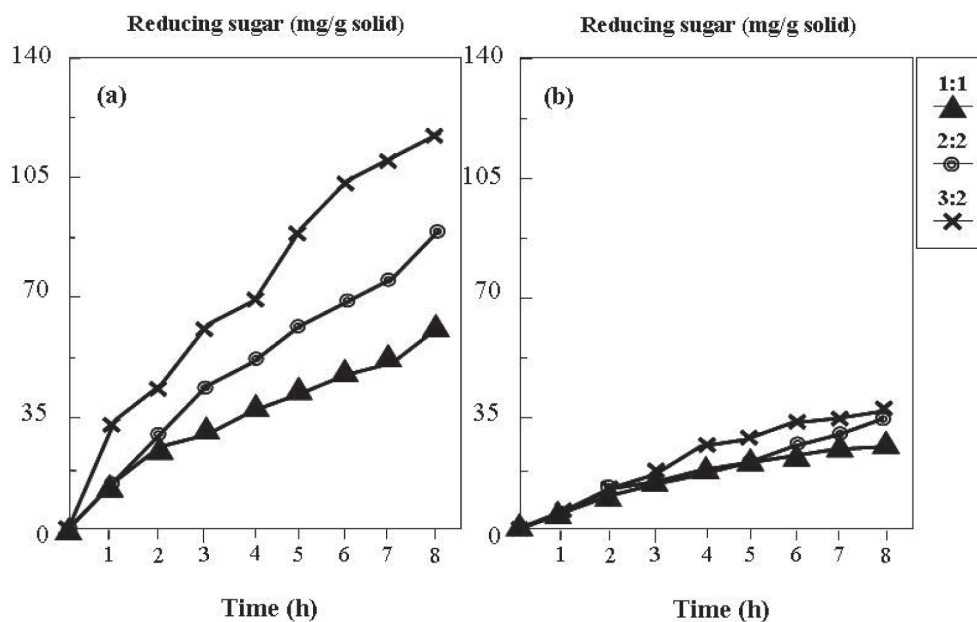
The mixed enzymatic digestion of uncooked coat waste in 8 h liberated 53 mg reducing sugars per gram of substrate (Figure 3a). Increasing amounts of both enzymes (2:2) per gram of substrate did not increase double the amount of reducing sugar. A synergistic effect could be clearly seen when the PG was increased to a ratio of 3:2, with a substantial increase to 116 and 301 mg reducing sugars per gram of solid waste in 8 and 72h, respectively. No synergistic effects were observed when the commercial Pectinex was used. Only 20~34 mg reducing sugars per gram of solid waste were detected in 8 h, even when the the ratio of the commercial pectinase was increased. Using *Rhizopus* PG and *Aspergillus* glucoamylase at the ratio of 1:1 and 3:2, was 2~3 and 4~5 times more efficient than the commercial pectinase, respectively (Figure 3b).

### Renewable products from solid fiber remaining after enzymatic utilization of cassava coat solid waste

The solid fiber that remained after enzymatic digestion was utilized in two value-added products, a charcoal, enzyme-digested cassava fiber (EDCF) and a composite board (Figure 4). EDCF had a calorific value of 3,555 cal/g that was comparable to the charcoal made from corncobs and rice straw (Table 1). The composite board had a fine appearance and good strength, with a possible use in furniture making and is of interest to the Board of Investment of Thailand (BOI). It was noted that less glue was used to make the composite board.

### DISCUSSION

Utilization of cassava is concerned mainly with the conversion of starch and pulp, but not with the peeled-off coat and the non-starch

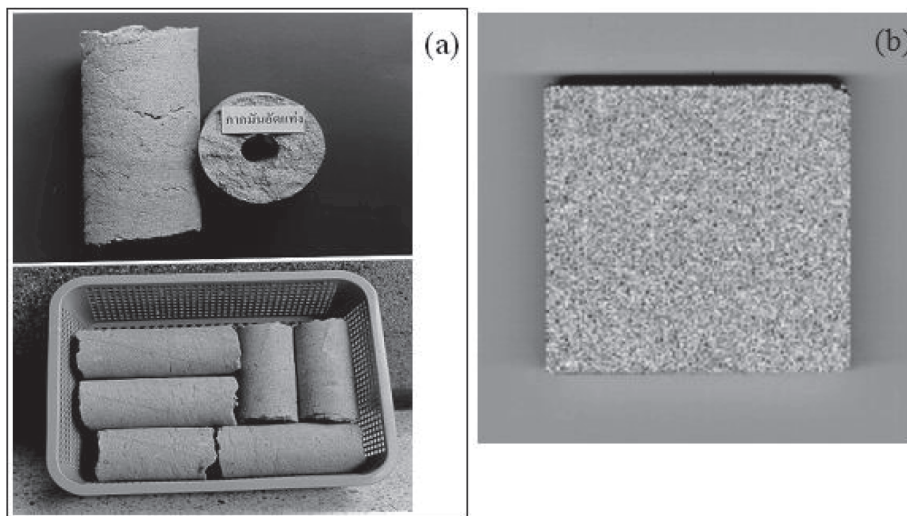


**Figure 3** Reducing sugars liberated from the digestion of raw cassava solid waste with the mixed enzymes of glucoamylase from *Aspergillus niger* J8 and pectinase from *Rhizopus stolonifera* 26R (a) or PectinexUltra SP-L, a commercial enzyme (b) at different ratios within 8 h. Ratio 1:1,  $\blacksquare$ ; 2:2,  $\circ$ ; 3:2,  $\blacktriangle$ .

fiber remaining in the solid waste. To the best of our knowledge, our search of the literature has not found any report on the renewable utilization of these solid wastes. Two fungal enzymes, glucoamylase and polygalacturonase, from fungi isolated in Thailand were used to utilize the raw starch in the solid waste. Advantages of the two enzymes are their capability to digest raw cassava starch and their stability over a wide pH range and at high temperature. High yields have been achieved from agricultural solid substrates (Lotong

*et al.*, 1987; Chitradon *et al.*, 1992, 1993, 1996a, 1996b), which have resulted in low cost investment and have been practical for Thai industry.

*Rhizopus* PG showed promising activity in enhancing the digestion of the cassava tuber and coat when used with *Aspergillus* glucoamylase. The *Rhizopus* PG had a synergistic effect on amyolytic activity. No such effect was observed when an imported commercial PG was used. Only a small amount of the two enzymes was effective. A combination of the two fungal



**Figure 4** Value-added products made from solid fiber waste remaining after processing with the mixed enzymes and cassava-coating solid waste: (a) EDCF; (b) Composite board.

**Table 1** Energy properties of enzymes-digested cassava fiber (EDCF) compared with other agricultural sources.

Property	EDCF	Firewood	Corncoobs	Sawdust	Rice straw
Calorific value (cal/g)	3555.00	4224.00	3851.00	2996.67	3740.00
Fixed carbon (%)	3.50	24.40	22.30	14.01	17.55
Moisture (%)	12.00	8.70	9.80	12.23	6.80
Ash (%)	2.60	0.69	4.80	22.29	20.55
Volatile matter (%)	81.90	62.00	62.80	51.40	63.55
Carbon (%)	38.80	41.80	36.40	22.90	-
Nitrogen (%)	0.24	0.17	0.52	0.60	-
Oxygen (%)	51.90	50.30	51.50	49.80	-
Sulfur (%)	0.02	0.05	0.14	0.57	0.16
Hydrogen (%)	6.50	7.00	6.60	5.60	-

enzymes in utilizing raw cassava starch and solid waste has not been reported in commercial applications, although there has been a report on the use of a multi-enzyme of  $\alpha$ -amylase with pullulanase and protease to enhance corn utilization (Lantero and Shetty, 2004). The present study is the first reported use of multi-enzymes that successfully enhanced the utilization of raw cassava starch, in particular directly from its tuber.

The non-carbohydrate fiber that remained after the enzyme digesting process was made into two value-added products, EDCF and a composite board. The EDCF proved to be a good source for alternative energy with a high calorific value comparable to corncobs and rice straw. The composite board had a practical use in furniture manufacturing involving a cheaper process using less glue. The present study produced a complete cycle of utilization of cassava, that benefited cassava starch manufacturing and solved environmental and energy problems.

### CONCLUSION

This study showed the success of using microbial enzyme technology in the renewable utilization of cassava waste. Using PG of *Rhizopus stolonifers* 26R and glucoamylase of *Aspergillus niger* J8 produced with microbial technology in Thailand, it was shown that the effective and renewable utilization of cassava solid waste from starch processing was possible. The two enzymes showed a synergistic effect on raw starch digestion that was not evident with the commercial pectinase. Since the fungi come from a natural resource, the enzymes that are capable of digesting raw starch could be used to reduce costs and time in the cooking step and produce a high yield using cheap agricultural substrates, while its high specific activities towards raw cassava meant that only a small amount of enzymes was needed per gram of waste. These benefits resulted in a low cost investment.

The importance of utilization of the cassava coating was not only that sugars were obtained from further fermentation, but also that there was a renewable utilization of the fiber that remained after enzyme digestion. The present study showed the possibility of producing value-added products from the final waste (composite board) and also to utilize it as a source of energy (EDCF), both with acceptable properties. Furthermore the technology utilized was developed in Thailand with a low cost of investment and so was plausible for an industrial application.

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