

# Theobromine Content in Cocoa Pod Husk (*Theobroma cacao*) Fermented by *Aspergillus spp.* in Different of Chop Sizes and Fermentation Times

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## Abstract

*Theobromine is one of alkaloid in cocoa pod husk (CPH). Fresh CPH has high theobromine content reach 0.40% (400.000 ppm) which is fresh CPH portion in ruminant ration more than 30% to be affected of health. This research aim was to know the change of theobromine content of CPH with different chop sizes were fermented in the different time of incubation by Aspergillus spp. Three species of Aspergillus spp. were used in this research i.e. A. niger; A. oryzae and A. ficuum BPT. There were 4 chop sizes i.e.; irregular size (A<sub>1</sub>), 1x5 cm (A<sub>2</sub>), 3x5 cm (A<sub>3</sub>), and 5x5 cm (A<sub>4</sub>). The incubation times were; 0, 5, 7, and 9 days, with three replications. The solely parameter was theobromine content which were prepared according to AOAC (1990) and analyze using HPLC (Chen et al., 2008). The result of research showed that CPH fermented using A. niger, the lowest of theobromine content for chop size A<sub>2</sub> and incubated 9 days was 38.088 ppm. CPH fermented using A. oryzae had the lowest of theobromine content in A<sub>2</sub> and 9 days only 36.215 ppm. CPH fermented with A. ficuum had the lowest of theobromine content in A<sub>2</sub> and 9 days reach 42.914 ppm. It was concluded that the best of CPH fermented for reducing of theobromine content for 1x5 cm (A<sub>2</sub>) chop size with 9 days fermentation, fermented by A. oryzae.*

*Keywords: theobromine, cocoa pod husk, Aspergillus spp., chop sizes, incubation times*

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## Introduction

Cocoa pod husk (CPH) utilization is expected to be capable of feed fulfilling the ruminant animal requirement yearly, so the animal will not be lacking of nutrients for its life and production. This condition has reason because cocoa trees have fruit in

a long year which is CPH abundant available in harvest season. Utilization of fresh CPH as feed supplement has limiting factor which one alkaloid is theobromine. CPH has high of theobromine content or 3,7-dimethylxanthine (Mahyuddin and Bakrie, 1993; Aregheore, 1999). The high concentration theobromine affected the health of animal (Golding, 1985). The theobromine content of CPH about 0.17-0.20% (Wong and Hassan, 1988), while in cocoa beans reached 1.5-3.0%, and in dry cocoa beans reached 1.8% (Willson, 1999).

Decreasing of theobromine content can be done with fermentation method using microbe agent such as fungi. Some researchers used *A. niger* as decomposer of CPH in solid fermentation process (Yamaoka-Yano and Mazzafera, 1999; Huq, 2006) or *A. oryzae* (Lubis *et al.*, 2002). They found that CPH theobromine content were reduced due to the role of enzyme activity that secreted by *Aspergillus spp.*

Based on those results, in this research we used *A. niger*; *A. oryzae* and *A. ficuum* in solid fermentation with CPH as a substrate. The aim of this research was to decrease theobromine content of CPH in Laboratory scale that subsequently the fermented CPH would used for alternative forage, and also we studied about storage time of fermented CPH as feed.

## Materials and Methods

This experiment was fermented CPH using three of *Aspergillus spp.* i.e. *A. niger*, *A. oryzae* and *A. ficuum*, in four types of chop sizes i.e. irregular size ( $A_1$ ) as control, 1 x 5 cm ( $A_2$ ), 3 x 5 cm ( $A_3$ ), and 5 x 5 cm ( $A_4$ ) and four kinds of incubation times i.e. 0, 5, 7 and 9 days. CPH was air dried for 8 hours after chopping, while powder fungi were used as much as 1% and then added urea 1% of CPH weight. Urea dissolved with sterile water in ratio 1 : 10 parts. Fresh CPH used 1 kg every treatment which this was spread in plastic box (the first coating). CPH sprayed with urea and inoculated of *Aspergillus spp.*, the next with same procedure was done for the second and third coatings.

After completion of fermentation process, CPH was removed and to be air-dried for 6 hours. The weighing of CPH and to be continued with samples drying using oven in temperature of 55 °C for 4 days. About 100 g of fermented CPH was dried using freeze dryer for 4 days. Dried CPH was ground with the mortal which was surrounded by ice to maintain optimal temperature and theobromine content, and then followed by analysis of theobromine content of CPH. Analysis of theobromine content was recommended by EFSA (2008) using High-Performance Liquid Chromatography (HPLC).

## Results and Discussion

Theobromine as alkaloid compound cause bitter in cocoa beans and CPH. Al-

kaloid is organic component from plant which has contain of nitrogen, commonly as alkalis and it has biology activity. The alkaloid in plant has function for attack of pest (Urich, 1994). Fermentasi treatment can decrease CPH theobromine content. CPH non fermented has higher of theobromine content (Table 1).

Table 1. Theobromine content of cocoa pod husk fermented by *A. niger* (ppm)

Incubation times (days)	Treatment of chop sizes				Average
	A1	A2	A3	A4	
0	157.560	134.410	171.554	186.193	162.429
5	76.008	71.093	78.857	81.778	76.934
7	38.545	38.103	46.680	52.697	44.006
9	38.477	38.088	43.837	45.727	37.507
Average	77.648	70.424	85.232	91.599	

Note: A<sub>1</sub>= irregular size, A<sub>2</sub>= 1 x 5 cm, A<sub>3</sub>= 3 x 5 cm, A<sub>4</sub>= 5 x 5 cm.

The finding results showed that fermentation process affected the decreasing of CPH theobromine content. In *A. niger* fermentation treatment, there was an effect on CPH theobromine content due to differences of chop sizes and incubation times. Reducing of CPH chop size was parallel to reducing of theobromine content. The A<sub>2</sub> and A<sub>4</sub> chop sizes showed average of the lowest and the highest of theobromine content, respectively. On the other side, increasing of incubation time was inversed with decreasing of average of theobromine content. The lowest average of theobromine content was achieved through fermentation at 9 days of incubation (reduced 76.91%). From this experiment, the lowest of theobromine content (38.088 ppm) was achieved in A<sub>2</sub> chop size when incubated during 9 days.

Fermentation using *A. oryzae* in treatment of chop sizes and incubation times showed effect to decreasing of CPH theobromine content that was presented in Table 2. Reducing of CPH chop size had correlation with reducing of theobromine content. The A<sub>2</sub> chop size showed average of the lowest theobromine content, while A<sub>4</sub> was the highest theobromine content. The longer incubation time supported reducing theobromine content. The lowest average of theobromine content was occur at 9 days fermentation (reduced 23.81%). From this experiment, the lowest of theobromine content (36.215 ppm) was achieved in A<sub>2</sub> chop size when incubated during 9 days.

The finding results showed that fermentation CPH using *A. ficuum* affected the decreasing of theobromine content (Table 3). Decreasing of CPH chop size had relation with decreasing of theobromine content. The A<sub>2</sub> chop size showed average of the lowest for theobromine content. On the other side, increasing of incubation time was inversed with decreasing of average of theobromine content. The lowest

Table 2. CPH theobromine content KBK nonfermented and fermented with *A. niger* (ppm)

Incubation times (days)	Chop sizes				Average
	A1	A2	A3	A4	
0	181.891	191.058	195.084	197.083	191.279
5	62.904	64.337	67.120	75.050	67.353
7	53.275	53.926	53.600	58.360	54.790
9	46.016	36.215	46.880	53.096	45.552
Average	86.022	86.384	90.671	95.897	

Note: A<sub>1</sub>= irregular size, A<sub>2</sub>= 1 x 5 cm, A<sub>3</sub>= 3 x 5 cm, A<sub>4</sub>= 5 x 5 cm.

Table 3. CPH theobromine content KBK nonfermented and fermented with *A. ficuum* (ppm)

Incubation times (days)	Chop sizes				Average
	A1	A2	A3	A4	
0	175.409	156.430	214.776	223.609	192.556
5	68.566	69.884	81.920	91.207	77.894
7	62.146	66.348	75.749	88.566	73.202
9	45.052	42.914	58.360	80.048	56.593
Average	87.793	83.894	107.701	120.858	

Note: A<sub>1</sub>= irregular size, A<sub>2</sub>= 1 x 5 cm, A<sub>3</sub>= 3 x 5 cm, A<sub>4</sub>= 5 x 5 cm.

average of theobromine content was achieved through fermentation at 9 days of incubation (decreased 28.09%). From this experiment, the lowest of theobromine content (36.512 ppm) was achieved in A<sub>2</sub> chop size when incubated during 9 days.

The average for theobromine content in CPH non fermentation (0 day) was 192.556 ppm (192.556 mg/kg, kg). This value was lower from reported by Haryati and Harjosuwito (1984); Wong and Osman (1988) about 0.17-0.20% (1,700-2,000 mg/kg), but EFSA (2008) reported higher reach 0.40% (4,000 mg/kg). Different of theobromine content for non fermented CPH was caused by different treatment of chop size. This research, CPH was chopped and air dried during 8 hours which was decrease water content and followed by decreasing of theobromine content. The cocoa bean had theobromine content higher reached 1.5-3.0% (15,000-30,000 mg/kg) (Harrison, 2001), while dry beans had theobromine content lower only 1.8% (18,000 mg/kg) (Willson, 1999). The CPH theobromine content was the lowest among 3 varieties of *Aspergillus spp.* after fermentation process that was *A. niger* in A<sub>2</sub> with incubation of 9 days (45.052 ppm).

Reducing of CPH theobromine content caused by decomposing activity of *Aspergillus spp.* which was during fermentation process to result heat (increasing

of substrate temperature). Beside that occurred hydrolysis in CPH substrate. In this step, theobromine dissolve in cell liquid and came out together with evaporation process. Rohan (1963) and Alamsyah (1991) were reported that theobromine content reduce during fermentation process because soluble in cell liquid and diffusion in cocoa bean nib. Diffusion process will stop if occur balance of theobromine content in cocoa bean. Decreasing of CPH theobromine content was relatively low between *A. niger* compared *A. ficuum*. This case had relation with ability *A. niger* to utilize theobromine as energy source. According to Asano et al. (1993) and Hakil et al. (1999) that *A. niger* had ability to utilize theobromine as energy source through methylxanthines degradation.

## Conclusions

The chop size of 1x5 cm and incubation times of 9 days affected decreasing of CPH theobromine content. The best of CPH fermented for reducing of theobromine content for chop size 1x5 cm (A<sub>2</sub>) with 9 days fermentation, fermented by *A. oryzae*.

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