GOOD MANUFACTURING PRACTICES ASSESSMENT
AND SHELF-LIFE ANALYSIS OF CHILI PASTE
MUSHROOM IN CHAIYO FARM

BRIAN NARANATHAN
STATEMENT LETTER OF MANUSCRIPT AND SOURCE OF INFORMATION

I declare the truth that this manuscript entitled *Good Manufacturing Practices Assessment and Shelf-life Analysis of Chili Paste Mushroom in Chaiyo Farm* is my own work with guidance of the advisors and has not been submitted in any form at any college, except Bogor Agricultural University and Prince of Songkla University. Sources of information derived or quoted from published and unpublished works of other authors mentioned in the text and listed in the List of References at the end of this manuscript.

Bogor, January 2015

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ABSTRACT

BRIAN NARANATHAN. Good Manufacturing Practices Assessment and Shelf-life Analysis of Chili Paste Mushroom in Chaiyo Farm. Supervised by SUBARNA and BENCHAMAPORN PIMPA.

Mushroom as food product has big potential to be used. The nutritional content of mushroom that suitable for low energy diet and good source of protein become the reason of mushroom popularity. *Schizophyllum commune* has been known as one of edible mushroom that has functional properties. In Southern of Thailand, this mushroom becomes one of the famous food that been sold commercially with chili or curry paste seasoning. In this research, we assessed the Thailand’s GMP application of the producer. Besides that, this research also measured the shelf-life of chili paste mushroom product in room temperature and made a comparison with the addition of mixed preservative (propyl-parabens 500 ppm and calcium propionate 1000 ppm). From the GMP application, Chaiyo Farm as a producer still needs great numbers of improvement due to a lot of nonconformities. The longest shelf-life of product, chili paste mushroom, was estimated less than six days with addition of preservative at room temperature.

Keywords: GMP, calcium propionate, propyl-paraben, *Schizophyllum commune*
GOOD MANUFACTURING PRACTICES ASSESSMENT AND SHELF-LIFE ANALYSIS OF CHILI PASTE MUSHROOM IN CHAIYO FARM

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PREFACE

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1. INTRODUCTION

1.1. Background

Mushroom is a macrofungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand (Chang and Miles, 1992). Nowadays, utilization of mushroom in food industry has been developed. The protein content and functional compounds of mushroom become the reason of mushroom popularity.

Member of *Pleurotus* genus has been recognized as commercial important commodity for edible mushroom. *Schizophyllum commune* nutritional composition in dry basis consists of carbohydrates (61.1%) protein content (16.0–27.0%), ash (6.9%) and fat (< 2.0 %). Besides that, the energy content of 100g *Schizophyllum commune* provides only 1.4–4.4% of the daily energy requirement, and it means it can be used in a low-energy diet due to the low energy level (Cheung, 2008).

The various benefits of taking mushroom diet, especially *Schizophyllum commune* has already assessed. It can be good as protein source and also could help to inhibit lipid peroxidation by antioxidant properties (J.H. Yang *et al.*, 2002), and become antitumorigenic properties by schizophyllan (Lavi *et al.*, 2006).

*Schizophyllum commune* is a popular edible mushroom. In Thailand, *Schizophyllum commune* usually cooked with curry or chili paste. This food is well-known in Southern part of Thailand. Chili paste mushroom is one of cooking product from *Schizophyllum commune*. This product has soft and moist texture, usually was sold in the plastic cup as packaging.Chili paste mushroom ingredients consist of mushroom, chili, lemon grass, garlic, and onion. This product usually eats with rice and sold as souvenir from south of Thailand (see Figure 1).

Chaiyo Farm, one of Chili paste mushroom producer sells this product in front of their mushroom plantation and sometimes in the food exhibition. The Chaiyo Farm owner faced a problem to determine the shelf-life of this product. The owner wanted to know how long this product is still appropriate to be sold.

![Figure 1. Ready to eat chili paste mushroom](image)

The shelf life is an important attribute for all food products. It can be defined as the time between the production and packaging of the product and the point at which it becomes unacceptable to the consumer (Forsythe, 2007). Food deterioration as one of the reasons unacceptability, occurs progressively during storage, and is caused mainly by the activity of microorganisms that thrive in the product (McMeekin and Ross, 1996). Microbial spoilage often limits the shelf lives of many foods (Kakiomenour et al., 1995)

Hurdle technology is therefore the application of two or more controlling factors to products in order to control or inhibit microbial growth (Tucker, 2007). One of hurdle technology is application of chemical preservatives. Chemical preservatives or antimicrobial chemicals are used in food in relatively small doses either to kill undesirable microorganisms or to prevent or inhibit their growth (Ray, 2005).

Parabens compounds have been reported to have multiple bioactivities, including as active antimicrobial agents (Soni et al., 2005). They can either be used singly or in combination to exert an optimal antimicrobial effect. The use of parabens as food preservers is attractive because they meet several of the criteria of an ideal preservative in that they: possess a broad spectrum of antimicrobial activity, are stable at a broad pH range, are sufficiently soluble in water to produce the effective concentration in aqueous phase, and are heat stables such that they can safely be autoclaved and still maintain their antimicrobial activity. They are also known to have very low toxicity and are biodegradable (Msagati, 2012). According to FDA (2013), the ingredient is used in food at level not to exceed good manufacturing practices. Current good manufacturing practices result in a maximum level of 0.1 % in food.

Another preservative that can be used in high pH food is propionate. Gram-negative bacteria are inhibited by propionate but its action against yeasts is weak and therefore, the yeasts used in bakery products are not inhibited (Tucker, 2007). Calcium propionate and Sodium propionate are GRAS for miscellaneous and general-purpose usage in the USA with no limitation other than good manufacturing practices (Smith and Lily, 2011).

Beside from the usage of preservatives, the assessment of production place based on GMP in Chaiyo Farm could be useful to prevent contamination that makes the product shelf-life short and unsafe for consumers. Good Manufacturing Practices (GMP) shall be implemented in food industry to ensure the quality and safety of food product. GMP according to Thaheer (2005), is a guideline to produce food products in the process that has fulfilled the requirement to gain desired food product and in accordance with the demands of consumers. The aspects for implementation of GMP consist of several aspects: 1) location and building; 2) tools, machinery, and production equipments; 3) control of production process; 4) sanitation; 5) cleaning and maintenance; 6) personnel and employee hygiene (Thailand Ministry of Public Health. 2012).
1.2. Research Objectives

These research objectives are:

1. To measure the shelf-life of cooked *Schizophyllum commune* in chili paste mushroom
2. Assessed the production place according to Good Manufacturing Practices (GMP) criteria. After that, this research also would give suggestions to improve the quality of product and solving the problems in Chaiyo Farm.
2. MATERIAL AND METHOD

2.1. Schematic overview of study

This study was conducted on November 2013. The study location was in Chaiyo Farm, Suratthani, Thailand. All the samples that collected from Chaiyo Farm were analyzed in the laboratory of Food Science and Technology, Faculty Science and Industrial Technology, Prince of Songkla University, Suratthani Campus, Thailand. A schematic overview of the experimental study is shown in Figure 2.

![Flow diagram of the methodology used in the study.](image-url)
2.2. Making of Chili Paste Mushroom

The ingredient for making chili paste mushroom consists of chili, lemon grass, garlic, red onion, mushroom (*Schizophyllum commune*) and soybean oil. The mushroom had already sorted before using, but it was not being washed due to decision of the Chaiyo Farm owner. See appendix 1.

For the beginning to make the chili paste mushroom, all the ingredient should be roasted in cooking pan separately. The mushroom was roasted about ten minutes and the spices (chili, lemon grass, garlic, and red onion) were being roasted until the smell of the ingredients came out (approximately 2 or 3 minutes). After that, the spices would be crushed and blended in blender.

The mixing of roasted mushroom and crushed spices were followed by addition of soybean oil. The mixture was cooked together about 35 minutes. Preservative was added when the cooking time almost finished. After that, the cooked mushroom with preservatives was cooled for 20 minutes.

2.3. Chaiyo Farm GMP Assessment

This step aims to provide information on the current hygienic and manufacturing practices adopted in the factory before the improvement of GMP. The internal audit was carried out by an audit checklist. Audit checklist was made as Primary GMP Thailand Guideline.

2.4. Sample Collection

As much as 3 kg of final products were taken randomly for microbiological, chemical, and sensory analysis. These samples were packaged in plastic cup with cap. The final products that be analyzed were 1.5 kg normal Chili paste mushroom and 1.5 kg Chili paste mushroom with food preservative (prophyl-paraben and calcium propionate). Each cup has 50 gram of sample. Every analysis took one cup of sample. Sample was used at 10 hours after arrived in the laboratory.

2.5. Microbiological Analysis

The microbial analysis that used in this study are total aerobic bacteria (Plate-Count Agar), total yeasts & molds (Malt Extract Agar). From the several batch of food product, samples were collected in 40 cup packages by random. From each cup of samples, it was weighed approximately 5 gram sample aseptically. After that, It put into sealed plastic bag. Dilution water (buffered-phosphate water) 45 ml was added into plastic bag, then it stomached manually by hand. This becomes the 1:10 dilution. Furthermore, the dilution was mixed into a 90 ml dilution blank to make the 1:100 dilutions. This procedure was repeated to prepare serial dilutions of $10^{-1}$. All dilutions were shook 25 times (FDA, 2001a).
2.5.1. Total Aerobic Plate Count (Bacteriological Analytical Manual Chapter: Aerobic Plate Count)

Butterfield’s Phospate-Buffered Dilution Water

Stock Phosphate Buffer Solution was made by dissolving 34 g potassium dihydrogen phosphate, KH$_2$PO$_4$ in 500 ml distilled water. Then, the solution was adjusted to pH 7.2 with 1 N NaOH, and diluted to 1 liter. 1.25 ml stock phosphate buffer solution was taken and mixed with distilled water to 1 liter. After that, the mixture was dispensed in bottle that would provide in 99 ml and/or 9 ml. Before used, it was sterilized by autoclave at 121°C for 15 minutes (FDA, 2013).

Plate Count Agar (PCA) Media

In making PCA, the ingredients were 5 g tryptone, 2.5 g yeast extract, 1.0 g glucose, 15.0 g agar. All the ingredients were dissolved into 1 liter distilled water. The value of pH solution was adjusted at 7.0 by 1N NaOH. After that, The media was sterilized by autoclave 121°C, for 15 minutes (Askar and Treptow, 1993).

Plating and incubation of sample

Using separate sterile pipets, the dilutions of $10^{-2}$, $10^{-3}$, $10^{-4}$ of food homogenate were prepared by transferring 10 ml of previous dilution to 90 ml of diluents. Those dilutions were shook for 25 times in 30 cm (1 ft) arc within 7 s. Then, it was pipeted 1 ml of each dilution into separate, duplicates, appropriately marked petri dishes. Every petri dishes were added 12-15 ml plate count agar (cooled to 45 ± 1°C) to each plate within 15 min of original dilution. The sample dilution was mixed with agar medium thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on flat level surface. After agar solidified, it was inverted and incubated promptly for 48 ± 2 h at 35°C.

Reported all aerobic plate counts (2) were computed from duplicate plates. The counted plates outside the normal 25-250 range were not used due to give erroneous indications of the actual bacterial composition of the sample. Plates with 25-250 CFU were counted with this formula:

$$N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2)] \times (d)}$$

where:

- $N$ = Number of colonies per ml or g of product
- $C$ = Sum of all colonies on all plates counted
- $n_1$ = Number of plates in first dilution counted
- $n_2$ = Number of plates in second dilution counted
- $d$ = Dilution from which the first counts were obtained (FDA, 2001b).
2.5.2. **Total Yeasts and Molds (Bacteriological Analytical Manual: Yeasts, Molds and Mycotoxins Method)**

*Malt Extract Agar (MEA) Media*

In making MEA, the ingredients that needed were 20 g malt extract, 20 g glucose, 1.0 g peptone, 20.0 g agar, 1.0 liter distilled water. The ingredients were mixed, heated to dissolved agar and sterilized at 121°C for 15 min. Media was tempered to 45°C and poured into the plates under aseptic conditions (FDA, 2001c).

**Plating and incubation of sample**

Spread-plate method was used for plating in this analysis. Aseptic pipet was used to take 0.1 ml of each dilution on pre-poured, solidified MEA agar plates. After that, the inoculum was spreaded with a sterile, bent glass rod. Incubation of the plates was taken in the dark room at 25°C for 5 days. Plates were not stacked higher than 3 and did not invert. The plates were undisturbed until counting. The counting of plates was started after 5 days of incubation. Counted plates was containing 10-150 colonies and reported results was in colony forming units (CFU)/g or CFU/ml based on average count of triplicate set (FDA, 2001d).

2.5.3. **Culture Settling Plate Technique**

This method was used to measure the microbiological air quality of Chaiyo Farm processing area and laminar flow in PSU laboratory. Media PCA and MEA were exposed on the air, in the processing area and laminar flow for 15 minutes. Then it was incubated as same as final product procedures. To calculate the number of microbial (total aerobic bacteria or total yeast & mold), formula below was used:

\[ N = \frac{C_{\text{aver}} \times 60 \text{ min}}{15 \text{ min}} \times \frac{1 \text{ week}}{1 \text{ hour}} \times \frac{1}{168 \text{ hours}} \times \frac{1}{\text{plate wide (cm}^2\text{)}} \]

where:

- \(N\) = Number of colonies per ml or g of product (cfu/week/cm\(^2\))
- \(C_{\text{aver}}\) = Average of all colonies on two plates (Salustiano *et al.*, 2003).

2.5.4. **Rinsing Test**

*Ringer Rinse Solution*

To prepare Ringer solution, as much as 2.15 g sodium chloride, 0.075 g potassium chloride, 0.12 g calcium chloride, 0.5 g sodium thiosulfate were dissolved in distilled water up to 1 liter. The solution was sterilized in autoclave for 15 minutes, at 121°C (Askar and Treptow, 1993).

*Rinsing test method and calculation*

It used rinse method to determine microbiological quality of packaging. As much as 20 ml Ringer rinse solution was added to a packaging cup and recap it aseptically. The cup was shaken for 10 minutes. It was taken 1 ml of the rinse solution and put into a plate. It was carried out in two replicates. Desired medium (PCA and MEA) was poured into the plate as much as 15 ml. PCA plates were incubated promptly for 48 ± 2 h at 35°C, whereas MEA plates were incubated in the
dark at 25°C for 5 days. To calculate the number of microbial (total aerobic bacteria or total yeast & mold), formula below was used:

\[ N = C_{\text{aver}} \times \text{rinse solution volume} \]

where:
\( N \) = Number of colonies per cup (cfu/cup)
\( C_{\text{aver}} \) = Average of all colonies on two plates, (Askar and Treptow, 1993).

2.6. Physical analysis

2.6.1. Measurement of pH value
pH-meter Docu-pH+ meter (model) Sartorius was used to measure pH value. Before used, the calibration was carried out by buffered-water with pH value 4, 7, and 10.

2.6.2. Water Activity (aW)
Water activity of sample was measured five replication using AquaLab® Model Series3. The weight of sample that was used for one measurement approximately about 2 – 3 grams. Before measuring the sample, calibration of the equipment was done by using distilled water for the maximum value.

2.6.3. Color Measurement
Miniscan XE Plus® Model No.45/0-L was used to measure the color of sample in 0, 3, and 6 days. This instrument defines color numerically in terms of its lightness or “L” value, “a” value for greenness (-) and redness (+) and “b” value blueness (-) and yellowness (+) Illuminant D65 and 10 Observer were used in the active view option. The weight of sample that was used for analysis was 25 gram for each replicates. Each sample was measured ten replicates.

2.6.4. Direct Observation
Direct observation was conducted by the researcher for detecting all physical changes that make the product was not appropriate anymore. In this observation, the visible growth of yeast and moulds was became the major concern. On the 0, 3rd, and 6th day, all the food samples was observed to check the changes in odor, physical appearance, and the presence of slimy surface.

2.7. Statistical analysis
Experimental data were processed by multivariate ANOVA and Tukey as post-hoc analysis with statistical significance at 95%. Analysis was conducted by using SPSS software (SPSS Student Version 16.0 for windows) (Priyatno, 2011).
3. RESULT AND DISCUSSION

3.1. Good Manufacturing Practices Assessment

Good Manufacturing Practices (GMP) assessed by filling the requirement form and compared with their facility (see appendix 2 and 3). The result of every requirement was divided into two option, those are conformity and non-conformity. The result of assessment showed in Table 1.

Table 1. Conformity and Non-conformity of Chaiya Farm assessed GMP

<table>
<thead>
<tr>
<th>Assessed Aspects</th>
<th>Conformity (%)</th>
<th>Non-Conformity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location and production buildings</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Tools, machinery and production equipment</td>
<td>33.33</td>
<td>66.67</td>
</tr>
<tr>
<td>Control of production process</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Sanitation</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Cleaning and maintenance</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Personnel and employee hygiene</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td><strong>AVERAGE</strong></td>
<td><strong>20.59</strong></td>
<td><strong>79.41</strong></td>
</tr>
</tbody>
</table>

3.1.1. Location and Production Building

GMP audit of production building at Chaiya Farm showed that home industry still need a lot of improvements. The production location of Chaiya Farm was exposed directly onto the road and it was not closed working area, so the dust from the road could be easily got into the production area. The production area was also connected with the owner house so there were lot of unnecessary materials and the pets of owner (a dog, a cat, and birds) was staying nearby the production place.

To improve the location and production building of Chaiya Farm, some corrective action was needed. It needs to make a room or a building that will be used to make the product. This room should be used only to make the product, so the number of people and irrelevant things or materials can be controlled. Besides that, making a production room will reduce dust and contamination from the road and residential areas.

3.1.2. Tools, Machinery, and Production Equipments

Tables and surfaces used during food production were made from ceramics, so this was conformed as one of GMP point, due to its ability as non-rusting material. But, there were also non-conformed points for the tools and equipment that was used for making the product. The design of equipment and
3.1.3. Control of Production Process

All the raw materials and ingredients were sorted, washed, and rinsed, except mushroom. In the process, mushroom was not washed. It was only sorted. To wash the ingredients, food handler used water from the sink. When the raw materials were ready, they started to cook the product without washing their hand before it.

In the cooking process, it was started with roasting the mushroom and chili sauce separately. After that, it was mixed together and cooked with soybean oil for approximately 30 minutes. When the product almost finished, food handler also had habit to take the mushroom product for testing by hand. It could be a source of contamination as well. She shall use a clean and sanitized spoon to test the final product taste.

After cooking process was done, the mushroom product was cooled in room temperature about 20 minutes then packed. Area to cool and pack the mushroom was near the road. It was right next to the stove for cooking process. The contamination from air might be high. So, cooling and packaging process should be carried out in enclosed space.

Hand washing is important to prevent contamination in food from the worker. The contaminations caused by hands are the second-leading cause of foodborne illness and hand washing is the single most effective means to prevent that (Cramer, 2013). The worker need to wash their hands before starting work, after handling raw materials, after using the toilet, after handling waste, after cleaning duties, after eating or smoking, after blowing nose/sneezing/coughing, and after handling money.

The owner of Chaiyo Farm did not wash the mushroom before cooking the product. She said it was used to prevent increasing the water content of products. Raw materials should be washed or cleaned before processing, it is necessary to remove soil or other contamination on it (Smith and Hui, 2004). If it cannot be washed, the owner should apply Good Agricultural Practices (GAP) for their mushroom farm. The GAP implementation will make sure the quality and safety of raw mushroom suitable for human consumption.

3.1.4. Sanitation, Cleaning, and Maintenance

Chaiyo Farm cleaned their production area just one time after the cooking process was done. Production table was cleaned by using wiping cloth that only cleans it from physical debris. The equipments that would be used in the production were not inspected and maintained properly, they just washed it after cooking and the place to store the equipment was rarely cleaned.

According to United States Food and Drug Administration (1996), sanitation of food-contact surface including utensils and equipment, shall be cleaned as frequently as necessary to protect against contaminant of food. The cleaned portable equipment and utensils should be stored in a location and manner that protect food-contact surface from contamination.
Furthermore, US Food Code (1996) explained that cleaned equipment and utensils, laundered cloths shall be stored in a clean, dry location that is not exposed dust or other contamination, at least 15 cm (6 inches) above the floor. Draining position of clean equipments and utensils shall allow air drying and covered. Utensils/ items that are kept in closed packages may be stored less than 15 cm (6 inches) above the floor on dollies, pallets, racks, and skids.

3.1.5. Personnel and Employee Hygiene

The Owner of Chaiyo Farm had a rule to prohibit the sick employee to come for working, especially the contagious one. The owner checked it by asking about their health condition every day before the production started. When the production about to start, the worker had to remove all of their accessories. Unfortunately, Chaiyo Farm did not have production clothes, hairnet, and shoes that specifically used only in the production room. The husband of owner, sometimes comes to production place while he was smoking.

Smoking and sick worker should not come into production place because many bacteria are found in the mouth and on the lips. During sneeze, cough, and smoking, some bacteria are transferred to the air and may land on food that being handled (Marriott and Gravani, 2006). The production clothes are needed to reduce the risk of contamination. On arriving at work, food handlers are likely to have contaminated clothing, footwear and hair. The clean clothes, shoes, and hairnet should be used before entering the production place and the contaminated outer street clothing should be stored in the changing room (Lelieveld et al., 2014).

3.2. Product Evaluation

3.2.1. Physical Evaluation

Water Activity

Water activity of chili paste mushroom on the zero day (10 hours after processing) was around 0.886 for the non-preservative and 0.896 for the one with preservative. Both of samples water activity was decreased on the third day, the preservative samples was around 0.887 and non-preservative was around 0.861. Finally, on the sixth day, all of the samples water activity was increased again, 0.911 for preservative samples and 0.880 for non-preservative samples (see Figure 3). Even so, the changes of water activity were not significant in storage and interaction factor (p > 0.05). On the other hand, the addition of preservative did affect the water activity of food product significantly (p < 0.05) and the statistical analysis is shown in appendix 8. This problem may caused by the cooking heat that was used to cook this product was different, because the addition of preservative will not affect the water activity of product (Smith and Lily, 2011).
pH of Samples

From the ANOVA statistical analysis in appendix 8, the pH of samples did not show any significant differences (p > 0.05) due to preservatives addition and interaction factor. The data showed that the pH was affected by storage time (p < 0.05). From the post-hoc, it showed that pH was likely to increase on the sixth day. The pH of chili paste mushroom was measured on the zero, third and sixth day with following result: 5.68, 5.68, and 5.84 for non-preservative samples and 5.72, 5.65, and 5.77 for the preservative samples (see appendix 4).

According to Homel (2014), strong fermentative yeast can metabolize some weak acid such as citric and lactic acid, this metabolism will increase the food pH. Besides that, it can also be caused by proteolytic bacteria such as Pseudomonas which can produce ammonia that tend to raise pH of food product (Ravishankar and Juneja, 2014). The increased pH value may cause harm for the food product, due to give the appropriate pH range which the pathogenic organism can grow (see Figure 4).

Color Measurement

The ANOVA (appendix 8) showed that chili paste mushroom with preservative and without preservative was showing significant differences (p <0.05) for all color parameters. The b* value of both of samples was different due to the storage times (p < 0.05). From the interaction of preservative with
storage time, the statistical analysis was also shown significance different in $a^*$ value ($p < 0.05$).

The redness ($a^*$ value) of preservatives samples was relatively stable, different with the non-preservatives samples that shown decreasing result (see Figure 5). This was caused by the preservatives in the samples could inhibit oxidation that will change the color of product (Kuleasan and Okur, 2012). This means, the red color stability of chili paste mushroom was affected by preservative. This is important because color will be the first quality attribute of food evaluated by consumers that provide the basic quality information for human perception, and has close association with quality factors such as freshness, maturity, variety and desirability, and food safety (McCaig, 2002).

![Figure 5. $a^*$ value of samples on the zero, third, and sixth day](image)

The result of $L^*$ value was 37.27, 35.64, and 36.54 for the preservatives samples from zero until sixth day and 31.52, 30.20, 31.64 for the non-preservatives samples. The $b^*$ value was about 19.55, 19.75, 21.93 for the preservatives samples and 15.49, 15.46, 17.77 for the non-preservatives samples. The $L^*$ and $b^*$ value difference was shown in Figure 6 and Figure 7. Actually, the addition of preservative did not affect color of food product, due to the nature of propylparaben and calcium propionate which are colorless in solution (Batt and Tortello, 2014).

![Figure 6. $L^*$ value of preservatives samples and non-preservatives samples](image)
From the post-hoc test (see appendix 8), the $b^*$ value of both of samples was only increasing on the sixth day (see Figure 8). According to Chen and Gutmanis (1968), the lipid of food product was dissolving beta-carotene and surrounded by protein globules that was concealing the color. But after the microorganisms break the lipid and protein cluster loosen, the beta-carotene was visible.

**Direct Observation**

Direct observation of product showed that after the third day, non-preservation samples were not able to be accepted due to the visible presence of molds. It shows in Figure 8 as white patches on the products. The result was different for samples with preservative addition, the molds growth still could not be seen.
On the sixth day, the samples with preservative were already containing visible molds that showed in Figure 9. It means the shelf life of product with preservative was longer than non-preservative. The addition of preservative could hold the growth of visible mold at least for three days.

3.2.2. Microbiological Evaluation

Total Aerobic Plate Count and Total Yeast and Mould

Analyses for microbiological content were performed with total aerobic plate count in Plate Count Agar media and total yeast and molds in Malt Extract Agar media. The samples were stored from 2 December 2013 to 8 December 2013 in room temperature and every three days these samples were analyzed. The result was showed in Table 2.
Table 2. Total Aerobic Plate in PCA (cfu/gr) and Total Yeast and Molds in MEA (cfu/gr)

<table>
<thead>
<tr>
<th>Media</th>
<th>Non-Preservative</th>
<th>Preservative</th>
<th>Packaging</th>
<th>Environment</th>
<th>Laminar Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA</td>
<td>Spreader</td>
<td>Spreader</td>
<td>&lt; 1 cfu/cup</td>
<td>11 x 10^2</td>
<td>&lt; 1 cfu/week/cm^2</td>
</tr>
<tr>
<td></td>
<td>Spreader</td>
<td>Spreader</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.54 x 10^3</td>
<td>7.23 x 10^3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.20 x 10^4</td>
<td>7.00 x 10^3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.26 x 10^5</td>
<td>7.35 x 10^5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEA</td>
<td>Spreader</td>
<td>Spreader</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total aerobic plate count of chili paste was resulting spreader, it was suspected to be caused by *Bacillus spp*. Almost all of bacteria in the genus *Bacillus* are implicated as human foodborne pathogen. *Bacillus* is thermal tolerance bacteria by forming endospores, these organisms may dominate as spoilage organism after the food product has undergone mild heat-treatment process. Contamination of *Bacillus spp* in food generally is considered to be via the entry of soil, air, and water (Jenson, 2014).

On the other hand, total yeast and molds shows countable results. From the zero day, both of samples had a similar result in 7 x 10^3 cfu /gr. The difference started to show in the third day, the number of yeast and mold in the non-preservative sample increased up to 10^6 cfu /gr and the one with preservative still in the 7 x 10^3 cfu /gr. On the sixth day, the number of yeast and mold colonies in the preservative samples was outnumbered the non-preservative. The rapid growth in the preservative samples may result of the calcium propionate presence. According to Smith and Lily (2011) calcium propionate can be used as a yeast nutrient, and it does not have any inhibition activity toward yeast growth. Propyl-paraben, one of the preservative which used to prevent growth of yeast and mold in sample, may not work properly. Lou and Yousef (1999) explained that propyl-paraben activity can be affected by the presence of oil. When food product has high oil content, propyl-paraben will be dissolved in oil and reduces the availability to inhibit microorganisms in water phase.

The result of chili paste mushroom in Chaiyo Farm was not appropriate for consumption. According to Codex Alimentarius Commission (1972), the maximum limit yeast and molds in ready-to-eat food is not more than 300 cfu / g. This may be caused by the contamination level in the production area was too high. To reduce this number, processing of chili paste mushroom should use microbiological quality approach.

Packaging of product and laminar flow results show about less than 1 cfu/cup and 1 cfu/week/cm^2 for the total aerobic plate count. Besides that, the air quality of production plant had total aerobic plate count as much as 11 x 10^2 cfu/week/cm^2 and spreader for the yeast and mold. The result of production plant air quality should be not more than 3 x 10^1 cfu/week/cm^2, according to American Public Health Association (APHA) standards (Salustioano et.al., 2003). This air quality could contaminate the product during cooling process, hot filling may be used to reduce the risk.
3.3. **Standard of Production Process**

Based on the data of this research, the production process in making of chili paste mushroom should be had standard for every process steps. This standard hopefully will help Chaiyo Farm to improve the quality and safety of the product.

**Storage and Sorting Raw Materials**

The raw materials were stored in the refrigerator before it will be used. This was helping the producer to extend the shelf-life of raw materials. The optimal temperature storage of mushroom is 0°C with normal O₂ (Chakraverty *et al.*, 2002). Besides that, the producer also has to use the raw materials that were stored first to avoid using expired ingredient.

Even though storing raw materials in refrigerator will keep the quality of raw materials longer, it still needs to be sorted before use. Sorting process become important to remove poor quality raw materials that can affect the final product. The sorting process also helps to procure materials whose properties most closely match with the requirement.

**Roasting and Stir-Frying Process**

Roasting and stir-frying are thermal processing in making of chili paste mushroom. The main reason of heating food is inactivating pathogenic and spoilage microorganism. This process also induces physical changes and chemical reactions, which in turn affect the sensory characteristics, such as color, flavor and texture (Brennan, 2006).

From the data of physical analysis, chili paste mushroom can be contaminated by pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*. Due to *Bacillus cereus* ability to form endospores, it becomes the most heat resistant bacteria in this product. *Bacillus cereus* one of bacteria in *Bacillus* genus, which is the common contaminate in cooked product. It also should be noted that spices often contain large number of aerobic spore-bearing bacilli including *Bacillus cereus*. *Bacillus cereus* is a pathogenic bacteria by producing toxin that will give diarrhea and vomiting symptoms (Bottone, 2010).

To reduce the *Bacillus cereus* in chili paste mushroom, the owner should look carefully at the temperature of cooking and cooling process. The value of D for *Bacillus cereus* is at 100°C range from 2.2 to 5.4 minutes (Batt and Tortorello, 2014). This means if Chaiyo Farm wants to reduce 1 log cycle of *Bacillus cereus*, they should make the product temperature at 100°C from 2.2 to 5.4 minutes. This D value is already surpass the yeast and mold D value, which usually at range of 60°C to 85°C (LDz-base, 2007) and this heating process will also reduce the number of yeasts and molds in the chili paste mushroom. The addition of water in the cooking process also can help to distribute the heat, so it will help to reduce the number of pathogenic bacteria.

**Cooling and Packaging of Product**

Cooling process become dangerous due to unsanitary production area of Chaiyo Farm. The cooling process should be carried out in the room that...
microbial content less than $3 \times 10^1$ cfu/week/cm² and it also should be done as fast as possible. After the cooling process is finished, chili paste mushroom should be packaged and stored in the refrigerator before it will be taken into the shop.
4. CONCLUSION AND RECOMMENDATION

4.1. Conclusion

Chaiyo Farm as the producer of chili paste mushroom was not conformed with Thailand’s Good Manufacturing Practices. It could only pass almost 21% of Thailand’s GMP standard. Furthermore, preservative addition did not affect the pH of chili paste mushroom. The preservatives also did not affect the microorganism on the sixth day due to the high oil content in it. Color and water activity from both of samples was different caused by the cooking heat was different. There were also found a significant difference between the zero day and sixth day mushroom on the b* value due to lipid breakdown. The preservatives also could help to maintain the redness of product. For the shelf life of product, visible molds growth became the limit by direct observation method. Non preservative samples were only stand less than 3 days in the room temperature and the samples with preservative could be kept up less than 6 days.

4.2. Recommendation

The application of GMP was recommended for Chaiyo Farm. It can help to improve the product quality and shelf life. The usage of hot filling method should be studied to reduce contamination risk. Further research was needed to determine specific molds that grow on product. If the specific molds were known, the preventive and corrective action can be done more efficient. Besides that, the usage of another preservative also should be known, especially preservative that can work in oil.
Bogor Agricultural University (Institut Pertanian Bogor)
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1. Hak Cipta Ditunduk Undang-Undang.
2. Hak Cipta Cipta Milik IPB (Institut Pertanian Bogor)

2. Hak Cipta Ditunduk Undang-Undang.
3. Hak Cipta Ditunduk Undang-Undang.
4. Hak Cipta Ditunduk Undang-Undang.

Bogor Agricultural University
APPENDIX


Mushroom from refrigerator

Sorting

Roasting (10 minutes)

Soy oil

Stir-frying (30 minutes)

Food preservative added, Cooling (20 minutes)

Preserved Chili Paste Mushroom

Spices (Chili, lemon grass, garlic, red onion)

Roasting (2-3 minutes)

Crushing

Chili paste

Cooling (20 minutes)

Packaging

Non-preserved Chili Paste Mushroom

Packaging
Appendix 2. Chaiyo Farm’s Good Manufacturing Practices assessment result.

<table>
<thead>
<tr>
<th>No.</th>
<th>Aspect Assessed</th>
<th>The requirements</th>
<th>C</th>
<th>NC</th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Location and production buildings</td>
<td>Buildings and their surrounding locations shall be clean and free from accumulated, unused materials, including those which may attract animals, insects, and/or pathogenic microorganisms.</td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Buildings shall be located away from areas that are exposed to high amounts of dust.</td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Buildings shall not be located near any repugnant areas.</td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Buildings shall not be located in dirty or flooded areas and shall have a well-drained pipeline connected to public sewage pipelines. In the case that food production buildings are located in unsuitable areas and do not adhere to phrases 1.1.1-1.1.4, there shall be standard measures in place to protect food products from, and eliminate insects and other carriers of infection and dust, as well as other sources of contamination.</td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The production building and surrounding area shall be clean and maintain in good sanitation.</td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Buildings and production areas shall maintain in good sanitation by eliminating all unused or irrelevant materials from production areas.</td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Production areas shall be isolated from residential areas.</td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All production areas shall have adequate lighting and ventilation for employees to be able to work to their highest potential.</td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Internal pipeline shall be provided to remove all waste water from manufacturing processes to the public sewage system.</td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protective measures shall be provided ensuring that no animals or insects pass through the production area or come in contact with food products</td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>2</td>
<td>Tools, machinery and production</td>
<td>Tools and equipment shall be appropriately designed, considering contamination may occurred and ease and thoroughness all over of cleaning for equipment, tools and install area.</td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td></td>
<td>Equipment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Production vessels or equipment which come into contact with food shall be made of non-hazardous materials, cause no harm to consumers and be easy to clean.</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Tables and surfaces used during food production shall be made of non-rusting materials, be easy to clean, not react with food products and not be harmful to consumers’ health. Specifically, the production area should have tables and surfaces of suitable height for ease of cleaning, to perform measures to protect food products from, and for removing contaminants.</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ingredients and raw materials used in production shall be clean, of good quality, and suitable for human consumption. Wash and rinse raw materials when necessary to remove dirt and contaminants. Store raw materials in a suitable environment to reduce spoilage and deterioration, and rotate raw food materials that are on stock to reduce chances of using expired ingredients. If food additives will be used, it is necessary to follow the notification from the Ministry of Public Health, Re: Food Additives.</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Every step involving raw materials and production ingredients including receiving, transferring, preparation, production, packaging, and transportation shall be controlled in good sanitation.</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vessels for packaging and transportation of ingredients in food production shall be suitable and shall not contaminate food products.</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water to be used in food production and processing shall be clean and of good quality as defined by the Ministry of Public Health, Re: Drinking Water and its Sanitary Utilization.</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ice and steam utilized in food processing which may come in contact with food and/or be incorporated into food products shall be complied with the notification from the Ministry of Public Health, Re: Ice and Drinking Water and their Sanitary Utilization.</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Production, storage, transfer, and transportation methods of food products shall protect food products from contamination and deterioration.</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Any water used in the factory shall be clean and treated according to government standards.</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adequate amounts of lidded trash cans and proper trash elimination systems for all types of trash must be available.</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>There must be an adequate number of toilets and sinks proportional to the number of workers, and these</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
facilities must be maintained in sanitary conditions. It should be regularly ensured that all sinks and toilets are in proper working order. Sinks for hand washing shall be fully equipped. Toilets shall be isolated from production areas and should not be directly exposed to production areas.

<table>
<thead>
<tr>
<th>C</th>
<th>Cleaning and maintenance</th>
<th>Facilities must be maintained in sanitary conditions. It should be regularly ensured that all sinks and toilets are in proper working order. Sinks for hand washing shall be fully equipped. Toilets shall be isolated from production areas and should not be directly exposed to production areas.</th>
</tr>
</thead>
</table>

| C | Adequate amounts of fully equipped hand washing sinks in the production area must be provided. |
| NC | Proper and effective sewage systems for waste water well as dirt gutters for waste material which will not contaminate any part of the food production process or food products, must be instated. |

<table>
<thead>
<tr>
<th>C</th>
<th>5 Cleaning and maintenance</th>
<th>Production buildings shall be clean and maintained at all times.</th>
</tr>
</thead>
</table>

| C | Tools, machinery and production equipment shall be inspected and maintained for efficient use at all times. |
| NC | Chemical agents for washing and/or chemicals for production shall be stored and used under safe conditions and be isolated from production areas. Additionally, they must all have clear, correct labeling. |

<table>
<thead>
<tr>
<th>C</th>
<th>6 Personnel and employee hygiene</th>
<th>Workers and personnel in production areas shall not be infected with any communicable or contagious disease as prescribed in the Ministerial regulation, or have any wound which may contaminate equipment or food products.</th>
</tr>
</thead>
</table>

| C | Wear clean and proper clothes, suitable for work. If a coat is worn, it shall also be clean. |
| NC | Wear appropriate shoes for the production facility, for example have shoes specifically to be worn in the production facility, or place shoes in sanitizing chemical before entering the production facility to prevent contaminants from entering the facility. |

| C | Shall not wear jewelry while working and keep their hands and nails healthy and clean at all times. |
| NC | Shall keep clean nails and wash their hands and nails before work and after any kind of contamination. |

| C | Shall wear a hat, head covering, or hairnet at all times in the production facility. |
| NC | All workers and personnel shall be warned against and prevented from expressing inappropriate behavior in the production facility. Such behavior includes smoking and spitting. |

| C | All workers and personnel not involved with food production shall adhere to procedures like food handlers when in the production facility. |

C = Conformity ; NC = Non-Conformity ; NA = Not Available.
Appendix 3. Chaiyo Farm’s production building and manufacturing practices.

<table>
<thead>
<tr>
<th>Place/ Activity</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage area for packaging</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>Cooking Process</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Packaging Process</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>Production area</td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Appendix 4. Chili paste mushroom physical analysis result.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day</th>
<th>Water Activity</th>
<th>Color Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>L*</td>
</tr>
<tr>
<td>Preservative in room temperature</td>
<td>0</td>
<td>0.8964</td>
<td>37.27</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.887</td>
<td>35.64</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.911</td>
<td>36.54</td>
</tr>
<tr>
<td>Non-preservative in room temperature</td>
<td>0</td>
<td>0.8862</td>
<td>31.52</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.861</td>
<td>30.20</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.880</td>
<td>31.64</td>
</tr>
</tbody>
</table>

Appendix. pH Value of Chili Paste Mushroom

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Non-preservative chili paste mushroom in room</td>
<td>5.68 ± 0.055</td>
</tr>
<tr>
<td>preservative chili paste mushroom in room</td>
<td>5.72 ± 0.015</td>
</tr>
</tbody>
</table>

Appendix 5. Total plate count on PCA medium.

<table>
<thead>
<tr>
<th>Sample</th>
<th>0</th>
<th>3</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preservative</td>
<td><img src="https://example.com/image1.png" alt="Image" /></td>
<td><img src="https://example.com/image2.png" alt="Image" /></td>
<td><img src="https://example.com/image3.png" alt="Image" /></td>
</tr>
<tr>
<td>Non-preservative</td>
<td><img src="https://example.com/image4.png" alt="Image" /></td>
<td><img src="https://example.com/image5.png" alt="Image" /></td>
<td><img src="https://example.com/image6.png" alt="Image" /></td>
</tr>
</tbody>
</table>
### Appendix 6. Total yeast and mold in MEA medium.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day</th>
<th>0</th>
<th>3</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preservative</td>
<td></td>
<td><img src="image1.jpg" alt="Image" /></td>
<td><img src="image2.jpg" alt="Image" /></td>
<td><img src="image3.jpg" alt="Image" /></td>
</tr>
<tr>
<td>Non-preserved</td>
<td></td>
<td><img src="image4.jpg" alt="Image" /></td>
<td><img src="image5.jpg" alt="Image" /></td>
<td><img src="image6.jpg" alt="Image" /></td>
</tr>
</tbody>
</table>

### Appendix 7. Environment processing plant and laboratory microbial.

<table>
<thead>
<tr>
<th>Place</th>
<th>MEA</th>
<th>PCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment of Chaiyo Farm</td>
<td><img src="image7.jpg" alt="Image" /></td>
<td><img src="image8.jpg" alt="Image" /></td>
</tr>
<tr>
<td>Packaging Product</td>
<td><img src="image9.jpg" alt="Image" /></td>
<td><img src="image10.jpg" alt="Image" /></td>
</tr>
<tr>
<td>Laminar Flow</td>
<td><img src="image11.jpg" alt="Image" /></td>
<td><img src="image12.jpg" alt="Image" /></td>
</tr>
</tbody>
</table>
Appendix 8. Statistical analysis for physical attributes of chili paste mushroom.

<table>
<thead>
<tr>
<th>Source</th>
<th>Dependent Variable</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>L</td>
<td>137.274^a</td>
<td>5</td>
<td>27.455</td>
<td>28.442</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>19.146^b</td>
<td>5</td>
<td>3.829</td>
<td>21.174</td>
<td>.000</td>
</tr>
<tr>
<td></td>
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R Squared = .922 (Adjusted R Squared = .890)
R Squared = .898 (Adjusted R Squared = .856)
R Squared = .852 (Adjusted R Squared = .790)
R Squared = .694 (Adjusted R Squared = .567)
R Squared = .574 (Adjusted R Squared = .397)

Tukey HSD

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Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 1.460.
### pH

#### Tukey HSD

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Sig. = .563 1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .003.
AUTHOR BIOGRAPHY

Brian was born on 8th December 1990 in Yogyakarta, Indonesia. He is the third son with two elder brothers and one younger brother from Mr. Djoko Minarso and Mrs. Lanneke Tjahja. He finished his elementary school in Tarakanita Yogyakarta, junior high school in Stella Duce 1 Yogyakarta, and senior high school in Kolese De Britto Yogyakarta. In 2009, the author continued his further study in Bogor Agricultural University as a Food Science and Technology student. During study, he joined some organizations and activity in campus such as Food Processing Club (FPC), Orde Keramat, and BAUR. Brian also joined DSDC IFT competition and got a runner-up position together with his team in 2012. In his last year study time, he became a finalist in IUFoST Student Fighting Hunger competition and joined a student exchange program AIMS (Asean International Mobility for Student) in Prince of Songkla University, Suratthani, Thailand. He did his final project which entitled “Good Manufacturing Practices Assessment and Shelf-life Analysis of Chili Paste Mushroom in Chaiyo Farm” during the exchange program in Prince of Songkla University.