Physical and Chemical Characteristics of Sulawesi Cocoa Beans 2 (*Theobroma cacao L*) Fermented and Unfermented Result

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Abstrak

Penelitian ini bertujuan untuk mengetahui karakteristik fisik dan kimia biji kakao kering klon BR 25 hasil fermentasi dan tanpa fermentasi. Perlakuan pada penelitian ini yaitu perlakuan fermentasi (Perlakuan A) dan perlakuan tanpa fermentasi (Perlakuan B). Perlakuan fermentasi dan tanpa fermentasi diolah dari biji kakao basah dengan jumlah biji 10 kilogram setiap perlakuan. Perlakuan fermentasi menggunakan kotak fermentasi dari styroform, sedangkan perlakuan tanpa fermentasi langsung dijemur dibawah sinar matahari setelah dikeluarkan lendir (pulpnya). Parameter uji biji kakao kering fermentasi dan tanpa fermentasi adalah kadar air, pH, kadar lemak, protein, polifenol, aktifitas antioksidan (IC50), dan ALT. Hasil penelitian menunjukan bahwa pada biji kakao fermentasi mempunyai kadar air 5,77%; pH 6,41; kadar lemak 55,63%; protein 14.36%; kandungan polifenol 4,62%; aktivitas antioksidan (IC50) 21,96%; dan nilai ALT 1,0 x 10^2 koloni/gram. Pada biji kakao tanpa fermentasi mempunyai kadar air 6,52%; pH 5,20; kadar lemak 59,63%; protein 17,83%; kandungan polifenol 2,06%; aktivitas antioksidan (IC50) 62,23%; dan nilai ALT 1,1 x 10^2 koloni/gram dan untuk kadar air biji kakao fermentasi dan tanpa fermentasi rata-rata memenuhi standar SNI yaitu 7,5%.

***Kata kunci**: biji kakao; fermentasi; tanpa fermentasi; karakteristik*

Abstract

This study aims to determine the physical and chemical characteristics of fermented and unfermented BR 25 clone dry cocoa beans. The treatment in this study was the fermented treatment (Treatment A) and the unfermented treatment (Treatment B). The fermented and unfermented treatments were processed from wet cocoa beans with 10 kilograms of beans for each treatment. The fermentation treatment uses a box of Styrofoam, while the unfermented treatment is directly dried in the sun after removing the mucus (pulp). The parameters for fermented and unfermented dry cocoa beans were water, pH, fat content, protein, polyphenols, antioxidant activity (IC50), and ALT. The results showed that fermented cocoa beans had a moisture content of 5.77%, pH of 6.41; fat content of 55.63%; protein of 14.36%; polyphenol content of 4.62%; antioxidant activity (IC50) of 21.96%; and ALT value of 1.0 x 10^2 colonies/gram. Unfermented cocoa beans have a moisture content of 6.52%, pH of 5.20; fat content of 59.63%; protein of 17.83%; polyphenol content of 2.06%; antioxidant activity (IC50) of 62.23%; and ALT value of 1.1 x 10^2 colonies/gram and average moisture content of fermented and unfermented cocoa beans meet requirements SNI standards, namely 7.5%.

Keywords: cacao beans; fermented; unfermented; characteristics

INTRODUCTION

The cocoa (*Theobroma cocoa* L) is a plantation plant that is very beneficial for health. Cocoa in Indonesia is still an export commodity in the form of beans, which is around 83%, and cocoa plantations can be found in various regions in Indonesia, especially in South Sulawesi, namely in Polman, Palopo, Luwu, Pangkep, and Toraja. Cocoa pods cultivated in Indonesia can be divided into three major groups (types): Criollo, Forastero, and Trinitario. Cocoa in trading commodities is usually divided into Fine Cocoa and Bulk Cocoa. (Schwan *et al*., 2015).

Smallholder colonies substantially produce Indonesian cocoa products. Indonesian cocoa product has great eventuality in the world cocoa trade because cocoa sap from Indonesia have fairly advanced quality nutrient content and polyphenolic composites than cocoa sap from Pantai Gading. It can increase the competitiveness of Indonesian cocoa in the transnational request to come more good (Haryadi and Supriyanto 2012). Farmers handle the post-harvest cocoa pods and the fresh

cocoa beans process in two forms: dry fermented and unfermented beans. The unfermented dry cocoa beans will taste bitter and astringent, so they cannot produce a distinctive aroma of chocolate when processed, while the fermented dry cocoa beans have a robust and distinctive aroma, taste, and chocolate color due to biochemical changes in the cocoa beans (Apriyanto *et al*, 2016; Afoakwa *et al*, 2011)

Cocoa beans are generally divided into two, fermented and unfermented. Processing cocoa sap by drying is an important step in forming a distinctive cocoa flavor. Meanwhile, unfermented cocoa bean are reused without turmoil and are directly dried under the sun or with a drying machine. During the turmoil process, the sugar-high pulp subcaste that fleeces the cocoa bean is converted into ethanol, acetic acid, and lactic acid through a turmoil process by bacteria. Cocoa bean contain protein and carbohydrates, which are relatively high and contain enzymes that play a part in the turmoil of the bean. The biochemical process in cocoa bean during the fermentation process involves enzymes similar as protease and carbohydrase enzymes. It's these enzymes that convert and hydrolyze proteins into amino acids in the conformation of precursor flavors (Rodriguez-Campos *et al*. 2012).

Dried cocoa beans have lost most of their water and substrate content during the fermentation process in enzymatic reactions and microbial growth in the pulp. The presence of water can bring enzymes and substrates together in the beans so that the process of hydrolysis and oxidation of candidate compounds for taste, color, and aroma in cocoa beans can occur (Dan Sri Wedhastri 2013). The moisture content required in the fermentation of cocoa beans is more than 35%, and the substrate is a material overhauled by microbes during the fermentation process. The substrate in cocoa bean fermentation is sugar and citric acid contained in the pulp (Apriyanto *et al*. 2016). Reducing sugar content in dry-dried cocoa bean fermentation increased at the beginning, decreased in the middle, and remained stable until the end of the fermentation period (Afoakwa *et al*. 2011).

The conformation of acetic acid and lactic acid in the fermentation process causes the cocoa bean to be acidic; there's an increase in temperature from 45 ^{0}C to 50 ^{0}C, and it diffuses into the bean so that the cocoa bean come acidic, which ranges from a pH or acidity degree of 3.8 and 5.8. So that under conditions, optimal endogenous protease exertion will affect bean protein declination to produce colorful kinds of aroma precursors and factors of unpredictable andnon-volatile composites. Fermented cocoa beans have lower polyphenol content than unfermented cocoa beans. Unfermented cocoa bean have a veritably low or indeed missing chocolate aroma because there's no enzymatic process in the bean to produce aroma precursors. While the color of the cocoa bean becomes purple or dominantly slaty bean with fairly high water content with a weak cocoa flavor and very high source of antioxidants (Haryadi and Supriyanto 2012; Yumas, 2021; Afoakwa *et al*. 2011)

Fresh cocoa beans contain 12-18% polyphenolic compounds without fermentation. The polyphenol compounds in cocoa beans include catechins 33-42%, leucocyanidin 23-25%, anthocyanins 5%, and cocoa beans that have been processed into a fat-free cocoa powder containing polyphenols 5-18%. Polyphenol compounds in cocoa beans and powder are essential antioxidants in nourishing the human body. Dried cocoa beans contain quite high fat, namely 45-57%, water 6.5-7.5%, ash around 4.2%, starch 9.5%, and fiber 3.2% (Afoakwa *et al.* 2011)(Crozier *et al*. 2011).

The quality of fermented dry cocoa beans is determined by the acidity (pH) and acidity of the beans during fermentation. pH and seed acidity are related; the bean acidity value will increase when the pH shows a low value. To produce quality cocoa powder and cocoa fat, a good cocoa bean pH is between 5.2 - 5.8. Fermented dry cocoa beans that are good produce cocoa beans with a fermentation index or color index ≥ 1, while cocoa beans that are unfermented not optimally have a fermentation index < 1. Observation of the color of cocoa beans with fermented and unfermented treatment is the indicator of the color of the beans. Unfermented cocoa beans are red-purple, indicating high anthocyanin content. In contrast, fermented cocoa beans are brown due to reduced and degraded anthocyanin compounds from red-purple to red-brown. (Apriyanto *et al*. 2017).

The taste of cocoa beans is influenced by the fermentation and drying processes. The decomposition of protein, sugar, and polyphenol compounds by enzymes that form flavor compounds occurs in the fermentation process. So that if the fermentation can run perfectly, more and more of these compounds are unraveled. In the drying process, the compounds that make up the flavor react with each other through the Maillard reaction, which produces volatile components with a distinctive chocolate aroma, such as furans, esters, thiazoles, alcohols, pyrazines, and pyrroles. Perfectly fermented cocoa beans have a slightly crumbly texture or break easily. Moreover, the drying process also affects the texture of the cocoa beans. The optimal drying process will produce crunchy cocoa beans. However, uneven drying will result in only some of the cocoa beans being crunchy (Marpaung and Putri, 2019).

Unfermented cocoa beans are slaty, namely dry cocoa beans. When split inside, the beans are purple, and generally, the purple dye in unfermented cocoa beans is commonly called

anthocyanin. Anthocyanins are polyphenol compounds that produce a purple color; therefore, when extracted, unfermented cocoa beans have a high purple dye. In addition, the cocoa beans are purple, so dry cocoa beans without fermentation can also be used as a source of dyes. Therefore, dry unfermented cocoa beans are widely processed and used for functional food, cosmetics, pharmaceuticals, and ingredients for food processing for chocolate product diversification products (Haryadi and Supriyanto, 2012).

The characteristics of dry cocoa beans are essential in determining the quality and price of dry cocoa beans in the market. The better the characteristics of the beans, the higher the quality and price of the cocoa beans. This study aimed to determine the characteristics of the chemical content and physical observations of fermented and unfermented cocoa beans from Sulawesi cocoa beans clones 2 which can meet standards for raw materials for beverage products.

METODHOLOGY

Materials and Tools

The raw materials of the research are Sulawesi cocoa clones 2 from North Luwu regency, Sabbang village. The process equipment is a fruit crusher, a styrofoam fermentation box, drying racks, a depulper, a thermometer, scales, and plastic packaging. Laboratory equipment for physical and chemical analysis, namely petri dishes, 100 ml measuring cyclinder, 500 ml beakers. volume pipette, 100 ml volumetric flask, digital balance, UV Vis spectrophotometer.

RESEARCH METHOD

Treatment of cocoa beans is fermented and unfermented according to the method (Mulato *et al*., 2005). The treatment was started by placing 10 kg of wet cocoa beans. Wet cocoa beans are divided into two parts. The first part is for the fermented treatment (Treatment A) into a Styrofoam fermentation box., and the second is for the unfermented (Treatment B) and directly dried in the sun using drying racks. Cocoa bean processing scheme in Figure 1.

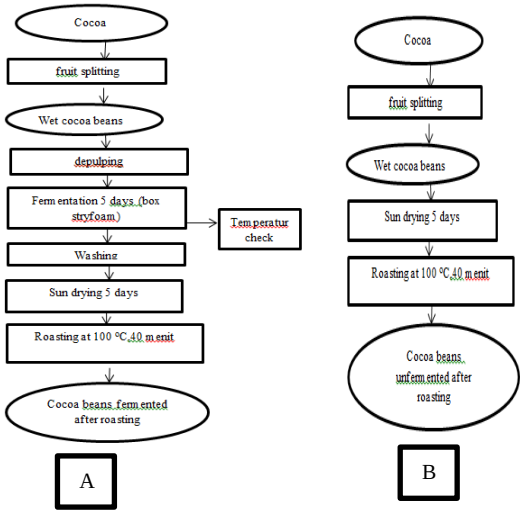

Figure 1. fermented (A) and unfermented (B) cocoa bean processing scheme.

Analysis of cocoa beans included moisture content, pH, fat content, protein, polyphenol antioxidant activity, and ALT value (colony/gram).

Moisture content (SNI 2323 :2008)

Determination of moisture content. A clean cup along with the lid were heated in the oven for 3 hours, then the cup was closed using a tweezer then cooled in a desiccator for 15 minutes. Subsequently, the cup was weighed as empty weight. Sample powder were weighed as much as 1-5 grams, respectively, then placed in the cup and weighed with the lid. The samples then heated in an oven at 105 ^{o}C for 3 hours then cooled in a desiccator, and then weighed again. The water content was calculated using the following equation:

$$\text{Moisture content (\%)} = \frac{W1 - W2}{W} \times 100\% \qquad (1)$$

pH Value (SNI 2323: 2008)

The pH value was measured based using a pH meter. The method is to take 10 grams of cocoa beans that have been blended, then put them in a 100 ml beaker then add 90 ml of distilled water. After that, it was stirred until homogeneous and filtered, then the sample was measured with a pH meter that had been calibrated in buffer 7. The measurements were carried out 3 times.

Fat Total (SNI 2323: 2008)

Measurement of fat content was carried out according to the BSN (2008) method, namely by hydrolyzing and extracting fat. In fat hydrolysis, ground dry cocoa beans are weighed 3-5 g into a 300-500 ml beaker, then 45 ml of boiling distilled water and 55 ml of HCl are added to the beaker.

Then shake the beaker, cover with a watch glass and bring to a boil slowly.

15 minutes. After that, the watch glass is rinsed with 100 ml of distilled water and the washing water is put into a beaker, then the sediment is filtered through fat-free filter paper. The beaker was then rinsed 3 times with distilled water through filter paper and the washing continued until it was Cl free (does not give white AgCl precipitate with the addition of 1 drop to 3 drops of AgNO3). The filter paper is then transferred along with its contents into an extraction lead or fat-free filter paper sleeve to dry for 6-18 hours at a temperature of 100-101 ^{o}C. ii. Fat extraction: The boiling pumpkin is dried for one hour in an oven at a temperature of 100-101 ^{o}C and weighed until the weight remains constant, then the boiling flask is connected to a Soxhlet extraction apparatus. After that, the extraction lead or filter paper sleeve is inserted into the soxhlet. The dried beaker and watch glass were rinsed several times with 150 ml of petroleum benzene and poured into the flask. Next, the material was refluxed for 4 hours with an extraction speed of around 3 drops per second. After the extraction is complete, the extraction lead is removed then n-hexan solvent is evaporated using an evaporator or by heating the flask in a water bath. Pumpkin and fat are dried in the oven at a temperature of 100-101 ^{o}C. After that, the material is cooled and weighed, the last remaining solvent after drying is evaporated by blowing air through a boiling pumpkin. Drying was repeated until the difference in fat weight measurements carried out successively was less than 0.05%. The way to express the fat content results is expressed as a percentage of weight per weight and calculated in dry weight with the following calculation:

$$\text{Fat Total (\%)} = \frac{M2 - M1}{Mo} \times KAx100\% \qquad (2)$$

Notes:
M0: weight loss sample (gram)
M1: boiling pumpkin and stone bone (gram)
M2: boiling pumpkin and stone bone and fat (gram)
KA: moisture sample (%)

Polyphenol analiysis(AOAC, 2010)

Weigh 1 gram of sample then dissolve it in distilled water and squeeze it into a 50 ml measuring flask. Each test solution (0.5 mL) was separately added with Folin-Ciocalteu reagent (5 mL, 1:10 in distilled water), Na2CO3 solution (4 ml, 1M) and left for 15 minutes. The test was carried out 3 repetitions. The absorbance was measured at its maximum wavelength of 750 nm using a UV-Visible spectrophotometer. Making a Calibration Curve Using Gallic Acid as a Comparative Solution. A total of 10 mg gallic acid was dissolved in 10 mL methanol: distilled water (50:50 v/v). Then a 2 dilution concentration series was made; 3; 4; 5; 6; and 7 µg/mL. Then added Folin-Ciocalteu reagent (5 mL, 1:10 in distilled water), Na_2CO_3 solution (4 mL, 1 M) and left for 15 minutes. The absorbance was measured at a wavelength of 750 nm using a UV-Visible spectrophotometer.

Antioxidant activity (AOAC, 2012)

Preparation of DPPH solution which weighed as much as 1.97 mg DPPH dissolved in ethanol to 25 mL and the obtained solution with a concentration of 0.2 mM. Ethanol extracts were diluted (concentration variation 25, 50, 75, 100, 125 and 150 mg/L). Each concentration of the solution as much as 1.5 mL pipette and added 0.75 mL of 0.2 mM DPPH. The mixture was homogenized and allowed to stand in the dark for 30 minutes. Uptake was measured by UV-Vis spectrophotometer at maximum wavelength is 517 nm DPPH. Tests performed three separate tests for each concentration of the sample solution. Reference solution used was BHT and ascorbic acid at a concentration of 2, 4, 6, 8 and 10 mg / L. Experiments performed three separate tests and calculated in the inhibition using the formula:

$$\text{Inhibition (\%)} = \frac{Abs\ blank - Abs\ Sample}{Abs\ blank\ x\ 100} \qquad (3)$$

Total Bacterial/ Total Plate Count Value/ALT (SNI 2323: 2008)

The sample preparation and dilution stages were carried out by pipetting 15 ml of the sample into a diluent bottle containing 125 ml of peptone buffer solution (1:10), then stirring until homogeneous. Dilution was carried out to 10-3. For instant ginger products, candied ginger and jelly, preparation is carried out by weighing a 15 g sample. then put it in a diluent bottle containing 125 ml of peptone buffer solution (1:10) then stir until homogeneous. Then dilutions were made up to 10-3, taking 1 ml of each dilution sample and put into a sterile petri dish. Next, 15-20 ml of liquid PCA media was poured into the petri dish. The petri dish is carefully rotated and moved horizontally or parallel (or forming a figure of eight) until the sample is thoroughly mixed. At the same time, a blank examination was also carried out by mixing the buffer into the media. The mixture in the petri dish was then allowed to freeze. The final stage is incubation by inserting all the petri dishes upside down into the incubator. Incubation was carried out at a temperature of 36 ± 1 ^{0}C for 24-48 hours. Calculation and recording of colony growth is carried out in colony forming units per gram.

RESULTS AND DISCUSSION

Moisture Content

Moisture content is the total amount of water contained in food ingredients expressed in percent; in other words, the moisture content is one of the physical properties of food ingredients which indicates the amount of water contained in the material per unit weight of the material (Nurdahlia, 2015). Moisture content influences cocoa beans' resistance to damage, especially during storage and transportation. Cocoa beans, which have a high moisture content, are very susceptible to attack by fungi and insects. Consumers dislike both because they tend to cause damage to the basic taste and aroma, which cannot be repaired in the following process. Unfermented and fermented cocoa beans show different values of moisture content. The moisture content of unfermented cocoa beans is 6.22%, and of fermented cacao beans is 5.77% fermented (Table 1.). The low moisture content in fermented cocoa beans is caused by the destruction of the pulp in the cocoa beans due to the microbial decomposition process so that the weight content of the beans is reduced, and facilitates the drying process. In addition to the pulp's destruction, the cocoa beans also die during the fermentation process. So, the permeability properties of the beans are damaged, making it easier for water to escape during the drying process (Sigalingging *et al*, 2020).

Table 1. Results analysis of fermented and unfermented cocoa beans

No	Analysis Test	Cocoa beans		
		Fermented	Unfermented	SNI Standard/Food Standard Cocoa
1.	Moisture (%)	5,77	6,22	7,5
2.	Fat Level (%)	55,63	56,83	45-57
3.	pH Value	5,21	6,41	5,5 - 6,8
4.	Polyphenol (%)	2,06	4,62	-
6.	Antioxidant Activity (IC_{50})	45,23	31,96	50
7.	Total Plate Number Test (koloni /gram)	$1,0 \times 10^1$	$1,10 \times 10^2$	-

Moisture content in food can have a significant impact on factors such as the product's taste and affects the processibility, shelf-life, usability and quality of a product. Accurate moisture content determination therefore plays a key role in ensuring quality for many industries including Food. The drying process interferes with biochemical reactions initiated during fermentation, leading to a reduction in the bitterness, astringency, and acidity of cocoa beans (Sigalingging *et al*, 2020).

Determination of the moisture content of cocoa beans is one of the benchmarks for the drying process to obtain good quality cocoa beans. Based on SNI 2323:2010, which requires the moisture content of dry cocoa beans is 7.5%. Changes in the decrease in moisture content in cocoa beans are due to the drying process to remove a certain amount of water so that the moisture content decreases or becomes smaller. According to Apriyanto et al (2016), the low value of water content in fermented cocoa beans is also caused by the process of releasing water content which diffuses out of the beans due to the heat during the fermentation and drying process, compared to unfermented cocoa beans which are directly dried in the sun. where the chemical content in unfermented cocoa beans does not decompose and the water component does not diffuse out into the bean shell so the water content is still quite high. The results of the average of unfermented and fermented cocoa beans meet the required quality standards, namely a maximum of 7.5%, so it can be said that the moisture content of cocoa beans is quite excellent and optimal.

pH Value

The pH of cocoa beans is one of the essential indicators during fermentation. The pH parameter can determine the success of cocoa bean fermentation. pH measurements were carried out to determine the acidity level of the beans. The results

of the pH analysis of fermented dry cocoa beans were lower, 5.21, compared to unfermented cocoa beans, 6.41 (Table 1). The low pH of fermented cocoa beans is due to the accumulation of acetic acid in the bean and epidermis during the fermentation process, resulting in an enzymatic reaction in the bean pieces, a sour aroma, and a decrease in the pH value during fermentation (Rosniati dan Kalsum, 2018; Misnawi,2004). During the fermentation, air enters the pile of cocoa beans accompanied by the entry of bacteria, namely acetic acid bacteria, resulting in acid penetration into the cocoa beans, causing a low bean pH value (Sigalingging, *et al*, 2020).

According to Haryadi and Supriyanto (2012), high temperature drying of cocoa beans has a big influence on the pH value. The higher the temperature, the lower the pH value of the cocoa beans. Drying in the sun generally provides a higher pH for cocoa beans because it is more protected from using high temperatures. drying using by machine at a temperature of 40 ^{0}C to 50 ^{0}C will give a pH value of 4.9 to 5.2, while drying in the sun gives a pH value of 5.1 to 5.2. This is also in line with the results of the pH value of fermented cocoa beans, which was 5.2 because the fermented cocoa beans were dried using a sun drying system.

The pH value of cocoa beans can be a parameter to see the acidity level of cocoa beans, especially fermented cocoa beans with acetic acid content. The acidity of cocoa beans is associated with a pH limit value of 5.0-5.8. If the pH of the beans is >5.8, it indicates that the fermentation of the cocoa beans is incomplete or excessive. Sour cocoa beans have a PH value of <5.0 and have a sour smell. The pH value obtained for fermented cocoa beans was 5.21, which is a good pH value. In line with research by Haryadi and Supriyanto (2012), the pH value most preferred by panelists was 5.2 to 5.8 so that the pH value showed a very strong linear relationship with total acid. The pH and temperature of fermented cocoa beans will activate the enzymes needed to form chocolate characteristics, flavour, aroma and color after roasting to get a strong chocolate taste, the pH of dry fermented cocoa beans is expected to be around 5.5 to 6.8. Meanwhile, in cocoa beans without fermentation the enzyme is inactive, the acidity is very low, the aroma becomes off flavor, the cocoa aroma is less strong, and the color of the cocoa beans is dark (Haryadi and Supriyanto, 2012).

Fat Level

Fermented and unfermented dry cocoa beans generally have a high-fat content level (Table 1). However, fermented beans have a slightly lower fat content than unfermented cocoa beans. The longer the fermentation time, the lower the fat content in dry cocoa beans, especially during drying and roasting. The fat content of cocoa beans also depends on the type and variety of cocoa fruit. The low-fat content is affected by the high-water content component. The requirement for cocoa bean fat content to be processed into chocolate products is a minimum of 50-51% (Marwati, 2013).

The results of the fat content analysis in unfermented and fermented cocoa beans were almost the same, namely in the range of 55.63 to 56.83%. The decrease in the value of the fat content of the last beans with high-fat content, which was in the range of 59 to 56%, indicates that the cocoa beans still contain high-fat content because they have not gone through the process of separating fat and fat content (Rosniati and Kalsum 2018).

Cocoa butter is the most expensive component of cocoa beans, so this value is used by consumers as a standard for determining prices. Piecemeal from the planting material and season, the fat content in both fermented and unfermented cocoa beans is influenced by processing treatment, type of factory material and seasonal factors. Cocoa beans that come from rainy season fertilization generally have a higher fat content. Meanwhile, the physical characteristics of post-processing cocoa beans, such as water content, fermentation level and skin content, influence the cocoa bean fat yield. Cocoa bean fat is a triglyceride which is a compound of glycerol and three fatty acids. Further 70% of glycerides consist of three monounsaturated compounds, namely oleodipalmitin, oleodistearin and oleopalmistearin . The fat content in fermented cocoa beans is very high. Cocoa butter is thin, yellow in color, solid and shows visible cracks at temperatures below 20 ^{0}C. The very sharp melting point is at 35 ^{0}C with melting or softening at a temperature of around 30 ^{0}C – 32 ^{0}C. (Misnawi *et al*., 2004)

The fat content of fermented and unfermented cocoa beans is influenced by planting material, season and processing treatment. The fat content of fermented cocoa beans (A) is lower than unfermented ones, this is due to several microbes involved in the fermentation process (mold and bacillus bacteria) which are able to produce lipase enzymes can break down fat so that the fat content of fermented cocoa beans reduce. Fermented cocoa beans have a low fat content because during the roasting process some of the fatty acids diffuse out of the cocoa beans so that the fat content is reduced. The size of the cocoa bean also greatly determines the fat yield. The larger the size of the cocoa bean, the higher the fat yield in the cocoa bean (Haryadi and Supriyanto, 2012).

Polyphenol Level

Cocoa beans and derivative products are containing in antioxidants, such as; catechins, epicatechins, and procyanidins. Procyanidin, which

includes polyphenolic compounds, is also found in grapes, vegetables, and tea. Polyphenols act as flavor precursors in cocoa and chocolate. During fermentation and drying, the complex chemical changes in the polyphenols result in the taste and color of chocolate and their concentration rapidly decreasing. Polyphenol compounds are essential components because these compounds determine the taste, color, and aroma of cocoa (Yuniar *et al*, 2018).

Polyphenols are abundant in storage cells that do not contain enzymes and then diffuse from

The analysis results of polyphenol levels in unfermented cocoa beans were (Table 1.) 4.62 and 2.06% in fermented cocoa beans. It happens due to the effect of processing cocoa beans, which still contain high fat, so polyphenols are still bound to fat groups, then after it becomes powder in the processing stage, there is an increase in polyphenol levels due to the release of fat. Polyphenol levels in fermented cocoa beans decreased during fermentation due to the decomposition of polyphenolic compounds into flavor and the formation of brown color. Then resulted in diffusing of polyphenol compounds out of the seed pieces, oxidation of polyphenols by polyphenol oxidase enzymes, polymerization of polyphenolic compounds to form tannin compounds, formation of complexes with proteins, and polysaccharides. The decrease in polyphenol content in cocoa beans and fermented powder is also due to roasting factors and changes in physical and chemical properties where the flavor compounds and the distinctive aroma of chocolate increase. (Misnawi *et al.* 2004)(Utami 2018).

Antioxidant Level

Antioxidant activity is the ability of antiradical compounds to capture free radicals. Antioxidants are essential to maintain the quality of food products. Antioxidants can inhibit the occurrence of various damages such as rancidity, changes in nutritional value, changes in color and aroma, as well as other physical damage to food products (Shadri *et al.*, 2018).

Natural antioxidants in the food system play a role in inhibiting and preventing the fat oxidation process so that they can function as preservatives. Natural antioxidant compounds can also function as free radical scavengers, ion chelators, and reducers of singlet oxygen formation. Natural antioxidants from fermented and unfermented cocoa beans such as cocoa flavonoids have the ability to provide stability against penoxyl free radicals due to the presence of hydroxyl groups in the cocoa beans so that able to increase antioxidant activity (Raharjo, 2006; Amic, *et al*, 2007)

storage cells to undergo polymerization, oxidation, and interaction with proteins. Polyphenols are a significant component of cocoa, approximately 10-20% (dry weight) of cotyledons, and contribute to the astringent taste of cocoa beans. Catechins have a total of 33-42% of all polyphenols, 92% of which are epicatechins, while 8% of other catechins are Catechin, Gallo Catechin and Epigallocatechin. More than 50% of the total polyphenols are leucocyanidins and tannin complexes, while anthocyanins only account for 5% (Caligiani *et al.* 2010)

Unfermented cocoa beans contain around 60% polyphenolic compounds which act as antioxidants because they contain flavonoid monomers and procyanidin oligomers in varying concentrations and have strong antioxidant activity.. The value of antioxidant activity in fermented cocoa beans has a higher value (45.23%) than in unfermented cocoa beans (31.96%) in Table 1. It shows that fermented and unfermented cocoa beans have powerful antioxidants because their value is below 50. A compound is said to be a powerful antioxidant if the IC50 value is less than 50, potent (50 - 100), or medium (100 – 150), and weak antioxidant activity is at a value of 151 – 200 (Salim 2018).

Total Plate Number Test (ALT)

The value of antioxidant activity in fermented cocoa beans has a higher value (45.23%) than in unfermented cocoa beans, which is 31.96%) (Table 1.). It shows that fermented and unfermented cocoa beans have powerful antioxidants because their value is below 50. A compound is said to be a powerful antioxidant if the IC50 value is less than 50, potent (50 - 100) or medium (100 - 150), and weak antioxidant activity is at a value of 151 – 200 (Atma 2016).

In general, the criteria for analyzing food products are the total microbial value or total plate count, total mold, yeast, and coliform bacteria. Certain products require analysis for the presence of pathogenic bacteria. Foods that require microbiological criteria include fresh products, processed products ready for consumption, and semi-finished products such as flour and food additives (Badan Pengawas Obat dan Makanan Republik Indonesia 2019).

The results of the ALT analysis of fermented cocoa beans were 1.01×10^2 colonies/gram and 1.02×10^2 colonies/gram without fermentation (Figure 6). The results of the analysis of fermented and unfermented cocoa beans produced in this study, on average, met the SNI quality standards for cocoa beans where the ALT value required for SNI 2323: 2008 cocoa beans was a maximum of 5×10^3 colonies/gram.

CONCLUSION

Based on the research results, it can be concluded that the physical and chemical characteristics of Sulawesi 2 cocoa beans, both fermented and unfermented with the parameters of moisture content, fat content, pH, and total plate number generally meet SNI cocoa beans 2323:2008. The results showed that fermented cocoa beans had a moisture content of 5.77%, pH of 6.41; fat content of 55.63%; protein of 14.36%; polyphenol content of 4.62%; antioxidant activity (IC50) of 21.96%; and ALT value of 1.0 x 102 colonies/gram. Unfermented cocoa beans have a moisture content of 6.52%, pH of 5.20; fat content of 59.63%; protein of 17.83%; polyphenol content of 2.06%; antioxidant activity (IC50) of 62.23%; and ALT value of 1.1 x 102. The polyphenol content of unfermented cocoa beans is higher than that of fermented cocoa beans, but both are powerful antioxidants because the IC50 value is below 50.

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