Journal of Animal and Veterinary Advances 9 (22): 2859-2862, 2010
ISSN: 1680-5593


# Protein Enrichment of Cassava Pulp Using Microorganisms Fermentation Techniques for Use as an Alternative Animal Feedstuff

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**Abstract:** This study was aimed to evaluate the optimal condition for improving protein content of cassava pulp through microbial fermentation under the various urea condition for further use as an animal feed. Cassava pulp were fermented with each pure strain of *A. oryzae*, *S. cerevisiae* or *C. utilis* using urea as Nitrogen (N) source (0, 0.25, 0.5, 0.75, 1.0 and 1.25%) for 7 days. Reducing sugar, crude protein, amino N and moisture were measured daily. Chemical analysis revealed that there was a significant increase ($p<0.05$) in the reducing sugar, crude protein and amino N of fermented cassava pulp compared to unfermented. Fermented cassava pulp with *A. oryzae* was found to enhance higher protein and amino N contents than *C. utilis* and *S. cerevisiae*. In which, the optimal condition to produce highest biomass of *A. oryzae* were 0.75% urea and fermented for 4 days this condition can be improved protein and amino N from 2.59 and 0.89% (unfermented) to 17.4 and 15.13%, respectively. It is suggested that the use of cassava pulp fermented with *A. oryzae* at 0.75% urea for 4 days can improve protein and amino N by up to 17.4 and 15.13%, respectively which would subsequently provide a good feedstuff for animals.

**Key words:** Cassava pulp, fermentation, *Aspergillus oryzae*, *Saccharomyces cerevisiae*, *Candida utilis*, improved protein

## INTRODUCTION

Thailand is currently the leading exporter of tapioca starch in the world and tends to have the productivity increase to reach 4 million ton per annum in the near future (TTSA, 2010).

Cassava pulp is the solid moist by product of cassava starch manufacture and it represents approximately 10-15% of the original root weight. Therefore, when cassava starch production increases, a large volume of by-product is also generated.

Cassava pulp which is composed of 70% starch is a valuable product to use as a feed for livestock. However, cassava pulp is extremely low in protein and high in fiber contents which limit its use in animals.

Khempaka *et al.* (2009) reported that dried cassava pulp can be used up to 8% in broiler diets, the higher inclusion levels resulted in decreased growth performance and nutrient digestibility.

Chauynarong *et al.* (2009) also reported that the major limitation of using cassava root meal in animal feed because of its low protein content and deficiency of essential amino acid. Therefore, it would be more valuable if this by-product is fermented with microorganisms to improve its nutritive value prior inclusion into animal diets.

An increase in the feed value of cassava pulp could be obtained by increasing its protein content through microorganism fermentation, Oboh *et al.* (2002) reported that cassava fermentation with *Aspergillus niger* can increase the protein content from 4.4-12.2%. In addition, the cultivation of microorganisms such as *A. fumigatus*, *A. niger*, *A. oryzae*, *A. arborea*, *S. cerevisiae*, *C. utilis* and *C. tropicalis* on low protein content feedstuffs has also been widely reported in the previous studies (Reade and Gregory, 1975; Chumkhunthod *et al.*, 2001; Oboh, 2006).

The objective of this study was conducted to evaluate the optimal conditions for improving protein content of cassava pulp through *A. oryzae*, *S. cerevisiae* and *C. utilis* fermentation under the various urea concentrations.

## MATERIALS AND METHODS

Fresh cassava pulp obtained from Korat Flour Industry Co., LTD, Nakhon Racthasima, Thailand was used in this study.

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**Microorganisms and inoculum preparation:** Three strains of microorganisms as follows: *Aspergillus oryzae* (3019), *Saccharomyces cerevisiae* (EC1118) and *Candida utilis* (5046) obtained from the Thailand Institute of Scientific and Technological Research (TISTR) were used in this study. *A. oryzae* was maintained on Potato-Dextrose-Agar (PDA) medium (HiMedia, India). The slants were grown at 30°C for 3 days and stored at 4°C. *S. cerevisiae* and *C. utilis* were maintained on Yeast-Malt-Agar (YMA) (HiMedia, India). Batch cultures were agitated on reciprocal shaker at 200 rpm at 30°C for 1 day and stored at 4°C. Prior inoculation microorganisms to substrate, the spores of *A. oryzae* were dislodged from PDA slant culture using 0.85% NaCl and diluted into $10^8$ cell $mL^{-1}$ under sterile condition. While the suspension of *S. cerevisiae* and *C. utilis* were centrifuged at 3000 rpm for 15 min at 4°C and the deposit were washed twice with 0.85% NaCl. The resulting cells were resuspended in 0.85% NaCl to obtain an approximate concentration of $10^8$ cells $mL^{-1}$.

**Fermentation procedure:** Three factors (microorganism, urea concentration and fermentation time) were performed to investigate the optimum condition for improving nutrient composition of cassava pulp by fermentation process. About 50 g of fresh cassava pulp was taken into a 250 mL Erlenmeyer flask and autoclaved at 121°C for 15 min. Nitrogen (N) source from urea at different levels (0, 0.25, 0.50, 0.75, 1.00 and 1.25%) was added to each flask. About 1 mL of each pure strain of *A. oryzae*, *S. cerevisiae* and *C. utilis* suspension (approximately $10^8$ cells $mL^{-1}$ of each) was submerged into substrate. Then thoroughly mixed and covered with aluminum foil before subsequently allowing fermented at 30°C for 7 days. The fermentation products were collected daily and used for chemical analysis.

**Sample analysis:** Crude protein and moisture contents of samples were determined according to AOAC (1990). The reducing sugars were determined using Dinitrosalicylic Acid (DNS) method (Miller, 1959). Amino nitrogen was measured as described by TISO (1983).

**Statistical analysis:** Data were analyzed as a CRD using repeated measurements in a factorial 3×6×8 with microorganism, urea concentration and fermentation time as the first, second and third factors, respectively (SPSS, 2004). The differences between means were determined by DUNCAN.

## RESULTS AND DISCUSSION

Cassava pulp contains low amount of protein and high fiber contents, it is therefore important to enhance its nutritive value to provide a valuable feed ingredient prior fed to animals. In this study, we performed the trail in order to evaluate the capability of *A. oryzae*, *S. cerevisiae* and *C. utilis* when inoculated to ferment with cassava pulp at various urea concentrations (0, 0.25, 0.50, 0.75 1.0 and 1.25%, respectively) for 7 days. The results show that reducing sugar content of cassava pulp had reached a maximum at 418 mg $g^{-1}$ after fermented with *A. oryzae* at urea level of 0.25% for 3 days and subsequently tended to decrease at the end of fermentation period (Fig. 1). While reducing sugar of cassava pulp fermented with *S. cerevisiae* and *C. utilis* remained very low at all of urea levels and fermentation times ($p>0.05$) (Fig. 2, 3). In general, microorganisms have a wide ranging capability to produce enzyme degradation starch into sugar and glucose (Sun *et al*., 2009). In this regard, fungal cellulase and amylase, particularly from *Aspergillus* species is widely used for the commercial enzyme production. Recently, *A. oryzae* has been reported to yield cellulase

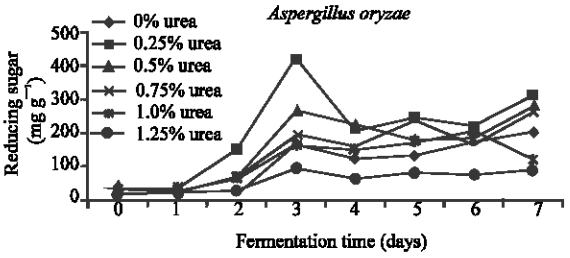


Fig. 1: Reducing sugar content of cassava pulp fermented with *A. oryzae*

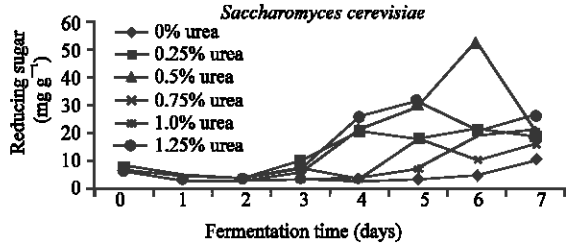


Fig. 2: Reducing sugar content of cassava pulp fermented with *S. cerevisiae*

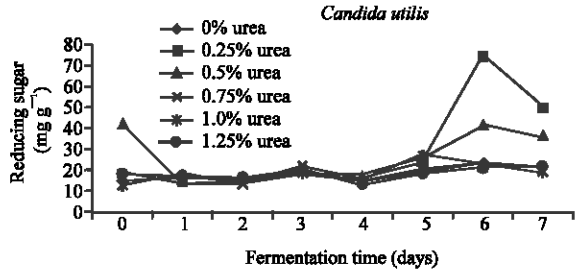


Fig. 3: Reducing sugar content of cassava pulp fermented with *C. utilis*

activity (Begum *et al*., 2009) which it is an important enzyme required for the catabolism of cellulose into smaller sugars. In addition, *A. oryze* also has an excellent capacity of α-amylase production under solid state fermentation using spent brewing grains (Francis *et al*., 2002) and wheat bran (Sivaramakrishnan *et al*., 2007). Therefore, with the highest reducing sugar content of cassava pulp fermentation with A. oryzae may indicate the capability of *A. oryzae* on producing enzymes, especially cellulase and amylase to hydrolyte glucosidic linkages in polysaccharide better than *S. cerevisiae* and *C. utilis*.

When considering the protein and amino N of cassava pulp fermentation produced from *A. oryzae*, *S. cerevisiae* and *C. utilis*, it was found that these contents were changed accordingly with the factors of microorganism, urea level and fermentation time. The results of chemical analysis revealed that protein and amino N contents of fermented cassava pulp were higher than unfermented ($p<0.05$). This phenomenon was due to the effects of microbial cell growth process (Belewu and Babalola, 2009) and N source from urea. Even the research literature on cassava pulp fermentation with microorganisms are not sufficient available, however a lot of information of fermented cassava has been widely reported.

Chumkhunthod *et al*. (2001) reported that cassava root fermented with *C. utilis* can increase crude protein up to 18.3%. In the study, we found that the highest level of crude protein produced from *A. oryzae*, *S. cerevisiae* and *C. utilis* were 18.11% fermented for 1 day at 1.25% urea, 21.21% fermented for 6 days at 1.25% urea and 19.18% fermented for 2 days at 1.25% urea, respectively.

However, this protein enhancement was included with a part of N from urea which is considered as a non-protein N and not useful for non-ruminant animals. In addition, it also has been stated that amino N is responsible for the true amount of cell growth in biomass production more efficient than crude protein.

From this measurement, it can be conclude that the optimum conditions for *A. oryzae*, *S. cerevisiae* and *C. utilis* growth in cassava pulp to perform the highest biomass were 0.75% urea fermented for 4 days, 0.5% urea fermented for 6 days and 1.25% urea fermented for 5 days, respectively.

In these conditions, *A. oryzae*, *S. cerevisiae* and *C. utilis* can produce protein and amino N from 2.59 vs. 0.89% (unfermented) to 17.4 vs. 15.1 and 10.0 vs. 7.58% and 16.82 vs. 9.2%, respectively. Over all, when all data was statistically tests, interaction of microorganism, urea level and fermentation time were found. It is laborious

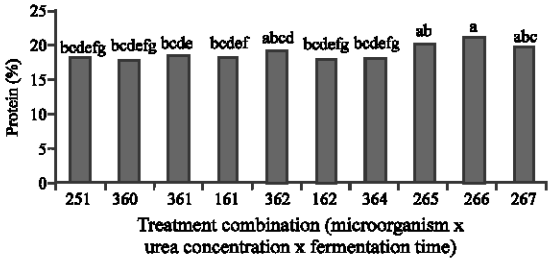


Fig. 4 : Protein contents of cassava pulp fermented with *A. oryzae*, *S. cerevisiae* and *C. utilis* at various urea concentrations during fermentation for 7 days, *Microorganism: 1 = *A. oryzae*, 2 = *S. cerevisiae*, 3 = *C. utilis*, Urea concentration: 1 = 0%; 2 = 0.25%; 3 = 0.5%; 4 = 0.75%; 5 = 1.0%; 6 = 1.25%, Fermentation time: 0 = day 0; 1 = day 1; 2 = day 2; 3 = day 3; 4 = day 4; 5 = day 5; 6 = day 6; 7 = day 7

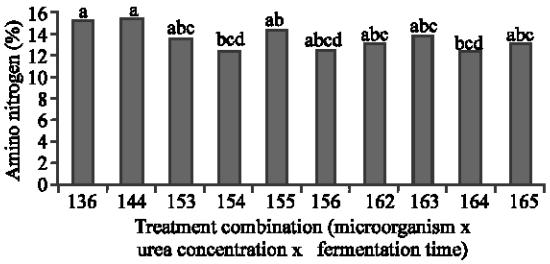


Fig. 5: Amino nitrogen contents of cassava pulp fermented with *A. oryzae S. cerevisiae* and *C. utilis* at various urea concentrations during fermentation for 7 days, *Microorganism: 1 = *A. oryzae*, 2 = *C. utilis*, 3 = *S. cerevisiae*, Urea concentration: 1 = 0%; 2 = 0.25%; 3 = 0.5%; 4 = 0.75%; 5 = 1.0%; 6 = 1.25%, Fermentation time: 0 = day 0; 1 = day 1; 2 = day 2; 3 = day 3; 4 = day 4; 5 = day 5; 6 = day 6; 7 = day 7

to demonstrate all of the interaction effects therefore, we represent only top ten of the best results of crude protein and amino N contents performed through fermentation process (Fig. 4 and 5). From Fig. 4 and 5, >1 condition did not show any significant difference to conclude the results.

## CONCLUSION

We have considered several factors in making a decision for obtaining the best beneficial outcome such as low cost and safe for animals. *A. oryzae* appeared to be more efficient by improving nutrient content of cassava pulp when compared to *S. cerevisiae* and *C. utilis*. This

could be attributed to the ability of *A. oryzae* to secrete cellulase and amylase enzymes into cassava pulp during fermentation process in an attempt to make use of the cassava starch as a carbon source. Apart from this, the increase in the amount of the microbial biomass in the form of single-cell proteins may possibly account for the increase in the protein content of the *A. oryzae* fermented cassava products (Akindahunsi *et al*., 1999).

Additionally, this result was according with the reducing sugar produced from *A. oryzae* which showed the higher quantity than *S. cerevisiae* and *C. utilis*. Therefore, these particular results can be conclude that the optimum condition of *A. oryzae* to perform the highest biomass were 0.75% urea fermented for 4 days which can produced crude protein and amino N 17.4 and 15.13%, respectively. It is suggested that the use of cassava pulp fermented with *A. oryzae* at 0.75% urea for 4 days can be improved protein and amino N from 2.59 and 0.89-17.4% and 15.13%, respectively which would subsequently be a good feedstuff for animals.

## ACKNOWLEDGEMENT

This research is supported by the Suranaree University of Technology with Korat Cassava Flour Industrial for supporting materials.

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