

Improvement of crude protein composition of cassava pulp by yeast fermentation with chicken manure

Simon Anthony Kayombo*, Pattaraporn Poommarin and Panida Duangkaew

Faculty of Animal Science and Agricultural Technology, Silpakorn University, Phetchaburi 76120, Thailand

ABSTRACT

***Corresponding author:**
Simon Anthony Kayombo
smnkayombo@gmail.com

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The aim of this study was to increase crude protein composition of cassava pulp by yeast fermentation with chicken manure. Two different fermentation experiments were conducted, the first with sugar activation under controlled temperature, and the second with sugar or molasses activation under uncontrolled temperature. The experiments were designed as a factorial arrangement in completely randomized design. Two factors, including treatment composition and fermentation time, were studied. The results revealed that the crude protein percentage of fermented cassava pulp increased in all treatments containing chicken manure. The highest level of crude protein composition was observed in cassava pulp fermented with yeast and chicken manure plus sugar activation for 20 days, which was higher than non-fermented cassava pulp. In conclusion, fermenting cassava pulp by yeast and chicken manure with sugar activation for 20 days could improve the crude protein content of cassava and may be used as an alternative ingredient for animal feeds.

Keywords: animal feeds; cassava pulp; chicken manure; *Saccharomyces cerevisiae*

1. INTRODUCTION

One of the constraints of monogastric animal production, especially in farms with poor resources, is high price and inaccessibility of feed. In commercial situations, feed cost accounts for 50 to 70% of the production cost (Spring, 2013). The biggest contributor to higher production costs is protein ingredients, which are more expensive compared to cereals (Manyelo et al., 2020). The increasing cost of conventional protein sources such as soybean, fish meal, sunflower seed cake, necessitates the need to find other alternative feeds to replace or decrease the crude protein content of conventional protein ingredients.

Cassava pulp is a solid moist fibrous material remaining after starch extraction from cassava roots. It is comparatively cheap, and its availability offers an

opportunity for utilization by the livestock industry because it may reach up to 30% of the whole original cassava roots depending on the efficiency of cassava starch processing factories (Ghimire et al., 2015). However, the nutritional problem of cassava pulp is its low crude protein of less than 2.2%, and a high fiber content of up to 26.9%, as reported by Heuzé et al. (2016). In addition, the presence of starch and cellulose fiber in cassava pulp makes it a good medium for growing fungi through fermentation (Boonnop et al., 2009; Sugiharto et al., 2017).

Fermentation is one of the less expensive ways of increasing protein and quality of feed ingredients. It entails a metabolic process involving the chemical breakdown of organic materials through the action of enzymes from microorganisms. *Saccharomyces cerevisiae* yeast is of interest and widely used in the fermentation of feed ingredients. Yeast fermentation of cassava pulp (YFCP) can

increase crude protein, true protein and lysine, but can also lower crude fiber and cyanide content (Kasprowicz-Potocka et al., 2016; Khampa et al., 2011). Also, the improvement may be more impressive if a source of nitrogen is used, such as urea, ammonium sulfate, diammonium sulfate, and peptone (Okrathok et al., 2018). Alternative nitrogen source from agricultural by-products can be further elucidated to explore the new materials and to reduce waste pollution.

Chicken manure has a crude protein content of 15.4% (Lanyasunya et al., 2006) to 31% (Trevino et al., 2002). It can possibly be a choice of organic nitrogen sources. It has been used directly as pig feed by some livestock producers in different countries. Nigerians use dried poultry manure to feed pigs. In Southeast Asia, farmers construct layer cages 1.5 meters above the pig pens, hence the excreta fall in pens and are consumed by the pigs (Flachowsky, 1997). The use of this waste at the recommended level does not show any negative effect (Adesehinwa et al., 2010). However, the chicken manure should be dried to remove harmful microbes (Trevino et al., 2002).

Therefore, this study intended to investigate the increase in cassava pulp crude protein composition by yeast fermentation with chicken manure as a nitrogen source

under different conditions of temperature, moisture, and sugar activation.

2. MATERIALS AND METHODS

2.1 Materials

Cassava pulp was bought from EK Animal Feed Store (Tha Yang, Phetchaburi, Thailand). *Saccharomyces cerevisiae* yeast (Lesaffre Saf-Instant®, France), ammonium sulfate (Crown, Terragro, Thailand), and table sugar were bought at a nearby store. Chicken manure was collected from layer pens at university farm. The trash was removed, dried in a hot air oven (60°C) for 3 days, and then finely ground using a pestle, mortar, and electric blender.

2.2 Experimental design

This experiment was designed as 5(A) × 3(B) and 2(A) × 3(B) factorial arrangement in completely randomized design in Fermentation 1 and Fermentation 2, respectively, with three replications. Factor A was arranged into treatments and factor B was set at different fermentation times (0, 10, and 20 days). Details of treatments with calculated total nitrogen (N) and crude protein are shown in Table 1.

Table 1. Treatments group with their hypothetical percentage of nitrogen and crude protein

Treatment	Composition (%)				Contribution of N from each ingredient (%)				Total N	% CP
	CPu	Y	AS	CM	CPu	Y	AS	CM		
Fermentation 1										
T1	100				0.32	0.00	0.00	0.00	0.32	2.00
T2	99	1			0.32	0.06	0.00	0.00	0.38	2.36
T3	97	1	2		0.31	0.06	0.42	0.00	0.79	4.95
T4	70	1		29	0.22	0.06	0.00	0.46	0.83	5.21
T5	70	1		29	0.22	0.06	0.00	0.46	0.83	5.21
Fermentation 2										
TS	70	1		29	0.22	0.06	0.00	0.46	0.83	5.21
TM	70	1		29	0.22	0.06	0.00	0.46	0.83	5.21

Note: AS = ammonium sulfate, CM = chicken manure, CP = crude protein, CPu = cassava pulp, N = nitrogen, T = treatment, TS = treatment with sugar, TM = treatment with molasses, Y = yeast. Nitrogen content of each ingredient used in calculation: CPu = 0.32, Y = 6.11, AS = 21.0, and CM = 1.6.

2.3 Preparation of cassava pulp fermentation

Cassava pulp fermentation was prepared by combining the methods of Huu and Khammeng (2014) and Nukreaw et al. (2019) with modification. In Fermentation 1, cassava pulp and chicken manure were weighted according to each percentage of treatment composition. Yeast solution (10% w/v) was prepared by mixing 12 g of yeast with 20% sterile sugar solution, stirring well for 30 min. Ammonium sulfate solution (20% w/v) was prepared in sterile water. Then, 10 mL of yeast solution were added into treatments T2, T3, T4, and T5 to obtain 1% yeast inoculation. Ammonium sulfate solution was added to T3 to get 2% of the treatment. All treatments were adjusted to 40% by the addition of sterile water, then thoroughly mixed. The treatments were each packed anaerobically in a plastic bag, and treatments T1 through T4 were stored in the incubator at 37°C, while T5 was stored at room temperature (28-33°C) for 0, 10, and 20 days. When reaching the specific fermentation time, the samples were placed in a freezer until analysis. Fermentation 2 (TS and TM) followed the same

procedures of Fermentation 1 (T5). The difference between the two was that TM was activated with 20% a solution of molasses as 4% of treatment weight, but TS was activated with table sugar as in Fermentation 1. Then, the moisture was adjusted to 30% instead of 40% and stored at room temperature (28-33°C).

2.4 Data collection

The data collected were dry matter, crude protein, crude fiber, gross energy, and pH of cassava pulp, chicken manure, and YFCP, following the AOAC (2000). The pH value was determined by dissolved samples in distilled water (1:10 ratio) and measured by a pH meter (Adwa AD12, Hungary).

2.5 Statistical analysis

The mean ± standard deviation of dry matter, gross energy, crude protein, crude fiber, and pH were statistically analysed using analysis of variance (ANOVA) and compared by Duncan's new multiple range test (DMRT) in the R program (significance was defined as $p < 0.05$).

3. RESULTS AND DISCUSSION

Prior to fermentation, the dry matter, crude protein, crude fiber, gross energy, and pH of ingredients, including cassava pulp and chicken manure used in the experiments, were analyzed (Table 2). Cassava pulp contained higher

dry matter, crude fiber, and gross energy, compared to chicken manure, which had higher crude protein and pH. The pH of cassava pulp was lower because storage and room temperature seemed to reduce the pH due to the production of acid by the fermentative natural microorganisms in the sample (Souza et al, 2013)

Table 2. Crude protein, crude fiber and gross energy of cassava pulp and chicken manure (on a dry basis)

Parameters	Cassava pulp	Chicken manure A
Dry matter (%)	87.03 ± 0.25	25.39 ± 0.68*
Crude protein (%)	1.99 ± 0.19	13.93 ± 0.29
Crude fiber (%)	15.63 ± 0.73	13.32 ± 1.48
Gross energy (kcal/kg)	3,456.67 ± 50.38	2,386.47 ± 24.31
pH value	4.67 ± 0.11	7.89 ± 0.05

Note: Data are expressed as mean ± standard deviation (n = 3), *chicken manure dry matter is on a fresh basis.

Percentage of crude protein (%CP) of YFCP under a controlled temperature of 37°C, showed that %CP was affected by the interaction effect among both factors ($p < 0.1$) and individual factors ($p < 0.001$) (Table 3). The highest CP of 6.41% was observed in the treatment with chicken manure stored in the incubator (T4) at 20 days, followed by 6.38% and 6.29% in the treatment with chicken manure stored at room temperature (T5) at 10 and

20 days of fermentation, respectively. Higher CP was found in the treatment containing a source of nitrogen (T3, T4, and T5), compared to those with no source of nitrogen (T1 and T2). The mean results within treatments showed T4 had the highest %CP of 6.04%. Also, there was an increase in crude protein at different fermentation times. The mean result within fermentation days showed that day 20 had a higher amount of CP (Table 3).

Table 3. Crude protein results of first fermentation of 5 treatments in 3 fermentation times under controlled temperature

Treatment (A)	Fermentation times (days)			Mean (A)
	0	10	20	
T1	2.08 ± 0.18 ^g	2.10 ± 0.16 ^{fg}	2.20 ± 0.36 ^{fg}	2.13 ± 0.22 ^e
T2	2.56 ± 0.04 ^e	2.40 ± 0.10 ^{ef}	2.54 ± 0.10 ^e	2.50 ± 0.1 ^d
T3	5.10 ± 0.20 ^d	5.10 ± 0.20 ^d	5.61 ± 0.11 ^c	5.28 ± 0.31 ^c
T4	5.80 ± 0.10 ^{bc}	5.91 ± 0.07 ^{bc}	6.41 ± 0.26 ^a	6.04 ± 0.31 ^a
T5	5.10 ± 0.10 ^d	6.29 ± 0.08 ^a	6.38 ± 0.28 ^a	5.92 ± 0.2 ^b
Mean (B)	4.13 ± 1.74 ^b	4.36 ± 1.83 ^b	4.63 ± 1.94 ^a	
<i>p</i> value				
A	<0.001			
B	<0.001			
A : B	<0.1			
% CV	3.9			

Note: Results are on a dry matter basis and are expressed as mean ± standard deviation (n = 3). The different superscript letters are statistically different by Duncan's new multiple range test ($p \leq 0.05$)

The results of Fermentation 2, whereby cassava pulp, yeast, and chicken manure with molasses (TM) or sugar (TS) activation were fermented in an uncontrolled temperature are shown in Table 4. Before fermentation, the crude protein was observed to be higher than in Fermentation 1. This was caused by the different batches of chicken manure collected. TS at 10 and 20 days of fermentation and TM at day 10 showed the highest crude protein value. Considering the effects of sugar and molasses, the results showed that sugar gave a significantly higher level of crude protein. The higher effect of fermentation time was observed at 20 days of fermentation.

Protein ingredients are one of the most expensive components of feeds in animal production. In the present work, cassava pulp was fermented with yeast in the presence of chicken manure, with activation by table sugar or molasses. The high percentages of CP in T3, T4, T5, TS, and TM during the different fermentation times were due to the addition

of ammonium sulfate and chicken manure as nitrogen sources.

In both fermentations, the crude protein of cassava pulp was increased. Interaction effects of both factors, time and treatment compositions, were observed in two fermentation batches. In Fermentation 1, the highest increase was 6.41% observed in the treatment containing chicken manure under a controlled temperature of 37°C (T4) and 6.38% in the treatment containing chicken manure (T5) stored at room temperature (Table 3). In Fermentation 2, both treatments (TS and TM) had comparable crude protein percentages at day 20 of fermentation; however, the percentage increase in TM was higher (2.15%), compared to 1.46% in TS (Table 4). The results suggested that fermentation with chicken manure seemed to produce a higher %CP and indicated that activation of soluble sugar was preferred. The effect of fermentation time was also observed. As fermentation

time increased, the crude protein level increased. This result of a time effect on fermentation matched with those of Hang et al. (2019) and Iyayi (2001), whereby the best

time for cassava pulp fermentation was between 14 and 21 days, respectively.

Table 4. Crude protein of second yeast fermented cassava pulp activated with sugar (TS) and molasses (TM) at room temperature

Treatment (A)	Fermentation times (days)			Mean (A)
	0	10	20	
TS	7.50 ± 0.90 ^b	8.65 ± 0.54 ^a	8.96 ± 0.72 ^a	8.70 ± 0.66 ^a
TM	6.17 ± 0.08 ^b	6.37 ± 0.35 ^b	8.32 ± 0.37 ^a	6.95 ± 1.06 ^b
Mean (B)	6.84 ± 1.4 ^b	7.51 ± 1.31 ^b	8.64 ± 0.62 ^a	
<i>p</i> value				
A	<0.001			
B	<0.01			
A : B	<0.05			
% CV	7.15			

Note: Results are on a dry matter basis and are expressed as mean ± standard deviation (n = 3). The different superscript letters are statistically different by Duncan's new multiple range test ($p \leq 0.05$)

The percentage of crude protein in Fermentation 2 was higher than in Fermentation 1. The low percentage of crude protein in the first fermentation may be caused by a higher temperature of 37°C. AL-Sa'ady (2014) discovered the optimal temperature for *S. cerevisiae* incubation is 30°C. Lower and higher temperatures decrease the enzymatic reaction of microorganisms and reduce growth. Also, it may be caused by the lower moisture content of 30% in the inoculant solution in the second fermentation, compared to 40% in the first fermentation. Low moisture reduced the porosity of the substrate, which limits the oxygen transfer within a substrate and led to low production of enzymes and growth as previously reported (AL-Sa'ady, 2014; Camacho-Ruiz et al., 2003).

The CP increase in Fermentation 2 is related to 8.54% (Kayombo et al., 2022). Also, the highest CP reached in the first and second fermentations of the present work were quite low when compared to 12% (Sengxayalath and Preston, 2017). This may be caused by a higher percentage combination of initial nitrogen source of 4.05% urea and 1% diammonium phosphate, and a higher percentage of 2.02% yeast, compared to 1% yeast and low initial nitrogen sources in chicken manure, which equated to 2% of ammonium sulfate in the present work. Likewise, the higher pH of chicken manure interferes with the fermentation process, which needs an acidic environment, as reported by Flachowsky (1997) and Serrano et al. (2004). However, the increase in the first fermentation (0.61%) was higher than the 0.04% increase, as revealed by Sugiharto et al. (2017), whereby *Rhizopus oryzae* fungi were used without a source of nitrogen. Additionally, the increase in the second fermentation was related to 2.05% (Hang et al., 2019), which used a combination of 2% urea and 1.5% diammonium phosphate as a nitrogen source. As well, related to 1.98% as reported by Kayombo et al. (2021), who used a related condition, the differences were 20% inoculant activator solution, a batch of chicken manure, and sugar activator solution, instead of the molasses activator of the present work. Moreover, the increase in percentage of crude protein in both fermentations was lower than 4.31% (Iyayi, 2001), whereby 2% of peptone was used as a nitrogen source for the yeast.

The increase in %CP may be caused by the action of yeast to utilize the carbohydrates of cassava pulp as an energy to produce some proteins using nitrogen from chicken manure. The *S. cerevisiae* cells have invertase and maltase enzymes, which can easily utilize simple carbohydrates (Batista et al., 2004; Parapouli et al., 2020). Also, yeast can use organic and inorganic nitrogen, because of the presence of three permease enzymes (MEP 1, MEP 2, and MEP 3), which facilitate the entrance of ammonia into the cell and linked with glutamate dehydrogenase, then combine with glutamine through glutamine synthetase (Jiranek et al., 1995; Magasanik and Kaiser, 2002). Besides the action of yeast, it was observed that treatments without chicken manure showed no difference in crude protein, compared to treatments with chicken manure, when increasing the fermentation time. Therefore, the increase of crude protein might be contributed by some associated microorganisms in chicken manure (Li et al., 2020). Incorporation of organic and/or inorganic nitrogen to amino acids and proteins could occur by the action of any microorganism presence in the fermentation batch. Hydrolysis of the carbohydrate portion of cassava to small sugar molecules and oxidation to energy by cellular respiration process could be sources of energy. The energy produced is used for synthesis of cell components, including nitrogen containing biomolecules such as proteins. For further clarification of this phenomena, the true protein should be directly examined from this fermentation. Also, the study of beneficial microorganisms in chicken manure might provide more information and further advantages.

4. CONCLUSION

Based on this study, fermentation of cassava pulp by yeast with chicken manure plus sugar or molasses activation could increase crude protein from 1.99% pure cassava pulp to 8.96% at 20 days. The higher initial crude protein source in fermentation contributed higher final crude protein. Both table sugar and molasses can be used as initial energy sources during the fermentation process of cassava pulp.

It should be noted that proximate analysis was used in this work to evaluate the increase in protein content in fermented cassava.

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