วารสารเทคโนโลยีชีวภาพ
Thai Journal of Biotechnology

ปีที่ 3 ฉบับที่ 1 กันยายน 2544
Vol. 3 No. 1 September 2001
ISSN 1513-3478

Bioconversion of Cassava Roots to High Protein Product for Animal Feed

Podjana Chumkhunthod[1], Sureelak Rodtong[2], Neung Teaumroong[1] and Nantakorn Boonkerd[1]*

[1]*School of Biotechnology, Institute of Agricultural Technology and* [2]*School of Microbiology, Institute of Sciences, Suranaree University of Technology, Nakhonratchasima 30000, Thailand*

This study was aimed at producing protein-enriched animal feed from cassava roots by the conversion of cassava using amylase-producing fungi. Mold and yeast which produce amylase were isolated from cassava wastes, khao-mak and various mold-brans (look-pang). It was found that the filamentous fungi strain no. SUT1 which most likely belongs to the genus Chlamydomucor was proved to be the best amylase producing strain. This fungi exhibited highest amylase activities at 2.32 units. Pretreatment of cassava was done by steaming and non-steaming. The cassava fermentation was conducted in solid state using urea as the nitrogen source. Under room temperature and uncontrolled pH, which stands commonly at between pH 5-7, steamed cassava was saccharified better than non-steamed cassava. Reducing sugars were obtained at 680.07 mg/g from steamed raw cassava after 5 days of fermentation when using inoculum in the form of look-pang. Then dry inoculum of mixed culture between Chlamydomucor SUT1 and Candida utilis was developed, it was found that the bacterial contamination was reduced in 5 log. The protein content from this fermentation condition which was amended with 1.0% urea was reached maximum at 18.3%. To reduce the production cost, non aseptic solid state fermentation in size of 540-L was recommended. After preliminary test, protein content could be obtained at 15.3% with composed of 11% amino nitrogen that was high enough to use for animal feed in further.

Keywords : Cassava, solid state fermentation, look-pang, khao-mak, protein-enriched feed

Introduction

Cassava is one of the most important root crops in the tropics. Thailand is one of the biggest cassava producers for the world. Such industrial applications include animal feed, alcohol and starch and food products such as tapioca and instant mixes. Besides export, it still remains abundant. Its level price over many years is impressive and makes it especially attractive as ones industrial raw material. Bacterial and fungal treatment has been used to enhance the value of crop and its residue (Charoensiri *et al.*, 1990; Fujio and Elegado, 1993 and Zeikus and Johnson, 1991). To utilize surplus cassava, cassava roots that are high in starch contents can be served as a carbon source and value added by fermentation over the human food and animal feed processing method. If protein content could be increased by microbial biomass, it could be used as animal feed, consequently the capital cost of animal feed could be reduced.

Cassava fermentation is very useful and could be developed to aid ensiling, reduce storage losses, increases its nutritional value by reduction of toxic compounds, better digestibility and the process can be done

* *corresponding authour : Tel (6644) 224752, Fax (6644) 224750, E-mail: nantakon@ccs.sut.ac.th.*

under non-aseptic conditions (Wolf, 1997).

SCP production and organic substance reduction in the effluent are the aims of most bioconversion processes. *Candida* species *C. utilis*, *C. arborea* and *C. tropicalis* are most successful for cell mass production (Balagopalan *et al.*, 1988 and Zeikus and Johnson, 1991). Although many reports demonstrated that biomass production by using solid state fermentation (Daubresse *et al.*, 1987; Yuthavong and Gibbons, 1994; Reade and Gregory, 1975 and Tani, Vongsuvanlert and Kumnuanta, 1986), the protein yield was still not high enough thus modification conditions of fermentation may be conducted to increase product yield. In recent years, there has been increasing interest in the use of solid state fermentation processes as alternatives to submerged fermentation such as batch, continuous and fed-batch fermentation etc. because it has lower energy requirements, produce less waste water and partly because of environmental concerns regarding the disposal of solid wastes (Lonsane and Ramash, 1990). Thus, solid state fermentation is considered to conduct in this study for bioconversion of cassava for biomass protein production.

This investigation was aimed at elucidation the extensive screening of potential amylolytic microorganism strains and developed high efficient dry inoculum preparation method of mixed cultures of combined between amylolytic strain and *Candida* species. This inoculum was used as starter cultures in food fermentation based upon starchy substrates such as rice and cassava roots. Solid state fermentation was also performed in saccharification process to enhance biomass protein yield in protein-enriched feed production.

Materials and Methods

Isolation and screening of starch utilizing strains

Microbial strains were isolated from cassava solid waste obtained from local factories, khao-mak and mold bran (look-pang). The starch utilizing microbial strains were isolated from the samples using a single colony isolation technique on cooked cassava starch agar medium (2% cassava starch, 0.3% yeast extract, 0.5% $(NH_4)_2SO_4$, 0.05% $MgSO_4.7H_2O$, 0.1% KH_2PO_4 and 1.5% agar; pH(6). The selected cultures were maintained on 1% starch agar slants for further screening. For the primary screening, the isolated microorganisms were cultured for amylase production by patching the cultures to raw starch agar plate surface and allowed them to grow for 3 days at 30°C. Amylolytic zones corresponding to enzymatic degradation of starch were detected by staining the plates with iodine solution (6.6g KI, 0.66g iodine in 165 ml distilled water). Colonies exhibited large clear zone were selected. The test strains were grown in 50 ml of starch broth containing 8% dried cassava, 0.2% $(NH_4)_2SO_4$, 0.025% $MgSO_4.7H_2O$, 0.05% KH_2PO_4, and 0.25% $CaCO_3$ by 200 rpm shaking at room temperature. Samples were collected after 36 hrs. After centrifugation, the supernatant was recovered as a source of secreted enzymes and frozen at -20°C until assayed. Amylase activities were assayed as described previously (Bernfeld, 1951 and Tan, Ferguson and Cariton, 1984). Specific activity was expressed as amylase activity per milligrams of protein. Protein is determined according to Lowry *et al.*, 1951. The selected strains were also cultured on starch agar slant for 72 hrs then 1 ml of cell or spore suspension which contained 108 cells or 108 spores/ml was inoculated into plate containing 50 g of autoclaved glutinous rice. Samples were collected after incubation everyday until 4 days. The extracellular amylolytic activity that produced during fermentation of glutinous rice was determined by measuring the amounts of reducing sugars produced from the substrate using DNS micro method (James, 1995). Physical properties and aroma changes were recorded. The best amylolytic strain was identified and used in further study.

Dry inoculum preparation (Look-Pang)

The selected strain was grown on 2% cassava starch slant at 30°C for 4 days. Before inoculation, 5 ml of distilled water was added then cell suspension was adjusted to approximately 10^8 cells/ml). To prepare the dry inoculum, rice was cleaned and then the moisture in the rice was allowed to equilibrate by tempering for several hours before further processing. The water was changed every hour to retard contamination of lactic acid bacteria. The grain was ground with a blender for 5-10 min. at high speed. The suspension was filtered through cheesecloth and starch was obtained after water removal. Several types of spices; licorice, cinnamon, rose scarlet, rhizome, garlic, ginger, long pepper, clover, galanga and pepper were used each at 0.1 percent of rice flour. Stiff dough was made from rice flours, spices, culture suspension and water. The dough was stored under room temperature for 2-3 hrs. After fermentation, the dough was formed into small loaves in diameters of approximately 1-1.5 cm, air-dried at room temperature. Inoculum balls were stored in paper bags and kept at 8°C before used as the inoculum for different trials. Shelflife of inoculum was always determined monthly by enumeration of viable cells.

Efficiency tests for cassava hydrolysation (lab scale)

The solid state fermentation was performed at room temperature in 250-ml beaker. 50 g of cassava cubes (1-1.5 cm^3) was added after treatment by steaming for 15 min. The inoculum powder at 0.4% was inoculated and mixed with substrates then incubated for a period of time. The fermentation product was determined for reducing sugars by DNS micro method to indicate amylase activity. For protein content, micro Kjeldahl method was carried out. To obtain the efficient fermentation before scale up in large scale using 540-L fermentor, the optimal conditions of fermentation were accomplished. Varying parameters such as types of substrate, urea concentration and inoculum sizes were tested in lab scale to find their effects on pH, moisture, reducing sugars, crude protein content or even amino nitrogen.

Biomass protein production (process scale up)

The large scale fermentation was conducted in solid state fermentor 540-L (60×150 cm, 60 cm in depth). Gelatinization was carried out by steaming for 15 min with 50 kg of processed whole fresh cassava root that was chopped into small cubes (3-3.5 cm^3). After gelatinization, it was left to cool then the nitrogen source solution of 1% urea and powder of dry inoculum were mixed by hand. Inoculum of mixed culture dry inoculum of *Chlamydomucor* SUT1 and *C. utilis* was added at 4% each. The cassava cubes were spread out in uniform 10-12 cm thick layer on the tray. Then the fermentation was undertaken in non-sterile conditions (pasteurization), non pH and temperature control for a period of time. Ventilation for this fermentation system could provide by opening the lid (natural convection). This was also sufficient to permit removal of excess heat that generated from culture's metabolism in the fermentor. Changes in protein, pH, moisture, reducing sugars and amino acids were measured everyday during the fermentation. The fermented cassava product was put out to sun dry on a tray before blended into flour for the purpose of animal feed production.

Results and Discussion

Screening of amylolytic culture strains

From 122 isolates of pure culture, it was found

*Table 1. Screening of starch hydrolysis strains by conventional method (Fermented rice making)

Culture strain	Hr	Appearance of fermented glutinous rice						
		Visible growth of culture	Softening	Starchiness		Odor		Reducing sugars (mg/g)
Standard	24		+	Dry		Fermented	++	
(Typical mold bran)	48		++	Wet	+++	Fermented	+++	
	72		++++	Wet	++++	Fermented	++++	425
Mold SUT1	24		-	-	-	-	-	
	48		++	Wet	++	Fermented rice	++	
	72		++++	Wet	++++	Fermented rice	++++	560
Rhizopus spp.	24	/ Gray spores mycelium	+	Wet	+	Fermented	+	
	48	/ full plate	+	Wet	+	Fermented rice	++	
	72	/ full plate	+	Wet	+	Fermented rice	++	325
Saccharomycopsis fibuligera	24		+	Wet	++	-	-	
	48		++	Wet	+++	Fermented	+++	
	72		+++	Wet	+++	Alcohol	++	85
Endomycopsis fibuligera	24		-	Wet	++	Fermented	+	
	48		++	Wet	+++	Fermented	+++	
	72		+++	Wet	+++	Alcohol	++	70

++++ high, +++ medium, ++ little, + very little, - no change

Table 2. Amylase activity of culture in 8% cassava broth

Culture Strain	Total Protein (mg)	Amylase	
		Total act. (U)	Specific act. (U/mg)
Mold SUT1	0.0800	2.32	31.08
Rhizopus spp.	0.0450	0.55	19.07
Penicillium sp.	0.0575	1.39	28.53
Endomycopsis fibuligera	0.0500	0.97	25.09
Saccharomycopsis fibuligera	0.0240	1.70	87.08

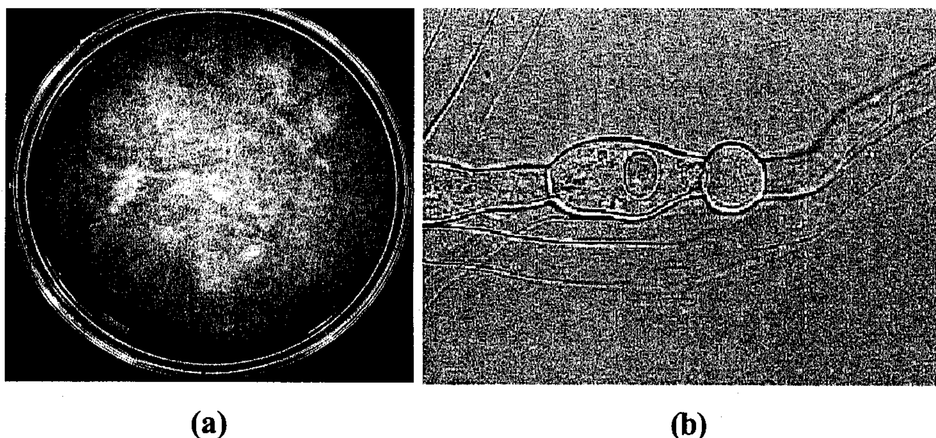
U = 1 unit of amylase defined as the amount of enzyme required to liberate 1μmole of maltose per min at 30°C, pH 6.9

that only 37 strains were able to grow on cassava starch agar medium and gave clear zone of starch hydrolysis when iodine test was performed. All of the selected strains were tested for their capability of starch hydrolysis by preliminary fermentation test with glutinous rice. Table 1. show results from starch hydrolysation test by conventional method (fermented rice making) compared with typical fermented rice (khao-mak) and also pure culture inoculation of mold and yeast that has been reported high abilities in amylase production as *Rhizopus spp.*, *Saccharomycopsis fibuligera* and *Endomycopsis fibuligera.*

The results showed clearly that mold SUT1 gave highest reducing sugars in fermented rice at 560 mg/g after pure suspension was inoculated in sterile plate containing 50 g of sticky rice after 3 days incubation. Appearance of fermented rice from pure culture of mold SUT1 inoculant was similar to typical fermented rice. When comparing with reference strains by observation of physical, chemical and microbiological changes of the product, for example; softening, starchiness and odor that are parameters showed efficiency of starch hydrolyzation, mold SUT1 could liquefy starch much faster than the others. Appearance changes of product were not occurred at the first day of incubation but starch hydrolysis showed clearly after two and three days of fermentation. The product was soften, wet and have smell like khao-mak such as acidity, alcoholic odor etc. and reducing sugars was detected in high level more than typical mold bran which used as standard about 24% higher but extremely higher than *Saccharomycopsis fibuligera* almost reached 88%.

From the Table 2, amylase activities were studied by culturing strains in cassava broth. Mold SUT1 performed highest amylase activities. Maximum total activity was detected at 2.32 units. Even specific amylase activity did not highest but less than *Saccharomycopsis fibuligera.* Mold SUT1 was still be the most interested because the total activity was higher that make it was considered to use in cassava fermentation.

When the filamentous fungal strain was observed by microscopy (Fig.1). It was found that mold SUT1 is in-group of non-septate mycelium fungal, spores are scattered on mycelium. Strain of this mold varies from ones without sporangia and possed only chlamydospores to one that produces considerable numbers of *abortive-type* sporangia that identified as *Chlamydomucor.* This genus had also been reported that found and plays important role in khao-mak manufacture (Chatisatiern, 1978; Pichyangkura and Kulprecha, 1977).



(a) **(b)**

Fig. 1. Morphology of Chlamydomucor SUT1 (a) Chlamydomucor SUT1 on PDA after 2 days incubation, (b) spores and mycelium of Chlamydomucor SUT1 under microscope (400X)

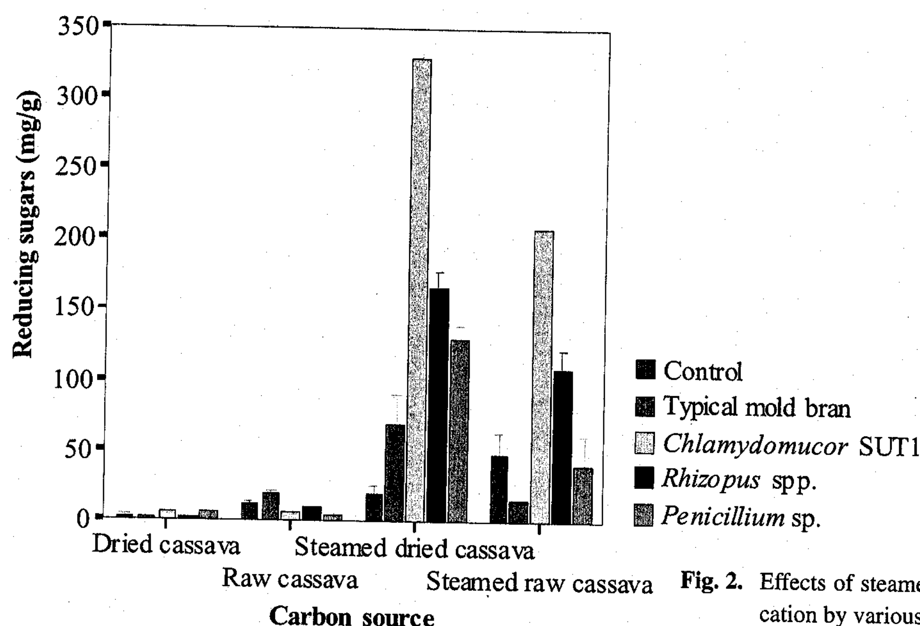


Fig. 2. Effects of steamed and non steamed cassava on saccharification by various strains after 4 days of fermentation

Effect of substrate and its pretreatment for conversion of cassava into glucose (saccharification)

Raw and dried cassava which had initial moisture content (before treatment) at 45-50% and 10-12%, respectively were used as substrates. The effect of raw and cooked starch on saccharification was studied by measurement of reducing sugars production obtained after 4 days cassava fermentation, steaming of raw materials was very effective to cassava utilization by microorganisms. Initial bacteria could be reduced in high amount by steaming for 15 min. This was useful for inoculated culture to grow in non-sterile conditions because competitive state was decreased at initial. Reducing sugars in non-steamed materials was very low (Fig.2). The maximum reducing sugars were produced at 327.44 mg/g dry wt. after 4 days incubation in steamed dried cassava by pure culture of mold SUT1. For steamed raw cassava, reducing sugars were increased much slower and reached 207.3 mg/g dry wt. at 4th day cultivation. From this part we can concluded that steamed cassava were suitable substrate in solid state fermentation of cassava than non-steamed materials.

Dry inoculum preparation

For dry inoculum preparation, it was revealed that after dry inoculum of mixed culture of *Chlamydomucor* SUT1 and *C. utilis* was developed, it showed higher efficiency than single culture dry inoculum of *Chlamydomucor* SUT1. Culture number of *Chlamydomucor* SUT1 could be detected in higher numbers at 106 CFU/g and cell numbers of *C. utilis* were obtained at 9.45(107 CFU/g in the inoculum. Very lower level of bacterial contamination was detected. This might be due to only single starch utilizing of mold SUT1 grew in the carrier (look-pang), some reducing sugars were accumulated and able to promote growth of other microbes. In contrast, when mixed cultures of *Chlamydomucor* SUT1 and C. utilis were developed, after the mold hydrolyzed starch, the yeast could use remaining glucose as carbon source for biomass production that promoted the lack of nutrient condition for other microbial contaminants. This was not only useful for overcoming the problem of contamination but *C. utilis* also beneficially provided some alcohol that enhanced flavor as well as protein yield of product at the same time. Another reason that could explain was the yeast *C. utilis* could reproduce themselves rapidly than mold. It performed high capacity in competition with bacteria contamination that resulted in 5.97 log CFU/g reduction of bacteria in look-pang.

Saccharification efficiency of Chlamydomucor SUT1- brans

For cassava fermentation with two types of substrate; steamed raw and steamed dried cassava, it was clearly shown that highest efficiency was obtained in steamed raw cassava (Fig. 3). Reducing sugars were assumed as oligosaccharides and simple sugars such as glucose and maltose (Gerhartz, 1990) and they were reached maximum at 680.07 mg/g dry wt. after 5 days of fermentation. While using steamed dried cassava as carbon source, maximum-reducing sugars produced at 380 mg/g dry wt. at 3 days after inoculation. Look-pang produced from single culture of *Chlamydomucor* SUT1 gave

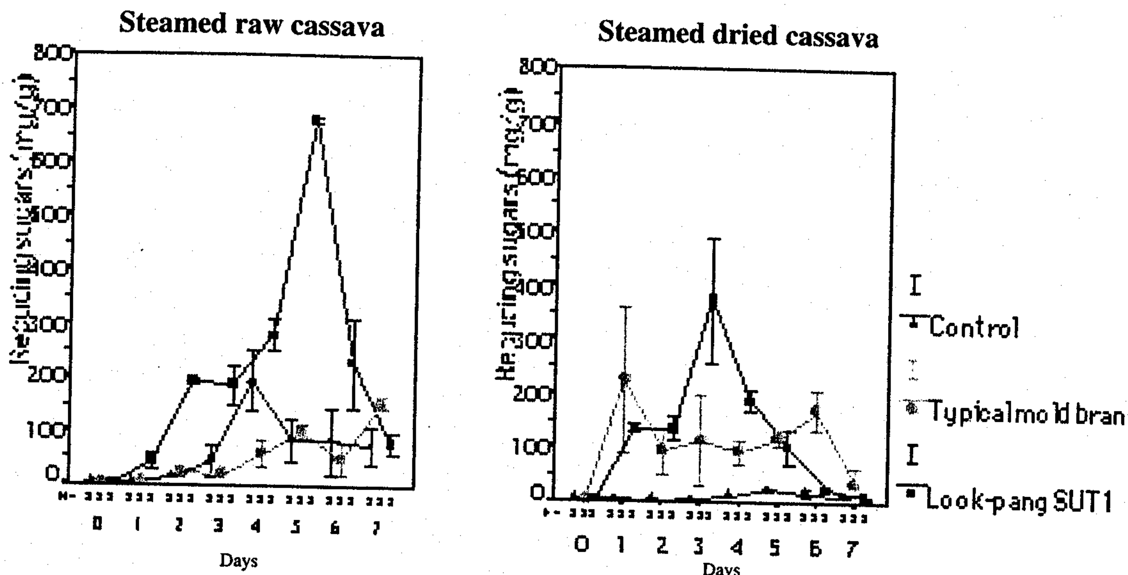


Fig. 3 Efficiency of dry inoculum *Chlamydomucor* SUT1 on saccharification of steamed raw and steamed dried cassava

higher efficiency than using pure culture. It was possible that when the microorganisms were growing in look-pang, they produced and released amylase. The retained enzyme in the look-pang could perform starch hydrolysis activity immediately at initial stage of fermentation (Lotong, 1992). These promoted saccharification.

If emphasize on types of substrate for look-pang SUT1, steamed raw cassava was better than steamed dried cassava due to raw cassava that prepared from whole fresh cassava root by chopping and steaming supposed to have lower impurities material than steamed dried cassava (steamed cassava chips). The steaming condition unable to get rid of all bacterial contaminant from initial so after fermentation all contaminants could reproduce themselves as fast as inoculated culture. Therefore, reducing sugars were lower in steamed dried cassava. Product from steamed raw cassava was appeared in white, soft texture, sweet and alcohol aroma as the previously report (Beuchat, 1991 and Chatisatienr, 1978).

Biomass protein production by solid culture of Chlamydomucor SUT1 and C. utilis

Results obtained from optimization conditions could be concluded that steamed raw cassava was the most suitable as carbon source and urea was considered to be the best nitrogen source at concentration of 1% (data not shown) by using dry inoculum of mixed cultures that necessary for further production.

Cassava fermentation was preliminary conducted in lab scale by using simple process, non-controlled pH and room temperature (30-35(C) incubation with initial moisture of 63%. The protein content of cassava that generally is about 2 to 3% dry wt. was raised to a maximum of 18.3% on basis of dry mass (Fig. 4).

This might be due to the loss of total solids and the synthesis of protein by the microorganisms (Cronk et al., 1977) after 6 days of cultivation. Almost all reducing sugars in the product were utilized by that time. Without pH control, pH level was slowly decreased from initial pH at 6.6 and maintained at pH 5.6 to 5.8 that was suitable for yeast growth. During fermentation the moisture was slowly increased, however, it remained at 60% to 75% throughout experiment. During the rapidly grown culture, crude protein content were reached maximum at 6th day and gradually decreased at the end of fermentation. Reducing sugars were observed at very low level less than when single strain inoculum was performed. This was due to the results of mixed culture that C. utilis could use reducing sugars while the starch was been hydrolyzing. It was also possible that steaming could be only partially hydrolyzed starch resulted in low starting sugars that were detected at initial stage. During the fermentation period the cassava was soften and a sweet/sour alcoholic flavor was developed.

Growth of culture during fermentation was also observed (Fig. 5). The results performed that *Chlamydomucor* SUT1 grew up rapidly in the first 3 days of the fermentation then its numbers were decreased at the 4th day. That correlated to the results of reducing sugars changed during running batch fermentation (Fig.4). These indicated that *Chlamydomucor* SUT1 was the culture that produced reducing sugars during the saccharification

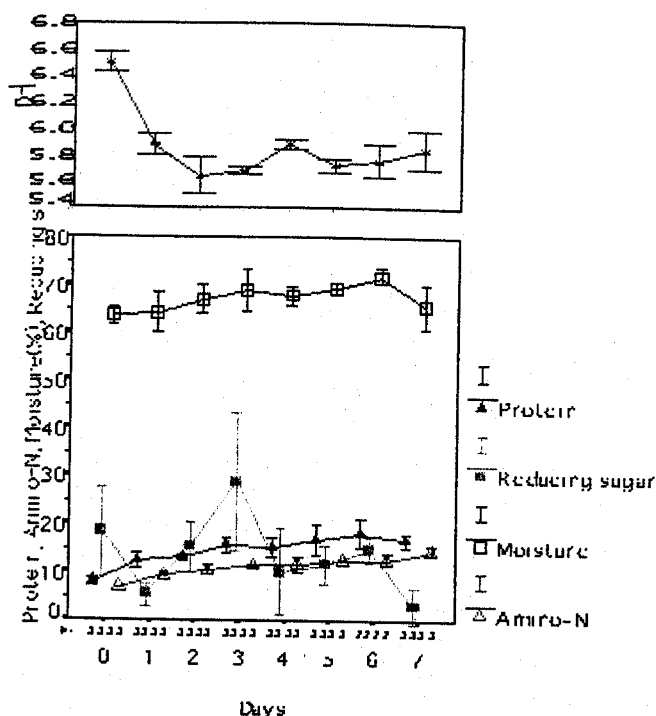


Fig. 4 Changes in reducing sugars, protein, amino nitrogen, pH and moisture content with time obtained from using 1% urea in beakers with 50 g steamed raw cassava by dry inoculum of *Chlamydomucor* SUT1 and *Candida utilis* at room temperature.

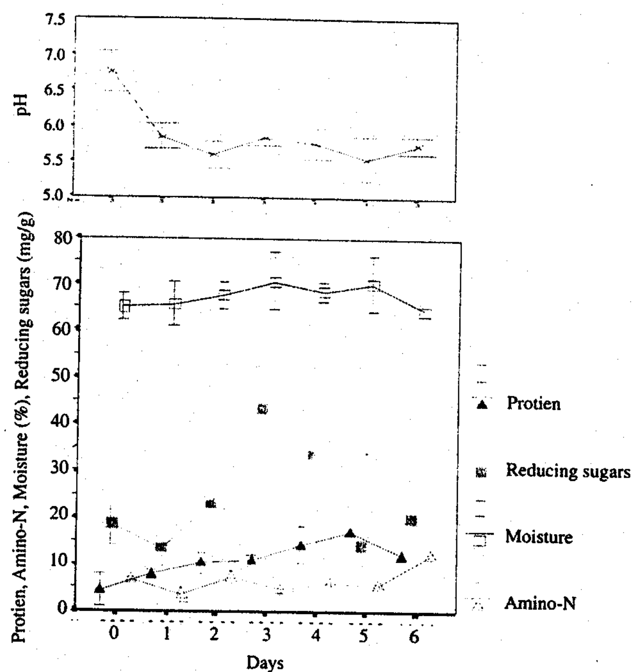


Fig. 6 Changes in reducing sugars, protein, amino nitrogen, pH and moisture content with time obtained from using urea concentration at 1% in 540-L fermentor with 50 kg steamed raw cassava by dry inoculum of Chlamydomucor SUT1 and Candida utilis at room temperature.

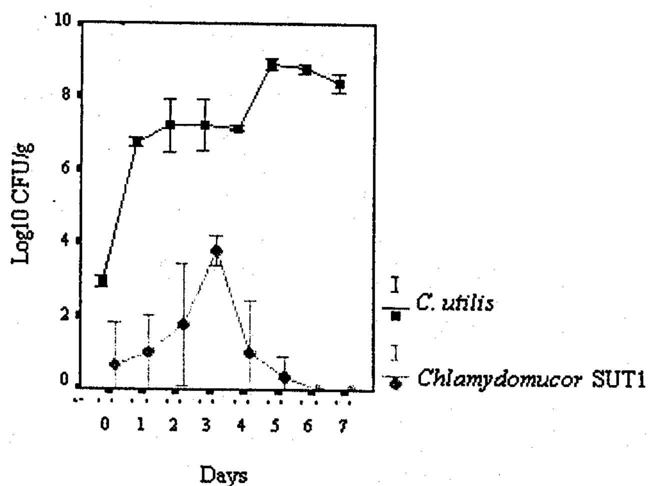


Fig. 5 Growth curve of *Chlamydomucor* SUT1 and *C. utilis* during cassava fermentation

which was following by the growth of *C. utilis* in biomass production. For *C. utilis*, the initial cell number was detected at 2.91 log CFU/g. It was increased and the maximum cell number was obtained at 8.90 log CFU/g at the 5th day of fermentation. This was responded to biomass cell that related to increasing crude protein yield in batch culture.

The results clearly suggested that mixing of dry inoculum powder before cultivation time could well promote more homogeneity of cultures which was directly effect on increasing crude protein content. The conversion of cassava starch to crude protein by mixed culture strains of *Chlamydomucor* SUT1 and *C. utilis* was comparable to that crude protein content level in similar published systems (Zvauya and Muzando, 1994; Reade and Gregory, 1975) and higher than that was reported by other authors who used raw cassava in solid state fermentation by culturing *Rhizopus* able to increase protein from 1.75-11.3% (Soccol *et al.*, 1994). Another solid state fermentation that cassava was processed by moistening, steaming and mixing with nutrient solution containing urea, KH_2PO_4, $MgSO_4.7H_2O$ the protein could be increased to 10.7% (Daubresse *et al.*, 1986). The method used in this research was more simply and less costly cost because no moisture adjustment, no pH control and cheap nitrogen source. Moreover, dry inoculum using in this study was proved to be the appropriate starter for bioconversion of cassava since only 0.4% of inoculum could be converted starch into almost 20% protein.

The biomass production was further evaluated in 540-L fermentor. The 1% of urea was added at the initial of batch culture with 6 days of incubation period as presented in a typical time course (Fig. 6).

The inoculum was increased from 0.4% to 4% to achieve the development of *Chlamydomucor* SUT1 and *C. utilis*. Fermentation was performed without controlling pH. Although the raw cassava had not been sterilized, no development of contaminant microorganisms was observed from its appearance and total plate count.

Changes in reducing sugars, protein, pH and moisture were observed in the same pattern of the experiments obtained from lab scale. The bioconversion was preceded and the highest reducing sugars were obtained at 32.37 mg/g dry wt. in the 3rd day and they were completely utilized after 5 days of incubation time. This activity correlated with crude protein yield that was reached at the same time that reducing sugars were depleted. At the end of fermentation amino nitrogen and crude protein content were in the same level. It could be notified that the last day of fermentation, almost all organic and inorganic substances such as urea was converted into organic substances like amino nitrogen indicated that protein converted from cassava. It was interested to find that after using dry inoculum of mixed culture coupled with 1% urea concentration which was optimized from lab scale, the maximum protein content was detected faster than expected at 15.3% on a dry basis which was composed of 11% amino acid in the 5th day. In general, the fermentation without pH control, the culture pH was usually rapidly falling below a level that the organism could tolerate. In contrast with this batch fermentation that pH was slightly dropped and remained at pH between 5.5-6.0. This was very beneficial because it was the optimal condition for fungal growth. This was possibly due to the use of urea as nitrogen source resulted in maintaining pH level. These encourage results make it possible to envisage numerous applications of mixed culture dry inoculum as starter to reduce contamination and shorten time of traditional food fermentation in solid substrate processes. For moisture content, almost 70% moisture was in the product indicating liquefaction of starch hydrolysis efficiency.

In conclusion, this work has demonstrated that the regular mold flora from look-pang in Thailand are *Chlamydomucor* SUT1, the same genus as found in fermented rice in other countries. It had high efficiency in glutinous rice as well as in cassava hydrolysation than others. Dry inoculum of mixed culture of *Chlamydomucor* SUT1 and *C.utilis* promoted fast starch hydrolysis in saccharfication that achieved high efficiency in biomass protein production. It was proved to be the appropriate tool for bioconversion of cassava since only 4% of dry inoculum could convert starch into higher crude protein in large scale that high enough to use as enriched protein feed for animal.

Acknowledgements

We would like to thank Korat Cassava Flour Industrial for supporting materials. We also thank Miss Apinya Rattanajit for her technical assistance. This research was fully supported by Suranaree University of Technology.

References

1. Balagopalan, C., Podmaja, G., Nanda, S. K. and Moorthy, S. N. (1988) Cassava in Food, Feed, and Industry. CRC Press Inc, Boca Raton.
2. Bernfeld, P. (1951) α, β-Amylase, pp. 379-428. *In* Nord, F.F. (ed), Advances in enzymology, Vol 12. Acadamic Press, New York.
3. Beuchat, L. R. (1991) Indigeneous fermented foods, pp. 507-543. *In* Rehm, H.-J., Reed, G., Puhler, A. and Stadler, P. (eds) Biotechnology: A multi-volume comprehensive treatise, Vol 9. Weinheim; VCH, New York.
4. Charoensiri, K., De-eknmkul, C., Assavaning, A., Varavinit, S. and Bhumiratana, A. (1990) Biomass protein produce from cassava using *Cephalosporium eichhorniae* 152 grown in an air-lift fermentor. Microbial. Utiliz. Ren. Res. 7, 330-335.
5. Chatisatienr, C. (1978) Selection of mold and yeast strains in Look pang for Khaomak Fermentation. M.S. Thesis. Kasetsart University, Thailand.
6. Cronk, T. C., Steinkraus, K. H., Hack, L. R. and Mattick, L. R. (1977) Indonesian tape ketan fermentation. Appl. Environ. Microbiol. 33, 1067-1073
7. Daubresse, P., Ntibashirwa, S., Gheysen, A. and Meyer, J.A. (1987) A process for protein enrichment of cassava by solid substrate fermentation in rural conditions. Biotechnol. Bioeng. XXIX, 962-968.
8. Fujio, Y. and Elegado, F.B. (1993) Food-processing enzymes from Rhizopus strains. The 5th Annual Meeting of Thai Society for Biotechnology. Bangkok, 25-27 November.
9. Gerhartz, W. (1990) Enzymes in industry production and applications. VCH, New York.
10. James, C. S. (1995) Analytical chemistry of foods. Blackie A&P, London.
11. Lonsane, B. K. and Ramesh, M. V. (1990) Production of bacterial thermostable α-amylase by solid-state fermentation: a potential tool for achieving economy in enzyme production and starch hydrolysis. Adv. Appl. Microbiol. 35, 1-47.
12. Lotong, N. (1992) Fermented food staters and processing technology. Deparment of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand.
13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.

(1951) Protein measurement with Folin phenol reagent. J. Biol. Chem. 193, 265-275.

14. Pichyangkura, S. and kulpeecha, S. (1977) Survey of mycelial molds in look pang from various sources in Thailand. Symposium on indigenous fermented foods, Bangkok, Thailand.

15. Reade, A. E., Gregory, K. F. (1975) High temperature production of protein. Enriched feed from cassava by fungi. Appl. Microbiol. 30, 897-904.

16. Soccol, C. R., Marin, B., Raimbault, M. and Lebeault, J. -M. (1994) Breeding and growth of *Rhizopus* in raw cassava by solid state fermentation. Appl. Microbiol. Biotechnol. 41, 330-336.

17. Tani Y., Vongsuvanlert, V. and Kumnuanta, J. (1986) Raw cassava strach-digestive Glucoamylase of *Aspergillus* sp. N-2 isolated from cassava chips. J. Ferment. Technol. 64, 405-410.

18. Tan, K. H., Ferguson, L. B.. and Cariton, C. (1984) Conversion of cassava starch to biomass. Carbohydrate, and acids by *Aspergillus niger*. J. Appl. Biochem. 6. 80-90.

19. Wolf, G. (1997) Traditional products, pp. 3-11. In Anke, T. (ed), Fungal biotechnology. Chapman & Hall, London.

20. Yuthavong, Y. and Gibbons. G. C. (1994) Biotechnology for Development: Principles and practice Relevant to Developing Countries. National science and technology development agency, Thailand.

21. Zeikus, G. and Johnson, E. A. (1991) Mixed cultures in biotechnology. McGraw-Hill, New York.

22. Zvauya, R. and Muzando, M.I. (1994) Some factors affecting protein enrichment of cassava flour by solid state fermentation. Lebensmittel. Wissenschaft und-Technologie. 27, 590-591.