SOLID STATE CULTIVATION OF THE SELECTED FUNGUS STRAINS FOR PROTEIN INCREMENT IN FRESH CASSAVA PULP

Titima Panchai[1*], Kamlai Laohaphatanalert[2], Paiboolya Gavinlertvatana[2], and Klanarong Sriroth[3]

Received: July 26, 2017; Revised: December 07, 2017; Accepted: December 13, 2017

Abstract

Solid state cultivation of the 3 selected fungal strains, *Aspergillus* sp. CASP01 and UN03, and *Trichoderma* sp. CASP02, was performed to increase the protein content in fresh cassava pulp. The fungi were isolated from cassava pulp, and exhibited over-production of cellulase, amylase, and pectinase. For increasing protein content in fresh cassava pulp, the addition of 1 of these 4 nitrogen sources, namely soybean meal, yeast extract, ammonium sulfate, and urea, at the equivalent concentration of 2% nitrogen, enhanced growth of *Aspergillus* sp. CASP01 and UN03, and at 1% nitrogen stimulated growth of *Trichoderma* sp. CASP02. Soybean meal was selected as the most suitable nitrogen source. From solid state cultivation of the selected 3 fungal strains in a packed bed bioreactor using fresh cassava pulp (600 g with 85% moisture content and 2% original crude protein) supplemented with soybean meal, *Aspergillus* sp. UN03 provided the highest protein increase (13.36% crude protein yield) after 36 h cultivation. The achieved results reveal the promising process for feed production and the management of solid waste in a value-added bio-product.

Keywords: **Cassava pulp, solid state cultivation, high protein content, *Aspergillus* sp., *Trichoderma* sp.**

---

[1] *Faculty of Biotechnology, Rangsit University, Pathum Thani 12000, Thailand. E-mail: titima.p55@rsu.ac.th*

[2] *Thai Orchids Lab Co., Ltd., Khannayao, Bangkok 10230, Thailand.*

[3] *Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok 10900, Thailand.*

[*] *Corresponding author*

Introduction

Cassava pulp is a by-product of the cassava starch industry produced from cassava root. Cassava (Manihot esculenta Crantz) is one of the most important industrial crops and is grown in several regions in Thailand. The production and yield were estimated to increase from 31.16 million tons in 2015 to 31.19 million tons in 2016 and 3437 kg to 3611 kg which were increases of 2.06% and 5.40%, respectively (Office of Agricultural Economics, 2016). About 17.16 million tons of cassava root are mainly used in the cassava starch industry and an enormous amount of solid waste (15-30% of processed root dry-basis) is generated (Chauynarong *et al*., 2015). Cassava pulp has become the major portion of the solid residue that is disposed of, approximately 5.15 tons per year (Ghimire *et al*., 2015). The fresh cassava pulp is usually difficult to dry due to its high moisture content and organic load which make the pulp spoil rapidly through microorganisms and it creates a bad odor resulting in an environmental problem (Sriroth *et al*., 2000). Currently, cassava pulp is classified as waste by the Department of Industrial Works, Thailand, and the regulations are that the manufacturer must not be in possession of the waste on the manufacturer's site for more than 90 days (Trakulvicheana *et al*., 2013). Therefore, there has recently been an urgent need to explore the most efficient way to utilize fresh cassava pulp into any value-added product.

Since cassava pulp still contains a high amount of some nutrients, for instance, starch (53.55-79.45%), fiber (4.84-13.59%), and trace amounts of protein, there is a lot of interest in utilization of cassava pulp as a cheap carbon source for the bioconversion process as well as for animal feed and fertilizer (Hermiati *et al*., 2011; Khempaka *et al*., 2009). However, with a high fiber and low protein content, the use of cassava pulp as a feedstuff for monogastric animals such as pigs and poultry is limited (Khempaka *et al*., 2014). Thus, it is necessary to add some protein-rich supplements such as soybean meal to improve its nutritional value. In the past few years, the animal feed industry has expanded greatly, resulting in it being more and more dependent on imported soybean meal. In 2017, the demand for imported soybean meal is set to increase by 8.65% and its price is also extensively high which has an adverse impact on the cost of animal feed (Khempaka *et al*., 2009; Thai Feed Mill Association, 2017). To reduce the feed production cost, a fermentation process is considered as an alternative tool to increase the protein content and fiber digestibility for livestock feed (Oboh, 2006).

Solid state fermentation (SSF) is one of the promising techniques for increasing protein in a solid or semi-solid substrate; it is not expensive and is easy to handle. Cultivation of microorganisms such as *Aspergillus* niger, Rhizopus oligosporus, and *Trichoderma* reesei on low protein material in solid state is widely known (Okpako *et al*., 2008; Yazid *et al*., 2017). Fungal fermentation is an attractive method to produce a single cell protein due to: (1) a fast growth rate, (2) a comparably good nutrition value, (3) provision of a high protein level, and (4) an inexpensive bioprocess (Iyayi and Losal, 2001). There are several reports of using SSF on various agro-wastes including dried cassava pulp with different fungus strains and the results support that this technique can elevate the protein level (Iyayi and Losal, 2001; Tesfaye *et al.*, 2013). Fermentation of sweet potato with Saccharomyces sp. IFO 1426 combined with a nitrogen supplement increased the protein content from 6.01% to 14.04% (Yang, 1988). For increasing protein in cassava pulp, several reports have focused on dried pulp rather than fresh pulp (Khempaka *et al.*, 2009; Thongkratok *et al.*, 2010). Thus, the understanding of enhancing the protein level in fresh cassava pulp is barely known.

A packed bed bioreactor consisting of a column of a cylindrical cross section, with a perforated base plate on the bottom which supports a bed of substrate could be applied for cultivating some fungal strains to increase protein in fresh cassava pulp. The bioreactor air is blown up through the base plate. In tray and drum-type bioreactors, the heat and mass

transfer effect is limited by conduction, convection, and diffusion. This limitation may be substantially overcome by forced aeration through the substrate bed in a packed bed bioreactor (Mitchell *et al*., 2006). The objective of this study was to increase the protein content in fresh cassava pulp using solid state cultivation of fungus strains isolated from cassava pulp which produce plenty of lignocellulase and amylase enzymes (Punhya and Laohaphatanalert, 2013).

Materials and Method

Raw Material

Fresh cassava pulp was supplied by Chorchaiwat Industry Co., Ltd. (Chonburi, Thailand). It contained 85.44% moisture, 16.34% crude fiber, 0.60% crude protein, 2.02% ash, and 58.35% starch. The fresh pulp was dried overnight at 50°C, then ground and passed through an 80 mesh sieve.

Broken milled rice used for inoculum suspension was purchased from a local market in Pathum Thani, Thailand.

Microorganisms and Inoculum Preparation

Aspergillus sp. CASP01 and UN03 and *Trichoderma* sp. CASP02 were isolated from fresh cassava pulp (Punhya and Laohaphatanalert, 2013) and maintained on potato dextrose agar (PDA) consisting of sweet potato, dextrose, and agar at 20, 2, and 1% (w/w). For inoculum preparation, the fungal strains were grown on a PDA slant for 7 days, then their spores were dislodged from the PDA surface using 0.05% Tween 80 under sterile conditions to enable approximately 1×108 spores mL^{-1}.

One hundred and twenty-five g of broken milled rice was soaked in water for 30 min and then autoclaved at 121°C for 15 min. The sterile medium was cooled down to room temperature and put in a plastic bag. Ten mL of the spore suspension (1×10^8 spores mL^{-1}) were inoculated into the sterile broken milled rice and incubated at room temperature for 5 days prior to use.

Solid State Cultivation of Fungi

Solid state cultivation of the selected fungi was performed in an aluminum circle tray for optimization of cultivation conditions and a packed bed bioreactor for increasing the fresh cassava pulp volume. The aluminum circle tray was constructed of clear aluminum with a diameter of 9 inch and a height of 2 inch. The packed bed bioreactor was an acrylic sheet with a length of 10 inch and an internal diameter of 6 inch, and the base plate had a height of 2 inch from the bottom.

Batch Experiment

The SSF process was performed using cassava pulp as a substrate under sterilized and non-sterilized conditions to compare the fungal capability to produce protein and to reduce energy consumption for large-scale processing. These conditions were used to evaluate the optimal media for the SSF. For the aluminum circle tray (9-inch diameter), fresh cassava pulp (300 g) was sterilized at 121°C for 15 min, and the inoculum at 10% (w/w, 3.5×10^6 spores g^{-1}) were mixed thoroughly to compare with the non-sterilized cassava pulp, and incubated on the aluminum circle tray (9-inch diameter) at room temperature for 7 days. The crude protein and moisture contents of all samples were analyzed.

Effect of Concentration of Urea as a Nitrogen Source on Protein Increment

Fresh cassava pulp (300 g), urea at 1, 2, 3, and 4% (w/w of nitrogen, N), and inoculum at 10% (w/w, 3.5×10^6 spores g^{-1}) were mixed thoroughly and incubated on the aluminum circle tray (9-inch diameter) at room temperature for 14 days. Thereafter, the crude protein was analyzed for all samples.

Effect of Different Types of Nitrogen Source on Crude Protein Content

Fresh cassava pulp (300 g), nitrogen (N) sources (inorganic nitrogen: urea and ammonium sulfate [$(NH_4)_2SO_4$], and organic nitrogen: yeast extract and soybean meal at 1, 2, 3, and 4% (w/w) of (N), and inoculum (10% w/w, 3.5×10^6 spores g^{-1}) were mixed

thoroughly and incubated on the aluminum circle tray (9-inch diameter) at room temperature for 14 days. Thereafter, the crude protein was analyzed for all samples.

Cultivation of the Selected Fungus Strains in Packed Bed Bioreactor

Fresh cassava pulp (600 g), soybean meal (2% N), and inoculum (10% w/w, 3.5×10^6 spores g^{-1}) were mixed thoroughly and incubated on the packed bed bioreactor at room temperature for 48 h. The samples were collected after cultivating for 12 h and every 6 h, and mixed every 12 h. Thereafter, the crude protein of all samples was analyzed.

Analytical Methods

The moisture content of the cultivated cassava pulp was determined by drying at 105°C overnight (method number 925.1, AOAC, 1995). The crude protein content was established directly by the Kjeldahl method using a conversion factor of 6.25 (method number 991.2, AOAC, 1995).

Aflatoxin analysis of the cultivated cassava pulp was determined with a DOA-Aflatoxin ELISA Test Kit (Thai Global Complex Co., Ltd., Bangkok, Thailand) performed by the Postharvest and Processing Research and Development Division, Kasetsart University, Thailand. The test kit was applied for the rapid detection of both the qualitative and quantitative aflatoxin B1 (AFB1) using a polyclonal antibody against the AFB1, according to the manufacturer's recommendation. AFB1-HRP (Horseradish Peroxidase) conjugate was used as the direct competitive test.

Statistical Analysis

All experiments were carried out in triplicate. Data were interpreted by one-way analysis of variance (ANOVA) using SPSS 21 software. The statistical significance was evaluated at the $p<0.05$ level. Duncan's multiple range test was applied for the mean comparison.

Results and Discussion

Solid State Cultivation of Fungi in Aluminum Circle Tray

Sterile and non-sterile cassava pulp with inoculation from 3 fungal strains was designed for enhancing the protein level of fresh cassava pulp using the SSF process. The purpose of testing on non-sterile cassava pulp arises from its advantage of no energy requirement for sterilization and simplicity to scale up. After 7 days of cultivation, the cassava pulp samples were harvested for crude protein and moisture analyses.

The moisture contents of the samples at 7-day cultivation were slightly lower than the originals ranging from 0.25-7.86% (Table 1). It was also found that all fungal strains showed their potential to increase protein levels in fresh cassava pulp. The increase of protein in the non-sterile fermented products was substantially higher than those obtained from

Table 1. Crude protein and moisture contents of fresh cassava pulp (300 g in aluminum circle tray, 9-inch diameter) after growth of different fungal strains for 7 days at room temperature

Substrate/Fungal strain	Moisture content (% w/w)		Crude protein content (% w/w)	
	0 day	7 days	0 day	7 days
Sterile cassava pulp:				
Aspergillus sp. CASP01	74.15±0.11	67.05±0.15^{e}	3.83±0.00	5.51±0.21^{d}
Aspergillus sp. UN03	76.10±0.31	69.65±0.18^{d}	4.05±0.26	5.60±0.09^{cd}
Trichoderma sp. CAS02	77.83±0.53	75.88±0.02^{b}	5.62±0.00	6.70±0.34^{b}
Non-sterile cassava pulp:				
Aspergillus sp. CASP01	73.36±0.55	73.11±0.19^{c}	3.98±0.27	6.41±0.23^{bc}
Aspergillus sp. UN03	75.72±0.11	67.86±0.88^{e}	4.36±0.03	4.87±0.33^{d}
Trichoderma sp. CASP02	79.93±0.12	78.73±0.10^{a}	4.08±0.10	7.88±0.12^{a}

Mean values with different superscripts within the same column are significantly ($p<0.05$) different.

the sterile ones. *Trichoderma* sp. CASP02 cultivated on non-sterile media recorded the highest increase of crude protein level of 3.80% (from 4.8 to 7.88%, w/w) followed by *Aspergillus* sp. CASP01 of 2.43% (from 3.98 to 6.41%, w/w). For the sterile treatments, the change of protein in the fermented product received from *Aspergillus* sp. CASP01 was slightly high when compared to the others at the value of 1.68% (from 3.83 to 5.51%, w/w). The lower protein contents of the sterile treatments were probably caused from a poor fungal growth rate due to a high viscous texture from gelatinization of the starch that remained in the cassava pulp after autoclaving. Apart from the insufficient nutrient transfer, the high viscosity of a culture also had an effect on the dissolved oxygen which, therefore, limited the biosynthesis and growth of the organism (Jones, 1998; Papagianni *et al*., 2001; Prabhakar *et al*., 2005; Mitchell *et al*., 2006).

In this study, unfortunately, from visual appearance, all the non- sterile experiments were totally contaminated with other microorganisms and were considerably inappropriate for use as animal feed. However, it was decided to use the sterile treatments for further study to avoid the effect of contaminants and even lower protein contents than for the non-sterile conditions were obtained.

Effect of Concentration of Urea as a Nitrogen Source

A nitrogen supplement urea with different concentrations was incorporated into fresh cassava pulp to improve its nutrition value and cell productivity. The increase in crude protein in cassava pulp after 14 days of cultivation with 3 fungal strains is presented in Figure 1.

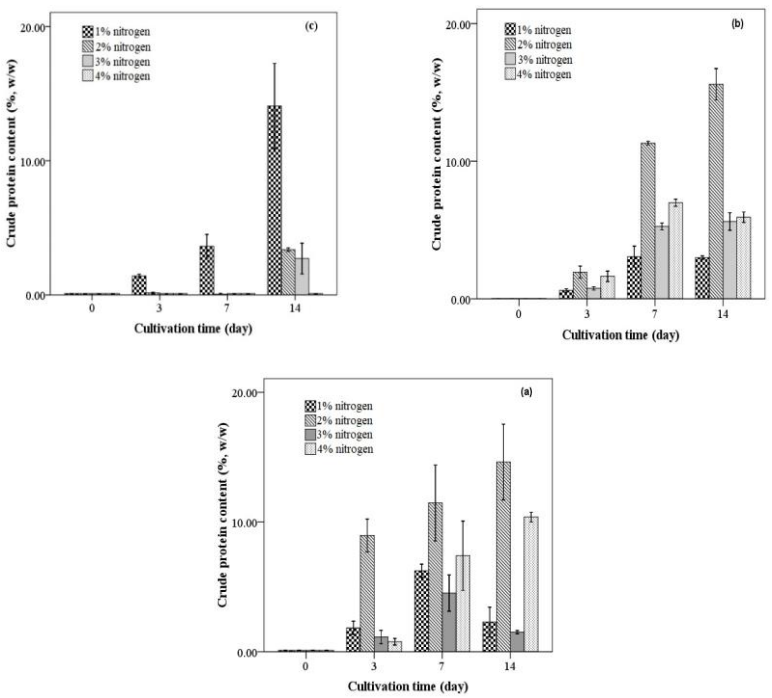

Figure 1. Effect of different concentrations of urea as a nitrogen source on crude protein content in fresh cassava pulp (300g in aluminum circle tray, 9-inch diameter) after inoculation with the 3 fungal strains for 14 days at room temperature: (a) *Aspergillus* sp. CASP01; (b) *Aspergillus* sp. UN03; and (c) *Trichoderma* sp. CASP02

It has been widely known that nitrogen is an important component of proteins and cell constituents; thus, a lack of nitrogen may cause slow or no growth or even the death of an organism, so it is necessary to balance the nitrogen in the media composition to promote cell reproduction (Niamke and Wang, 2003; Humar and Pohleven, 2005). The results clearly showed that a lower or higher nitrogen concentration had a negative effect on the cell productivity of the fungal strains. *Aspergillus* sp. CASP01 and UN03 showed the highest amounts of crude proteins (14.62 and 15.59%, respectively) after 14 days of incubation at 4.35% urea equivalent to 2% nitrogen. A similar finding was obtained from other studies which reported that cultivating A. oryzae and A. niger on cassava by-products could increase the protein level by 14.81 and 5.80%, respectively (Iyayi and Losal, 2001; Thongkratok *et al*., 2010). However, some studies found that a lower urea concentrationat 0.75% was suitable for fermenting cassava pulp with A. oryzae and could increase the protein content by 17.4% (Khempaka, 2010).

From Figure 1, *Trichoderma* sp. CASP02 was shown to be more favorable to urea than the other 2 strains and 2.17% urea equivalent to 1% nitrogen concentration was suitable for the growth of *Trichoderma* sp. CASP02 which gave the highest protein level by 14.08% after cultivating for 14 days. At higher amounts used, urea could be toxic and slow down the growth of *Trichoderma* sp. CASP02 (Ververka *et al*., 2007). This finding was comparable to supplemented corn stalk with 0.4% urea followed by cultivating *Trichoderma* reesei

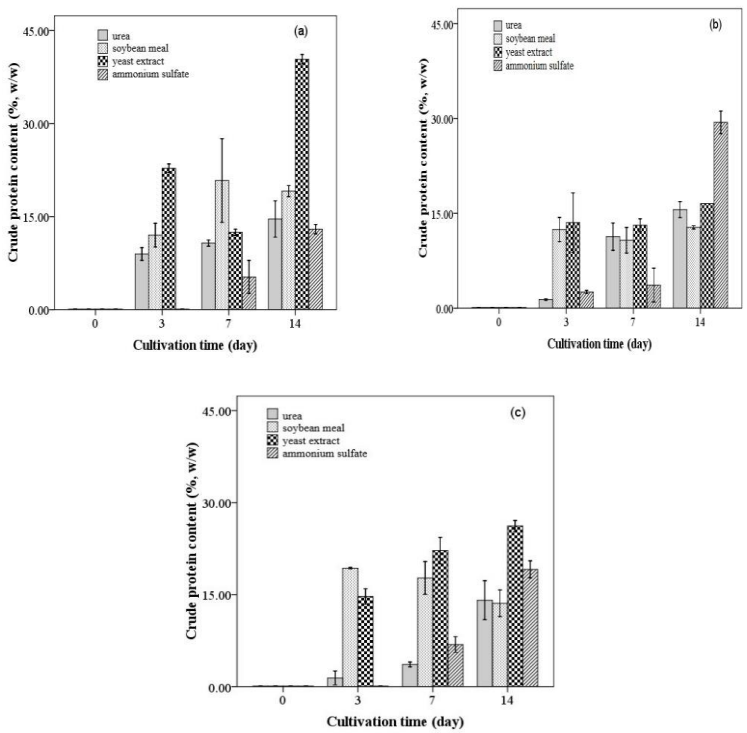

Figure 2. Effect of different nitrogen sources on crude protein content in fresh cassava pulp (300 g in aluminum circle tray, 9-inch diameter) by cultivating the 3 fungal strains for 14 days at room temperature: (a) *Aspergillus* sp. CASP01; (b) *Aspergillus* sp. UN03; and (c) *Trichoderma*

that could increase the protein level in cassava pulp about 7.14% (Omer *et al*., 2012).

Effect of Difference Types of Nitrogen Source on Crude Protein Content

Nitrogen is a major factor which limits fungal growth both quantitatively and qualitatively (Finlay *et al*., 1992). Different organic and inorganic nitrogen sources, namely yeast extract, soybean meal, urea, and ammonium sulfate at 2% nitrogen for *Aspergillus* sp. (CASP01 and UN03) and 1% for *Trichoderma* sp. CASP02 were studied for cultivation of the selective fungal strain using SSF. The changes of the crude protein contents from the various nitrogen sources' fermentation are shown in Figure 2.

In this study, results indicated that all selected fungal strains were able to use both organic and inorganic nitrogen sources. However, different nitrogen sources exhibited variations in the fungal growth which impacted on the increase in protein.

Yeast extract provided the highest increase of the crude protein contents of *Aspergillus* sp. CASP01 and *Trichoderma* sp. CASP02 which were 40.36 and 26.19% after 14-day cultivation, followed by soybean meal at 20.82 and 34.84% respectively. Ammonium sulfate, an inorganic nitrogen source, also showed a moderate increase of the protein content. This finding supports the theory that both *Aspergillus* sp. and *Trichoderma* sp. are classified in the Ascomycota division and utilize a broad array of nitrogen sources such as inorganic nitrogen and organic compounds, especially amino acids (Digby *et al.*, 2010), This phenomenon may be caused by the lower pH of inorganic nitrogen sources which consequently slows the growth rate of fungal strains (Costa *et al*., 2002). Surprisingly, a drastic change in the protein caused by *Aspergillus* sp. UNM03 was performed after being cultivated on medium supplement with ammonium sulfate for 14 days when compared to the others.

This outcome indicated that, mostly, organic nitrogens (yeast extract and soybean meal) yielded a better growth rate than inorganic nitrogens (data not shown) and consequently the maximum protein enrichment compared to inorganic nitrogen (ammonium sulfate). The effect of different nitrogen sources on growth in liquid medium was reported for Paxillus involutus that grew well with the addition of an organic nitrogen source (asparagine or aspartic acid) (Finlay *et al*., 1992) similar to the growth of *Aspergillus* oryzae, which suggested that peptone was a better nitrogen source than sodium nitrate (Ramachandran *et al*., 2004). Coprinopsis phlyctidospora on Asp-N media gave the

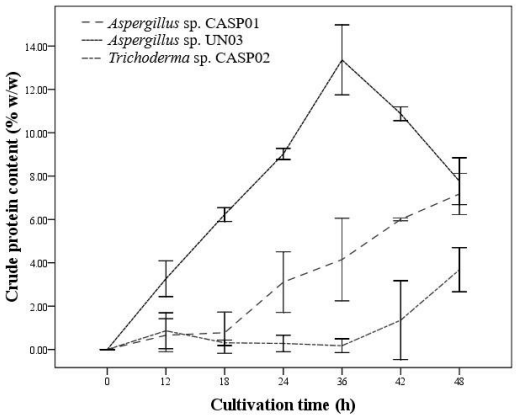

Figure 3. The increase in crude protein content of fresh cassava pulp (600 g in packed bed bioreactor) after cultivating the 3 fungal strains for 48 h

highest mycelial biomass when prepared in inorganic media (urea, NH4-N, NO3-N) (He and Suzuki, 2003). Finally, after calculating the raw material cost of different nitrogen sources, it was found that soybean meal was cheaper than yeast extract and also provided a satisfactory amount of protein level for feed. Thus, soybean meal was chosen as the sole nitrogen source for scaling up the experiment using a packed bed bioreactor.

Cultivation of the Selected Fungi in Packed Bed Bioreactor

The solid state cultivation of 3 selected fungal strains on a packed bed bioreactor was performed using sterile cassava pulp supplemented with soybean meal. Fermented products were evaluated for the crude protein content and the result is shown in Figure 3.

Aspergillus sp. UN03 showed the highest value of crude protein (13.36%) after 36 h of SSF, while *Aspergillus* sp. CASP01 and *Trichoderma* sp. CASP02 yielded only 7.18 and 3.53% crude protein content at 48 h, respectively (Figure 3). The cultivation process using a packed bed bioreactor revealed a lower crude protein content when compared to the aluminum circle tray (9-inch diameter) under the same conditions due to the heat produced from the fungal growth. However, there was a report which suggested that a packed bed bioreactor combined with a humidifier can be used for faster cultivation to minimize the effect of heat production from fungal metabolism and could increase crude protein content by 2.6-9.60% (Feng *et al*., 2007). Therefore, to solve the overheating problem, a humidifier should be used in combination.

After that, the cultivation sample of *Aspergillus* sp. UN03 was sent for analysis of aflatoxin B1 at the Postharvest and Processing Research and Development Division, Kasetsart University, Thailand. The result was also investigated and recorded a value of 18.0 ppb which was less than the Ministry of Agriculture and Cooperatives of Thailand's standard for cattle feedstuff (100.0 ppb).

Conclusions

Solid state cultivation of fresh cassava pulp with 3 fungal strains, *Aspergillus* sp. CASP01 and UN03, and *Trichoderma* sp. CASP02, resulted in the production of protein-enriched feedstuff. Optimal conditions when using the aluminum circle tray (9-inch diameter) for protein enrichment by solid state cultivation were given by *Aspergillus* sp. CASP01 and UN03 grown on fresh sterile cassava pulp supplemented with 4.35% urea equivalent to 2% nitrogen as a nitrogen source, while *Trichoderma* sp. CASP02 exhibited optimal growth on sterile cassava pulp supplemented with 2.17% urea equivalent to 1% nitrogen. Soybean meal was selected as the nitrogen source due to the satisfactory increases of protein and economic value. When using a packed bed bioreactor supplemented with soybean meal, *Aspergillus* sp. CASP01 and UN03, and *Trichoderma* sp. CASP02, it was found that *Aspergillus* sp. UN03 provided the highest protein increase (13.36% crude protein yield) after 36 h cultivation and a very low amount of aflatoxin B1 (18.0 ppb) was detected. Therefore, using the solid state cultivation technique of fungi to increase the protein level in fresh cassava pulp should be considered as an alternative choice for feed production as well as a sustainable way to manage solid waste in a value-added bio-product.

Acknowledgements

This research is financially supported by Rangsit University. We are grateful to Chorchaiwat Industry Co., Ltd, Chonburi, Thailand for the provision of facilities.

References

AOAC. (1995). Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Inc., Arlington, VA, USA.

Chauynarong, N., Iji, P., and Kanto, U. (2015). Variation in nutrient composition of cassava pulp from Thailand. Asian J. Poult. Sci., 9:203-212.

Costa, E., Teixidó, N., Usall, J., Atarés, E., and Viñas, I. (2002). The effect of nitrogen and carbon sources on growth of the biocontrol agent Pantoea agglomerans strain CPA-2. Lett. Appl. Microbiol., 35:117-120.

Digby, A.L., Gleason F.H., and Mcgee, P.A. (2010). Some fungi in the Chytridiomycota can assimilate both inorganic and organic sources of nitrogen. Fungal Ecol., 3:261-166.

Feng, J., Liu, X., Xu, Z.R., Lu, Y.P., and Liu, Y.Y. (2007). The effect of *Aspergillus* oryzae fermented soybean meal on growth performance, digestibility of dietary component and activities of intestinal enzymes in weaned piglets. Anim. Feed Sci. Tech., 134: 295-303.

Finlay, R.D., Frostegard, A., and Sonnerfeldt, A-M. (1992). Utilization of organic and inorganic nitrogen source by ectomycorrhizal fungi in pure culture and in symbiosis with Pinus contorta Dougl. ex Loud. New Phytol., 120:105-115.

Ghimire, A., Sen, R., and Annachhatre, A.P. (2015). Biosolid management options in cassava starch industries of Thailand: Present practice and future possibilities. Procedia Chem., 14:66-75.

He, X.M. and Suzuki, A. (2003). Effect of nitrogen resources and pH on growth and fruit body formation of Coprinopsis phlyctidospora. Fungal Divers., 12:35-44.

Hermiati, E., Azuma, J., Mangunwidjaja, D., Sunarti, T.C., Suparno, O., and Prasetya, B. (2011). Hydrolysis of carbohydrates in cassava pulp and tapioca flour under microwave irradiation. Indonesian J. Chem., 11:238-245.

Humar, M. and Pohleven, F. (2005). Influence of a nitrogen supplement on the growth of wood decay fungi and decay of wood. Int. Biodeter. Biodegr., 56:34-39.

Iyayi E.A. and Losal, D.M. (2001). Protein enrichment of cassava by-products through solid state fermentation by fungi. J. Food Tech. Africa, 6:116-118.

Jones, A. (1998). Large-scale of fungal inoculum. In: Environmentally Friendly Technologies for the Pulp and Paper Industry. John Wiley&Sons, Inc., Hoboken, NJ, USA, p. 481-504.

Khempaka, S. (2010). Utilization of Cassava Pulp as Feedstuff for Broilers. Research report. Suranaree University of Technology, Nakhon Ratchasima, Thailand.

Khempaka, S., Molee, W., and Guillaume, M. (2009). Dried cassava pulp as an alternative feedstuff for broilers: effect on growth performance, carcass traits, digestive organs, and nutrient digestibility. J. Appl. Poult. Res., 18:487-493.

Khempaka, S., Thongkratok, R., Okrathok, S., and Molee, W. (2014). An evaluation of cassava pulp feedstuff fermented with A. oryzae on growth performance, nutrient digestibility and carcass quality of broilers. J. Poult. Sci., 51:71-79.

Mitchell, D.A., Berovic, M., and Krieger, N. (2006). Introduction to solid-state fermentation bioreactors. In: Solid-State Fermentation Bioreactors: Fundamentals of Design and Operation. Mitchell, D.A., Krieger, N., and Berovic, M., (eds). Springer-Verlag, Berlin, Germany, p. 33-44.

Niamke, J.M. and Wang, N.M. (2003). Cellulose degradation by fungi. In: Fungal Biotechnology in Agricultural, Food, and Environmental Applications. Arora, D.K. (ed). Marcel Dekker, Inc., NY, USA, p. 363-374.

Oboh, G. (2006). Nutrient enrichment of cassava peels using a mixed culture of Saccharomyces cerevisae and Lactobacillus spp. solid media fermentation techniques. Electron. J. Biotechn., 9:46-49.

Office of Agricultural Economics. (2016). Cassava. In: Agricultural Statistics of Thailand 2016. Office of Agricultural Economics, Bangkok, Thailand, p. 20-25.

Okpako, C.E., Ntui, V.O., Osuagwu, A.N., and Obasi, F.I. (2008). Proximate composition and cyanide content of cassava peels fermented with *Aspergillus* niger and Lactobacillus rhamnosus. J. Food Agric. Environ., 6:251-255.

Omer, H.A.A., Alia, F.A.F., and Gad, S.M. (2012). Replacement of clover hay by biological treated corn stalks in growing sheep rations. J. Agr. Sci., 4:257-268.

Papagianni, M., Nokes, S.E., and Filer, K. (2001). Submerged and solid-state phytase fermentation by *Aspergillus* niger: effects of agitation and medium viscosity on phytase production, fungal morphology and inoculum performance. Food Technol. Biotech., 39:319-326.

Prabhakar, A., Krishnaiah, K., Janaun, J., and Bono, A. (2005). An overview of engineering aspects of solid state fermentation. Malays. J. Microbiol., 1:10-16.

Punhya, P. and Laohaphatanalert, K. (2013). Screening and isolation of cellulase amylase and pectinase producing microorganism from fresh cassava pulp. Proceedings of the RSU Research Conference; April 4, 2013; Pathum Thani, Thailand, p. 175-180.

Ramachandran, S., Patel, A.K., Nampoothiri, K.M., Francis, F., Nagy, V., Szakacs, G., and Pandey, A. (2004). Coconut oil cake - a potential raw material for the production of α-amylase. Bioresource Technol., 93:169-174.

Sriroth, K., Chollakup, R., Chotineeranat, S., Piyachomkwan, K., and Oates, C.G. (2000). Processing of cassava waste for improved biomass utilization. Bioresource Technol., 71:63-69.

Tesfaye, E., Animut, G., Urge, M., and Dessie, T. (2013). Cassava root chips as an alternative energy feed ingredient in broiler ration. Int. J. Poultry Sci., 12:298-306.

Thai Feed Mill Association. (2017). Soybean meal. In: Feed Business. Thai Feed Mill Association, Bangkok, Thailand, p. 14-20.

Thongkratok, R., Khempaka, S., and Molee, W. (2010). Protein enrichment of cassava pulp using

microorganisms techniques for use as an alternative animal feedstuff. J. Anim. Vet. Adv., 9:2859-2862.

Trakulvicheana, S., Chaipraserta P., and Songkasiri W. (2013). Environmental impact valuation model of cassava pulp utilization alternatives. Starch Update 2013: The 7th International Conference on Starch Technology; November 21- 22, 2013; Bangkok, Thailand, p. 117-123.

Veverka, K., Štolcová, J., and Růžek, P. (2007). Sensitivity of fungi to urea, ammonium nitrate and their equimolar solution UAN. Plant Protect. Sci., 43:157-164.

Yang, S. S. (1988). Protein enrichment of sweet potato residue with amylolytic yeasts by solid state fermentation. Biotechnol. Bioeng., 32:886-890.

Yazid, N. A., Barrena, R., Komilis, D., and Sánchez, A. (2017). Solid-state fermentation as a novel paradigm for organic waste valorization: a review. Sustainability, 9:224-252.