Quality of Chocolate Bar from Fermented Cocoa Beans from Lombok, West Nusa Tenggara

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Abstract. About 500 kgs cocoa beans have been fermented using box fermentation. The fermentation experiments were conducted at the Gangga district North Lombok, West Nusa Tenggara - Indonesia. The starter culture, which contains yeast, lactic acid bacteria, and acetic acid bacteria (10⁷/mL) was added to cocoa beans mass in box fermentation. The fermentation was conducted for 5 days and every 24 hours were mixed. After fermentation, the cocoa beans were dried for 5 days to reduce the moisture content using a sun drying. Dried fermented cocoa beans were processed to be a chocolate bar. The quality of cocoa liquor was determined using organoleptic score. Chocolate aroma, chocolate flavour, and caramelly score were 8.25. The texture and colour score were 8.00 and off flavours score was 0.00. Overall quality of cocoa liquor score was excellent. Analysis of proximate and microbiological properties was carried out to obtain the chocolate bar qualities. The result showed that quality of chocolate bar from fermented cocoa beans appropriate with SNI 7934:2014 about chocolate and chocolate products.

Keywords: Theobroma cacao L, box fermentation, starter culture, chocolate bar.

1. Introduction
Cocoa is a fruit seed from the tree of Theobroma cacao L., which can grow in tropical regions such as Indonesia, Central, and South America and West Africa [1,2,3]. One of the most preferred cocoa beans products is chocolate because it has a unique taste and has a positive effect on health due to it contains a number of antioxidants.

The fermentation of cocoa beans is still traditional and uncontrolled and an adequate process together with an appropriate selection and handling of raw materials. Drying and roasting can influence the character of chocolate [4]. Fermentation process involves complex microbial activities and biochemical changes that have been recently deeply reviewed by Schwan and Fleet [5]. The microorganisms responsible for the fermentation are yeast, lactic acid bacteria (LAB) and acetic acid bacteria (AAB). Recently, Ho et al. [6] elucidated the importance of yeast growth and activity for successful cocoa beans fermentation and their involvement in the development of chocolate aroma.

The fermentation process of cocoa beans is important to phase in producing aromatic compounds as well as bioactive components in cocoa and chocolate products [2]. The fermentation of cocoa beans in the process of reforming sugar and citric acid in pulp into organic acids by a consortium of microbial [1]. The resulting organic acids will induce the enzymatic reactions present in the seeds resulting in biochemical changes that will form the compounds that give rise to the smell, taste, and colour of the cocoa [2]. Without fermentation process, cocoa beans will taste bitter and astringent, so it
cannot produce a distinctive aroma of chocolate when processed into chocolate products such as brown powder or brown paste [2].

The fermentation process is divided into 3 stages; the initial stage (0-36 h) is an anaerobic stage, in which yeast will convert sugars to alcohol in low oxygen and pH conditions below 4. The second stage is the dominant role of lactic acid bacteria to convert sugar and some organic acids to lactic acid. The third stage is the role of acetic acid bacteria in converting alcohol to acetic acid. During the fermentation process, cocoa pulp as a substrate containing sugar and citric acid will be converted into organic acids. The resulting organic acids will diffuse into the seeds and induce enzymatic reactions to form the candidate compounds of flavour, aroma, and colour [2].

The quality of fermented cocoa beans is determined by the acidity (pH). Processing cocoa requires a pH of seeds between 5.2-5.8 to produce quality cocoa butter [7]. In the cocoa fermentation process, the pH and total acid values are closely related to the process of seed death followed by acid diffusion into the seeds and the chemical reactions that affect the quality of the cocoa beans.

After the fermentation process, the cocoa beans are dried until the moisture content is below 7%. During the drying process, microorganism activity is still in progress in the formation of specific aroma, texture and colour characteristics [1, 3]. This study aims to determine the quality of chocolate stem produced from fermented cocoa beans from Lombok, West Nusa Tenggara.

2. Material and methods

2.1. Fermentation of cocoa beans

The raw materials used are 500 kgs of Forastero cocoa beans from Gangga district North Lombok, West Nusa Tenggara. The culture starter used for the fermentation process consists of yeast, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) (10^7 CFU/mL). The yeast was grown in Malt Extract Broth medium (peptone 3 g/L, malt extract 30 g/L), LAB was grown in DeMan Rogosa Sharpe Broth medium (52.2 g/L), AAB were grown in medium Glucose, Yeast Extract, Peptone Broth (D-glucose 20 g/L, yeast extract 5 g/L, peptone 5 g/L). Each isolate was inoculated into the medium, and incubated for 48 hours at 30°C, with shaking 150 rpm.

Fermentation was conducted for five days, using box fermentation. The starter culture was added by spraying into cocoa beans mass, and then the fermentation box is covered with banana leaves and stacked with another fermentation box. Every 24 hours cocoa beans mass were mixed to get O2 and to uniform, the cocoa beans mass. After five days of fermentation, cocoa beans were dried in the sun for five days until the water content decreased to 7%.

2.2. Chocolate preparation and analysis

The dried beans were processed to milk and dark chocolate using the facilities from Indonesian Coffee and Cocoa Research Institute (ICCRI) – Jember, East Java, Indonesia.

Sensory analysis of milk and dark chocolate were performed using a consumer acceptance test. The tests were conducted with 20 expert panelists. Samples were taken as much as 120 g of roasted beans, roasting with a temperature of 116°C for 25 minutes. The cocoa beans are peeled and then the seeds are milled to a fine paste. The fine cocoa paste is then heated in 50°C heater and tested organoleptically by a trained panelist.

The taste of chocolate paste was done organoleptically using hedonic test by 10 trained panelists from ICCRI. Characteristics of chocolate flavour tested include flavour, taste, acidity, caramel qualities, bitterness, texture, colour, and overall appearance. Scale for taste organoleptic test using 5 scale that is: 0 = zero, 1-2 = weak, 3-4 = weak enough, 5-6 = strong enough and 7-8 = strong. Scale for scent assessment, acidity, caramel nature, bitterness, texture, colour and overall appearance using 6 levels of scale i.e. 0 = unfit for consumption, 1-2 = very bad, 3-4 = bad, 5-6 = neutral, 7-8 = good and 9-10 = very good.

2.3. Analysis of chocolate bar quality

Analysis quality of chocolate bars (milk and dark chocolate) includes chemical and microbiological analysis. This analysis aims to determine the suitability quality of chocolate product with the standard quality of chocolate based on SNI 7934: 2014 [8]. The chemical analysis included proximate content in the form of fat content, non-fat cocoa solids, total cocoa solids, and metal contaminants. The
microbiological analysis included i.e. the total plate count (TPC), the amount of contamination *E. coli*, *Salmonella* sp., mold, and yeast.

### 2.3.1. Fat content
Fat content was analyzed based on the test method contained in SNI. 01-2891-1992 concerning how to test foods and beverages [9].

### 2.3.2. Total cocoa solids
Cocoa solids in chocolate are cocoa products used in the manufacture of chocolate i.e. cocoa mass, cocoa powder, and cocoa fat. Total cocoa solids are calculated using the formula:

\[
\frac{[(CM-(k_{a\text{CM}} \times CM)) + (CP-(k_{a\text{CP}} \times CP)) + (CB-(k_{a\text{CB}} \times CB))] + [(S-(k_{aS} \times S)) + (CB-(k_{a\text{CB}} \times CB))] + (CM-(k_{a\text{CM}} \times CM)) + (MP-(k_{a\text{MP}} \times MP)) + (CP-(k_{a\text{CP}} \times CP)) + (MF-(k_{a\text{MF}} \times MF))] \times 100}\%
\]

Where:
- CM = mass cocoa content in chocolate formula (%);
- KaCM = moisture content of cocoa mass used (%);
- CP = powdered cocoa content in chocolate formula (%);
- KaCP = moisture content of cocoa powder used (%);
- CB = cocoa fat content in brown formula (%);
- KaCB = moisture content of cocoa fat used (%);
- S = sugar content in brown formula (%);
- KaS = moisture content of the sugar used (%);
- MP = powdered milk content in brown formula (%);
- KaMP = moisture content of the milk powder used;
- MF = milk fat content used in brown formula (%);
- KaMF = moisture content of milk fat used (%)

The fatty cocoa solid is calculated using the formula:

\[
\frac{[(CM-(k_{a\text{CM}} \times CM)) + (CP-(k_{a\text{CP}} \times CP)) + (CB-(k_{a\text{CB}} \times CB))] + [(S-(k_{aS} \times S)) + (CB-(k_{a\text{CB}} \times CB))] + (CM-(k_{a\text{CM}} \times CM)) + (MP-(k_{a\text{MP}} \times MP)) + (CP-(k_{a\text{CP}} \times CP)) + (MF-(k_{a\text{MF}} \times MF))] \times 100}\%
\]

Total non-fat cocoa solids (% b / b, bk) :

\[
\frac{[(CM-(f_{CM} \times CM)) - (k_{a\text{CM}} \times CM)) + (CP-(f_{CP} \times CP) - (k_{a\text{CP}} \times CP))] + [(S-(k_{aS} \times S)) + (CB-(k_{a\text{CB}} \times CB))] + (CM-(k_{a\text{CM}} \times CM)) + (MP-(k_{a\text{MP}} \times MP)) + (CP-(k_{a\text{CP}} \times CP)) + (MF-(k_{a\text{MF}} \times MF))] \times 100}\%
\]

Where:
- CM = mass cocoa content in brown formula (%);
- fCM = fat content of cocoa mass used (%); 
- kCM = moisture content of cocoa mass used (%);
- CP = powdered cocoa content in brown formula (%); 
- fCP = fat content of cocoa powder used; 
- kCP = moisture content of cocoa powder used (%);
- CB = cocoa fat content in brown formula (%);
- kCB = moisture content of cocoa fat used (%); 
- S = sugar content in brown formula (%);
- kS = moisture content of the sugar used (%);
- MP = powdered milk content in brown formula (%);
- kMP = moisture content of the milk powder used; 
- MF = milk fat content used in brown formula (%);
- kMF = moisture content of milk fat used (%)

### 2.3.3. Microbial contamination analysis

#### 2.3.3.1. Total plate count
The sample was weighed 25 g and put into an Erlenmeyer containing 225 mL diluent solution. The mixture is then shaken to homogeneous and diluted to $10^{-5}$. Each dilution level was taken 1 mL and inserted into a sterile petri dish by duplo and then added 12-15 mL of PCA liquid medium. The plate was then incubated for 72 hours at 30°C. Total plate count is calculated by the formula:

\[
\text{TPC} = \frac{C}{(1x\text{n1})+(0.1x\text{n2})x\text{d}}
\]

Where:
- C = number of colonies of each petri dish;
- n1 = number of Petri dishes of the first dilution calculated;
- n2 = number of Petri dishes from the second dilution;
- d = first dilution calculated

#### 2.3.3.2. Analysis of *Salmonella* sp.
Samples weighed 25 g and 225 mL of sterile BPW were then shaken for 2 min and incubated at 37°C for 18 h. A total of 0.1 mL of pre-enrichment culture was piped into 10 mL of RVS medium and 1 mL of other pre-enrichment cultures into 10 mL MKTT broth then vortexed to homogeneous. The RVS medium was incubated at 41.50 °C for 24 hours in a circulating water bath while MKTTn broth medium was incubated at 37 °C for 24 hours.
The incubated samples were shaken using a loop. The TT broth enrichment culture was streaked into a petri dish containing agar medium for XLD, HE, BS and RV, then incubated for 24 hours at 35 °C. The presence of Salmonella sp. characterized by the presence of pink colonies with or without black core on XLD media, blue to blue colored colonies with or without black core on HE medium, brown colonies, grey to black sometimes metal luster on BS media.

2.3.4. Metal contamination analysis
The analysis of metal contamination includes analysis of lead pollution (Pb), cadmium (Cd), tin (Sn), mercury (Hg) and arsenic (As) was performed by SNI 7934: 2014 methods on analysis of metal contamination on chocolate products [8].

3. Result and Discussion

3.1. The water content of cocoa beans
Fermented cocoa beans from Lombok, containing content of 8.2% water content, this value is higher than dry water content of dried cocoa beans according to SNI 2323: 2008 standard that is a maximum of 7.5%. Water content of cocoa more than 8% cause seeds susceptible to fungi and insects, thus increasing the risk of damage to the seeds, but if the seed moisture content of less than 5% will cause the seeds to break easily. Non-fermented cocoa beans will cause a sense of sprinkling and bitter and less sharp aroma in cocoa processed products thereby degrading the quality of cocoa. Quality of fermented cocoa beans from Lombok has a grade A.

| Parameter                        | Analysis result | SNI 2323:2008 |
|----------------------------------|-----------------|---------------|
| Moisture content                 | 8.2 (N.C)       | Max 7.5       |
| Life insect                      | Absent          | Absent        |
| Bean’s smell smoky/abnormal/other taints | Absent          | Absent        |
| Number of beans / 100 g          | 96 beans        | Grade A       |
| Foreign matters                  | Absent          | Absent        |
| Mouldy beans content             | 0.3 %           | Max.2         |
| Slaty beans content              | 15 %            | Max. 3        |
| Insect damaged beans content     | 0 %             | Max. 1        |
| Waste contents                   | 1.3 %           | Max. 1.5      |
| Sprouted beans content           | 1 %             | Max. 2        |

3.2 Analysis of cocoa liquor
The sensory analysis resulted that aroma, flavours, caramelly and creamy of cocoa liquor score were 8.25. Beckett [10] reported that the presence of milk content in chocolate would affect the properties of cream and soft texture on chocolate products.

| Characteristic          | Organoleptic Score* |
|-------------------------|---------------------|
| Aroma                   | 8.25                |
| Flavour                 | 8.25                |
| Acidity                 | 7.25                |
| Bitterness              | 7.75                |
| Astringent              | 7.25                |
| Caramelly               | 8.25                |
| Creamy                  | 8.25                |
| Sweetness               | 7.00                |
| Texture/colour          | 8.00                |
| Taints/Off flavours     | 0.00                |
| Preference              | 8.25                |
| Quality                 | Excellent           |

Score notes :
Notation for taste: 0 = nil; 1-2 = weak; 3-4 = moderately weak; 5-6 = moderately strong; 7-8 = strong.
Notation for preference and quality 0 = inconsumable; 1-2 = very bad; 3-4 = bad; 5-6 = neutral; 7-8= good; 9-10 = excellent.
Notation for taints: 0 = nil; X = weak; XX = strong.
The astringent and acidity of chocolate liquor score were 7.25. The presence of astringent and bitterness in the chocolate product caused by the presence of tannin and polyphenol in cocoa beans. Group of polyphenol compounds found in cocoa is flavonoids [11]. The bitterness of chocolate liquor has a score of 7.75. Cocoa has bitterness due to the presence of a natural substance such as alkaloid from theobromine types. The level of sweetness of chocolate liquor from fermented cocoa beans has a score of 7.00.

Texture and colour of chocolate liquor from fermented beans have score 8.00 and the off flavours have a score of 0.00. These results indicate that brown stems produced from fermented cocoa beans from Lombok has quite good quality. According to Pangabean et al. 2008, high-quality chocolate has texture smooth and buttery, it can melt with softly in a mouth [12].

### 3.3 Analysis of chocolate bar quality

Cocoa fat is dominated by triglycerides consisting of stearic acid (34%), palmitate (27%) and oleic (34%) which is solid at room temperature, melts at 37°C and provides a soft texture when chewed in the mouth [13]. Cocoa fat is a very important vegetable fat in the chocolate and candy industry because it has the unique physical characteristics of the triglyceride composition (TG) composed mainly of 55% POS, 5% POP and 20% SOS and has a melting point range of 32-35°C [14].

| Parameter                  | Unit       | Milk chocolate | Dark chocolate | SNI 7934:2014 |
|----------------------------|------------|----------------|----------------|---------------|
| Smell                      | -          | normal         | normal         | Normal        |
| Flavour                    | -          | normal         | normal         | normal        |
| Colour                     | -          | normal         | normal         | normal        |
| Fat                        | %          | 54.3           | 48.4           | ≥ 18          |
| Solid content non fat      | %          | 36.5           | 33.5           | ≥ 14          |
| Solid content              | %          | 80.3           | 73.8           | ≥ 35          |
| **Metal contamination**    |            |                |                |               |
| Lead (Pb)                  | mg/kg      | < 0.031        | < 0.031        | max.1         |
| Cadmium (Cd)               | mg/kg      | 0.04           | 0.03           | max.0.5       |
| Lead (Sn)                  | mg/kg      | < 0.8          | < 0.8          | max.40.0      |
| Mercury (Hg)               | mg/kg      | < 0.005        | < 0.005        | max.0.03      |
| Arsenic (As)               | mg/kg      | < 0.013        | < 0.013        | max.1         |
| **Microbial Analysis**     |            |                |                |               |
| Total Plate Count          | colony/g   | < 10           | < 10           | Max. 1 x 10⁴  |
| E.coli                     | APM/g      | < 3            | < 3            | < 3           |
| Salmonella sp.             | /25 g      | Negative       | Negative       | Negative      |
| Mold                       | colocy/g   | < 10           | < 10           | Max. 1 x 10²  |
| Yeast                      | colony/g   | < 10           | < 10           | Max. 1 x 10²  |

Solid fat content (SFC) is one of the typical parameters needed in the cocoa fat business. Chocolate industry requires this parameter as an indication of the nature of cocoa fat liquefaction in fat processing and its use in the food industry (confectionary) [15]. Table 3. Showed that non-fat cocoa solids and total cocoa milk solids are higher than dark chocolate. This is due to milk chocolate containing more milk than dark chocolate causing solid components derived from milk to affect the number of solids in chocolate produced.

In general, the quality of the chocolate stems produced from fermented cocoa beans from Lombok fulfills the criteria for chocolate bars according to SNI 7934: 2014 regarding chocolate and chocolate products. This was shown that chocolate stems produced from fermented cocoa beans from Lombok have good quality.
4. Conclusion
The fermented cocoa beans have a water content of 8.2%. The quality of cocoa beans is included in grade A (96 seeds / 100 g). The quality of the chocolate stems produced from fermented cocoa beans is very good and in accordance with SNI 7934: 2014.

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