Profile of bioactive compounds, antioxidant and aromatic component from several clones of cocoa beans during fermentation

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Abstract. Commonly cocoa beans containing various chemical components, nutrients, and bioactive compound. The main bioactive compounds in cocoa beans consisting flavonoids and methylxantines has an positive influence on the health of the human body. Besides the fermented cocoa beans also produce a distinctive of cocoa aromatic. One of the main factors influencing the formation of aroma, chemical content and bioactive compounds of cocoa beans is the fermentation process. The purpose of research is to determine the effect of fermentation duration of cocoa clones with different varieties on the profile of bioactive compounds, antioxidant activity and aromatic components (volatiles) that occur in cocoa beans during fermentation. The treatment was arranged in a Randomized Block Design of two factors, the first factor is cocoa clones (Sulawesi 2/S2 clones and local clones) and the second factor is fermentation time (0, 2, 4 and 6 days). The observed parameters were polyphenol content, antioxidant activity, flavonoid bioactive compounds (catechin and epicatechin) and methylxanthine (theobromin and caffeine), as well as aromatic components (volatile) of fermented cocoa beans. The results of the study was showed that cocoa beans from local clones providing polyphenols, antioxidant activity and bioactive compounds (catechin, epicatechin and theobromine) are higher than S2 clones. While the high caffeine content and distinctive aroma of the pyrazine compound were produced from S2 cocoa beans on 6 days fermentation. The average content of polyphenols, antioxidants and bioactive compounds in non-fermented cocoa beans is higher than fermented beans, but the resulting scent is not strong. The results of the research are expected to obtain information about the profile of bioactive compounds, antioxidants and aromatic components of cocoa beans especially derived from Pinrang district has the potential improving community health.

1. Introduction

Cocoa (Theobroma cacao L.) is the one of plantation commodities playing an important role in supporting national economic activities. South Sulawesi Province is the second largest cocoa producing province in Indonesia has a distribution of cocoa at six districts. One of them is in Pinrang district [1]. Several types of cocoa clones/ varieties have been widely planted in Pinrang district are: Sulawesi 1, Sulawesi 2, 45, M01 and local clones.

Commonly cocoa beans contain various chemical components, nutrients and bioactive compounds. Cocoa beans has a higher polyphenol content and antioxidant activity compared to green tea, black tea, red wine and blueberries. Polyphenol compounds in cocoa and chocolate have been widely studied containing various bioactive components that very beneficial for human health. The bioactive components contain many antioxidants, anti-cancer, anti-diabetes, anti-hypertensive, anti-
inflammatory properties, relieve stress, preventing dental caries, improve cognitive abilities, improve heart health and another function as aphrodisia[2–7].

Unprocessed fresh cocoa beans contain about 12-18% polyphenol compounds consisting of the main polyphenol groups namely: flavanols, anthocyanidins and proanthocyanidins where (+) - catechin and (-) - epicatechin are components of flavanol monomers [8–10] Cocoa products also rich in methylxanthine compounds such as: caffeine, theobromine and theophylline [11]. The methylxanthine compound giving contributes to the bitter taste in cocoa products. Pinilla et al., (2015) [12] reported the combination of caffeine and theobromine in cocoa has benefit as anti-tumor, anti-inflammatory and working as a protector from cardiovascular disease.

In addition to the content of these bioactive compounds, fermented cocoa beans also produce of cocoa aromatic. Fermentation is the main factor influencing the formation of these flavors [13,14]. During the fermentation process there is an overhaul of the sugar component into organic acids and volatile components which are precursor compounds of flavor, aroma and brown color [15,16]. Cocoa beans that are not fermented or imperfect fermentation have a concentrated bitter taste and can not produce a chocolate flavor when roasting [17,18].

The content of bioactive compounds, antioxidants and aroma in cocoa beans and their derivative products will vary depending on the level of fruit maturity, variety, place to grow and processing. One of the important processing of cocoa beans is the fermentation process. Fermentation is the center of processing of cocoa beans and absolutely necessary preparing wet cocoa beans into high-quality dry beans for consumption [9,19]. The perfection of the fermentation process was influenced by the weight of the cocoa beans, stirring, fermentation time, type of fermentation container, dimensions and aeration degree of the container and presence of yeast or bacteria that playing a process [20–23]., Menezes et al. (2016) reported that commonly Saccharomyces cerevisiae was used in fermentation of cocoa beans [24]. Based study of Widianto et al. (2013); Lima et al. (2011); Widyotomo (2008), Towaha et al. (2012); Tarigan and Tajul (2017) and Rahmi et al. (2017) state that perfect fermentation of cocoa beans resulting in better taste, good aromatic and color, because during fermentation the physical, chemical and biological changes occurring in the cocoa beans. Commonly changes of fermentation process occurring in pulp, seed coat and cotyledon [25–32]

Commonly fermentation process around the world using wood container. However, few researcher reported styrofoam as alternative as the tool of cocoa seed fermentation. Scollo et al. (2020) reported that over 1000 peptides have been detected from cocoa beans in all fermentation stages used styrofoam box.

The purpose of research is to determine the effect of fermentation duration of cocoa clones with different varieties on the profile of bioactive compounds, antioxidant activity and aromatic components (volatiles) that occur in cocoa beans during fermentation. Research on cocoa fermentation has been widely carried out, but never publication regarding the bioactive compounds and aromatic profile (volatile compound) profile of fermented cocoa beans using styrofoam container, especially from Pinrang district, South Sulawesi. Based the increasing consumption of Indonesian people on cocoa products, it is necessary to conduct research on the profile of the main bioactive compounds, antioxidant activity and aromatic components of cocoa beans variaety in South Sulawesi with great potential in improving public health.

2. Materials and Methods

2.1. Materials and Ingredients
The research supporting tools in the form of a styrofoam fermentation box measuring 75 x 40 x 32 cm with a capacity 40 kg of wet cocoa beans. The fermentation box used 3 box for each cocoa bean clones, sacks, drying basin. The another tools used in analyzed the sample are: erlenmeyer, analytical scale, oven, aluminum plates, desiccators, vortices, centrifuges, sonicators, UV-Vis spectrophotometers, 0.45 µm agilent PTFE microfilter filters, HPLC-UV, gas-mass spectrometry (GC-MS) chromatography and various glassware such as : test tubes, petridish, Erlenmeyers, porcelain
cups, stirring rods, funnels, measuring flasks, goblets, measuring cups, volumetric pipettes and drop pipettes.

The raw materials of the research are: fermented and non-fermented cocoa beans from S2 and local clones. Cocoa beans as research material from cocoa plantation at Batulappa area, Pinrang district, South Sulawesi. Some types of chemicals and supporting tools used in this activities: 80% acetone (Mallinckrodt, USA), Folin-Ciocalteu 50% reagent (Merck, Germany), n-hexane solvent, 20% Na₂CO₃, glacial acetic acid (Merck, Germany), gallic acid solution, Trolox standard (Sigma, Switzerland), distilled water, 300 mM acetate buffer solution pH 3.6, DPPH 104 mM reagent, methanol (Merck, Germany), theobromine (Sigma, USA), caffeine (Sigma, USA), (+) - catechins (Fluka 22110), and (-) - epicatechin (Sigma E1753), methanol HPLC grade (Merck, Germany) and water grade HPLC (Merck, Germany), filter paper, cotton, and aluminum foil.

2.2. Research Procedures
Fermentation of cocoa beans in the study was held in 6 days. Spontaneous fermentation of cocoa beans used styrofoam container with 40 kg capacity. The styrofoam container has a hole in the bottom used as a fermentation liquid discharge or change space for air in and out (aeration). Fermentation of cocoa beans was worked in anaerobic and aerobic. Anaerobic fermentation is carried out for the first 2 days then followed by aerobic fermentation for 4 days. Stirring of cocoa beans in aerobically after 48 hours on the 3 days until the 6 days. Drying of cocoa beans used sun drying was held for 6 - 7 days with a long irradiation of 7 - 8 hours per days. Drying was stopped after the water content of the cocoa beans reaches a maximum of 7.5%.

Samples for cocoa beans analysis from fermented and non-fermented from Sulawesi 2 clones and local clones. Samples in the form of dried cocoa beans with a moisture content of about 7%, weighing about 1 kg were taken 3 times for each clone, respectively. Samples were taken on the fermentation day 0 (non-fermentation), 2, 4 and 6 days before stirring.

2.3. Sample Analysis
Samples analyzed were carried out by removing the fat first in cocoa powder, then extracted using the method of Brcanovic et al., (2013). The polyphenol content analysis method uses the Folin-Calciteau method [33], antioxidant activity (IC50) using the DPPH method [34], bioactive compounds (Flavonoids and Methylxantin) using HPLC (Ramli et al., 2001), and aroma component analysis (volatile) was carried out using GC-MS QP 2010 Ultra (Shimadzu) [35].

The research was conducted used a factorial in Randomized Block Design (RBD) of two factors. The data was evaluated by ANOVA and continued with Duncan Multiple Range Test (DMRT) at 5% level to determine differences in mean values and analysis of interactions between treatment factors. The first factor is: the treatment of cocoa clones (S2 clones and local clones). The second factor is the length of fermentation treatment (0 day fermentation or non-fermentation, 2 days, 4 days and 6 days). Each treatment interaction was repeated in three times.

3. Results and Discussion

3.1. The Content of Polyphenols
The polyphenol compounds as chemical compounds has antioxidant properties very important in their role because they nourish the human body [36,37]. Polyphenol compounds is the important components because they determine the color, taste and aroma of chocolate. This is important factor in determining quality of cocoa beans. In general, good quality of cocoa beans has full brown color, strong taste and aroma. The polyphenol content in this study was showed in Figure 1.
Figure 1. Level of dried cocoa beans polyphenols during fermentation

The polyphenol content of cocoa beans was produced in the study ranged between 6.48-22.93 mg/g. Based on the data was showed the polyphenol content of cocoa beans decreased with increasing fermentation time. The result of variance was showed the cocoa clones and fermentation duration had a very significant effect (P<0.01) on the level of cocoa beans polyphenol produced, while their interaction showed the significant effect (P<0.05). The average polyphenol content from dried cocoa beans in local clones was higher than Sulawesi 2 clones (S2). The content of polyphenols in the cocoa beans before fermentation (F0) at the two clones was higher than the cocoa beans under the fermentation process. At the end of fermentation, the polyphenol content of cocoa beans dropped dramatically from 22.93 mg/g to 9.54 mg/g at the local clones, then S2 clones decreased from 10.71 mg/g on the second day fermentation to 6.48 mg/g at the end of fermentation. This is consistent with the research conducted by Aikpokpodion dan Dongo (2010) state that during the 5 days fermentation process there was a reduction in polyphenol compounds from 16.11% to 7.60% per day, resulting in a reduction in polyphenol compounds by 52.82%. The findings of Misnawi (2003) stated that there was a reduction in the polyphenol compound by 53.0% during the 5 day fermentation process of cocoa beans.

The longer process of fermentation caused greater loss of polyphenols in cocoa beans [38]. The decrease in the content of polyphenols during fermentation is due to the oxidation and condensation of polyphenols by polyphenol oxidase enzymes. The diffusion of polyphenols from cotyledon to the skin layer and the polymerization of polyphenol compounds especially epicatechin and proanthocyanidin formed tannin compounds and the formation of complexes with proteins and polysaccharides [39–41]. DiMattia et al., (2013) and Afoakwa et al., (2013) findings that the polyphenol polymerization reaction and the formation of complexes with other compounds, increasing the solubility of the polyphenols lost carried by the pulp fluid [42,43]. This process is desirable to reduce the astringent and bitter taste caused by the polyphenol compounds contain in the cocoa beans. While excessive overhaul is undesirable because they will produce processed chocolate products in tasteless condition [44]. According to Camu et al., (2008); Owusu, (2010) and Misnawi (2003) state the fermentation process able improve quality and forming a good chocolate flavor and aroma. In general, they will reduce the concentrate of bitter taste [45–47].

The changes in the composition of polyphenol during fermentation was characterized by a reduction in the purple color of the cocoa beans and increasing the intensity of the brown color. The higher polyphenol content in the cocoa beans will encourage the Maillard reaction with help of polyphenol oxidase produces cocoa color [48] The amount of polyphenol compounds in cocoa beans fermentation has varies number depending on the level of fruit maturity, clones/cultivars and the
environment in plantation of the cocoa plants grow, such as climate, soil types, presence of cacao insect pests and diseases [10,49,50].

3.2. Antioxidant Activity (IC$_{50}$)

IC$_{50}$ is a value that indicates the ability to inhibit the oxidation process by 50% a sample concentration. This IC$_{50}$ value was showed antioxidant activity in dried cocoa beans for inhibiting free radicals. The smaller IC$_{50}$ value indicates the higher antioxidant activity [51] IC$_{50}$ values generated from this study was showed in figure 2.

![Figure 2. IC$_{50}$ values of dried cocoa beans during fermentation](image)

The data from analysis was showed the length of the fermentation time was produced the higher value of the IC$_{50}$. The higher the IC$_{50}$ value meaning produced the lower of the antioxidant activity or free radical blocking. This data was showed that the antioxidant activity of cocoa beans decreased with increasing fermentation time. The results of variance was showed the factors of cocoa clones, fermentation time and the interaction of both had a very significant effect (P<0.01) on the produced of antioxidant activity (IC$_{50}$) in the cacao beans. The value of IC$_{50}$ in this study ranged from 14.39 to 88.67 mg/L. The average of antioxidant activity from local clones is much higher compared to S2 clones. In non-fermented cacao beans (F0) the IC$_{50}$ value produced in local clones was 14.39 mg/L while in clone S2 was 30.89 mg/L. During the fermentation process, the IC$_{50}$ value in cocoa beans increased until the end of the fermentation such as 53.37 mg/L in local clones and 88.67 mg/L in S2 clones. The increasing in IC$_{50}$ value indicates the antioxidant activity in cocoa beans is decreasing. This findings has strong related with study by Aikpokpodion dan Dongo (2010) [52] findings that the fermentation process in cocoa beans has an effect on decreasing the value of antioxidant activity. Antioxidant capacity was started from 96 to 79% from day 1 to 6 fermentation meaning decreased value of them based duration of fermentation.

The antioxidant activities derived from fermented cocoa beans is significantly lower than is not fermented [53]. According to Steinberg (2002) [54], DPPH radical inhibitory activity decreases with longer fermentation time. It means that the decrease in antioxidant activity occurs decrease in polyphenol content. Polyphenols are the main component of cocoa beans that play a role in antioxidant
activity. The flavonoid and phenolic compounds as the group of polyphenols are known to be antioxidants due to the hydroxyl attached to the structure [55].

3.3. Content of Bioactive Compounds (Flavonoids dan Methylxantin)

3.3.1. Flavonoids Compound (Catechins dan Epicatechins). The flavonoid compounds have been identified in the study are: catechin and epicatechin. The results analysis of the content of catechins and epicatechins found in fermented dried cocoa beans was presented in Table 1. The content of catechin and epicatechin compounds produced in dried cocoa beans is very volatile where during 6 days of fermentation. There is a fluctuation in concentration. The content of catechin compounds in cocoa beans produced in both clones is very high with ranging between 33.67 - 104.63 (mg/g). The highest of catechin content in S2 clones was produced on the second day fermentation of 57.41 mg/g and the lowest of 44.09 mg/g on 6 days fermentation. For the local clones, the catechin content produced very high approximately 104.63 mg/g in unfermented cocoa beans (F0). The catechin content dropped dramatically to 33.67 mg/g during the 2 days fermentation then increase again until the 6 days fermentation to 84.03 mg/g of dried cocoa beans. The epicatechin, catechin, flavanol and antioxidant capacity of fermented cocoa beans are significantly lower than cocoa beans not fermented [52,53].

However, these results very much different from the research conducted by Brćanovic et al., (2013) findings catechin levels of 0.03-0.18 mg / g and Belsak et al., (2009) [56] the number 0.04-0.33 (mg/g) in cocoa powder. Based this findings, it means processing of cocoa beans has an influence on changes of the composition in catechin compounds contain in the cocoa products. The catechin content will be reduced with increasing processing of dried cocoa beans after fermentation. The activities after fermentation such as: drying, roasting, pasting and pressing. The many processed of cocoa beans reduced the catechin content in cocoa products. Efraim et al., (2011) [57] state that during the fermentation, the polyphenol content decreased about 70%, and epicatechin as the main substrate of polyphenoloxidase will reduction of 90% from its initial concentration. At the same time, catechins forming complex of tannin and anthocyanins are hydrolyzed into self-polymerizing anthocyanins.

Table 1. The content of flavonoid compounds (catechin and epicatechin) in dried cocoa beans during fermentation

| Cocoa Clone | Phenolic Compounds | Fermentation Time (days) |
|-------------|--------------------|-------------------------|
|             | 0                  | 2          | 4          | 6          |
| Sulawesi 2  | Catechin           | 54.74<sup>a</sup> | 57.41<sup>a</sup> | 53.41<sup>c</sup> | 44.09<sup>d</sup> |
|             | Epicatechin        | 17.34<sup>a</sup> | 7.37<sup>d</sup>  | 15.09<sup>b</sup> | 9.71<sup>c</sup>  |
| Local       | Catechin           | 104.63<sup>a</sup> | 33.67<sup>d</sup> | 79.19<sup>c</sup> | 84.03<sup>b</sup> |
|             | Epicatechin        | 28.50<sup>c</sup> | 32.37<sup>b</sup> | 41.97<sup>a</sup> | 27.38<sup>d</sup> |

Notes : The numbers followed by the same letters on the same column are not significantly different based to the Duncan test at the α = 5%.

The content of cocoa beans epicatechin compounds in S2 and local clones was highly number (Table 1). In the cacao beans before fermentation (F0) on Sulawesi 2 clones was showed epicatechin content about 17.34 mg/g then decreased on the 2 days fermentation at 7.37 mg/g, then increase again on 4 days fermentation. In the end of fermentation, final result of epicatechin from S2 clones as 9.71 mg/g. Based finding research of Kim dan Keeney (1984) [58] and Kayaputri et al. (2014) [59] suggested that there was a decrease epicatechin in cocoa beans during the 2 and 3 days of fermentation, then increase in epicatechin at the skin of cocoa beans. In the end of fermentation at 6 days, occur decrease of epicatechin in the cotyledone and skin of cocoa beans. Bernaert et al., (2012) [45] state that during 2 to 3 days of fermentation of cocoa beans the epicatechin content began to decrease significantly. Hurst et al., (2011) [37] and Subagya (2009) [60]explained that the reduction in epicatechin caused by the phenomenon of epimerization in epicatechin into catechin due to changes in pH during the fermentation process. Higher catechin concentration supported the claim that
epicatechin epithelium has occurred into catechin. In fermentation, catechin level will increase, at the same time epicatechin level will decrease.

Epicatechin level in local clones also increased and decreased concentration in cocoa beans. Where the epicatechin content in cocoa beans before fermentation (F0) produced by 28.50 mg/g and reach 41.97 mg/g on the 4 days fermentation then decreased to 27.38 mg/g on the 6 days fermentation (Table 1). This incident accordance with research of Cruz et al., (2015) [61] was showed an increase in epicatechin content during the fermentation process. According to Cruz et al., (2013), the increase in epicatechin during the fermentation was related to the hydrolysis of tannin and procyanidin. The action of polyphenoloxidase is the process of producing and oxidizing each smaller phenolic compound.

3.3.2. Methylxantine Compounds (Theobromin dan Caffeine). In general, methylxanthines such as theobromine and caffeine as another group of bioactive compounds that also found in cocoa beans [38], Methylxanthes (caffeine and theobromin) have benefits for maintaining health [62]. Caffeine compounds have a stimulating effect on the brain, increase psychomotor reactions and blood pressure, theobromin acts as a muscle relaxant, diuretic, blood pressure reducer, cardiovascular protection and reduces the risk of coronary heart disease [63,64]. The content of methylxanthine compounds found in fermented dried cocoa beans was showed in Table 2.

Theobromine compounds produced in this study ranged from 13.98 to 36.37 mg/g. This number is quite high for the theobromin content of cocoa beans. The results of the analysis was showed that the theobromin content in dried cocoa beans in S2 clones decreased with increasing fermentation time. However, it is inversely proportional to the theobromin content in local clones increases with increasing fermentation time. The theobromine content of unfermented S2 cocoa beans is relatively greater than that fermented cocoa beans. The theobromin content in non fermented cocoa beans (F0) in S2 clones results about 18.03 mg/g then decreased until the 6 days fermentation to 13.98 mg/g. In contrast, the theobromin content at local clones was increased where the highest level produced at the end of fermentation amount to 36.37 mg/g and the lowest produced from cocoa beans before fermentation/non-fermentation amount to 24.67 mg/g. On the other hand, high methylxanthine content affected the sensory characteristics of cocoa beans, because sensory attributes such as concentrated bitter are highly correlated with methylxantine and phenolic compounds [57].

The decrease in theobromine content was caused in the fermentation because the process in high temperature with purpose killing the cotyledone of cocoa beans. At that time, the fermented liquid was formed and dissolve theobromin. The theobromin comes out of the cocoa beans to the skin. Based on this fact, well fermented of cocoa beans generally have theobromine levels less than 0.7% which go to the cocoa beans coat due to condensation reaction [65]. Sena et al. (2011) [66] reported that on the first day of fermentation, there was an increase in theobromine concentration at cocoa beans piece and a decrease in their coat. On the following day there was a decrease in concentration in the cocoa bean piece followed by an increase in its coat. A decrease in theobromine is expected with the aim of improving taste, but excessive decline also results in less good [67]. Fermentation of cocoa beans in local clones was showed increased in theobromine concentration. This incidence to stimulate the degradation of theobromine as a derivative of caffeine compounds. Microbes such as bacteria need a source of carbohydrates in the form of simple sugars derived from the degradation of cocoa beans. It need more time optimal degradation the theobromine. Cocoa beans are not fermented properly will have high theobromine and anthocyanin causing a bitter taste in cocoa beans [27].
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Table 2. The content of methylxanthine compounds (theobromine and caffeine) in dried cocoa beans during fermentation

| Cacao Clone | Methylxantine Compounds | Fermentation Time (days) |
|-------------|-------------------------|--------------------------|
|             |                         | 0       | 2       | 4       | 6       |
| Sulawesi 2  | Theobromine             | 18.03a  | 17.78b  | 14.4c   | 13.98d  |
|             | Caffeine                | 2.32b   | 2.33a   | 2.20c   | 1.97d   |
| Local       | Theobromine             | 24.67d  | 27.73c  | 35.88b  | 36.37a  |
|             | Caffeine                | 1.62a   | 0.33d   | 1.07c   | 1.14b   |

Notes: The numbers followed by the same letters on the same column are not significantly different based on the Duncan test at the α = 5%.

Table 2 was showed the caffeine content in cocoa beans on average decreased during 6 days of fermentation. The highest caffeine content produced from non-fermented cocoa beans (F0) in local clones is 1.62 mg/g while in S2 clones the highest caffeine content was produced in fermented cocoa beans at 2 days is not much different in the cocoa beans before fermentation around 2.32 mg/g. On the 6 days of fermentation, caffeine levels decreased to 1.97 mg/g in S2 clones and 1.14 mg/g in local clones. Caffeine is a compound has a low concentration in cocoa beans. Methylxanthine compounds decreased during the fermentation process to 30% of the initial methylxanthine content. This decrease is due to the diffusion of alkaloids from cotyledons [68]. The fluctuation in the content of bioactive compounds produced during fermentation is an unavoidable factor. The fluctuation occurred because several factors such as: plant genetic, soil and growth conditions, weather characteristics then processing of cocoa beans affected the profile and concentration of these compounds [10,16,69,70]. The infection impact by insect pests and fungal pathogens during the growth of cocoa plants also affected the concentration of bioactive compounds found in the cocoa beans produced by these plants. The finding of research by Aneja and Gianfagna (2001) was showed the plant tissue infected with fungi produced several biochemical defense mechanisms as evidenced by the higher content of methylxanthines and phenolic compounds. When plants attacked by potential pathogens, there is the activation of complex defense responses was showed by the expression of various genes, resulting in the synthesis and accumulation of secondary metabolites such as phenolic compounds [70].

3.4. Aromatic Components (Volatile) from Dry Cocoa Beans Fermentation

The component analysis of aromatic compounds in this study was carried out by adapting the method of Reineccius (1972) which analyzes presence pyrazin in cocoa beans. Pyrazin is the one component of the typical aromatic compounds in cocoa beans. The compound considered a large contribution and responsible to the aromatic and flavor in cocoa beans is pyrazine because it is non volatile (Jinap et al., 1994). The results of the cocoa beans aromatic extract then analyzed by gas chromatography-mass spectrometry (GC-MS) type QP 2010 Ultra Zhimadzu. The results of the analysis of aromatic compounds (volatiles) on cocoa beans fermented from local clones was showed in Table 3.

Table 3. Components of aromatic compounds (volatile) of fermented cocoa beans

| Number | Compounds                                      | Area of Concentration (%) |
|--------|-----------------------------------------------|---------------------------|
|        |                                               | Fermentation 2 days | Fermentation 4 days | Fermentation 6 days |
| 1      | Alcohol :                                     |                          |                      |                      |
|        | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol         | -                        | 1.98                 | -                    |
|        | 1,6-methanonaphthalen-1(2h)-ol, octahydro-4,8a,9,9-tetramethyl | 1.57                    | 2.79                 | -                    |
|        | 1,1,4,7-tetramethyldecahydro-1h-cyclopropa[e]azulen-4-ol | -                        | 2.16                 | -                    |
| 2      | Ketone :                                      |                          |                      |                      |
The results of the analysis in Table 3 was showed that the volatile compounds identified in the fermented cocoa beans consist of: alcohol, aldehyde, ester, some acids, purines, hydrocarbons, ketones and several other compounds. The way and time of the fermentation process is a crucial factor in the formation of cocoa aromatic and flavor precursor components [71]. Purine and caffeine compounds showed a decrease number in the concentration of the area with increasing fermentation time, whereas acidic, ketone and hydrocarbon compounds were identified only on the 6 days of fermentation. For alcohol group, more of them was identified in the 4 day fermented cocoa beans, while for the ester group was identified in fermentation 2, 4 and 6 days. However, more compounds found in 2 days of fermented cocoa beans such as: Dodecanoic acid, methyl ester, Tetradecanoic acid, Hexadecanoic acid and Octadecanoic Acid. Alcohol group will experience an increase during fermentation and decrease during the drying and roasting process. Ester group was related to fruit flavor, the content decreases with increasing fermentation time, while the acid content increasing follow the fermentation time [29,72,73].

The aromatic component of cocoa beans consisting of volatile compounds. There are mainly formed from the reaction between amine and carboxyl groups. The last two compounds is the result of
a breakdown of peptides and carbohydrates that are formed during the process of preparing cacao beans, especially during fermentation and drying [74]. In the fermentation process, acetic acid bacteria playing an important role in the formation of flavor precursor compounds [75]. Voigt et al., (1994) [17] added important information that the candidate compounds forming the distinctive aromatic of cocoa beans consist of hydrophobic amino acids, hydrophilic peptides and reducing sugars. Flavor-forming compounds react with each other through the Maillard reaction to produce volatile components and produce distinctive chocolate flavors, including alcohol, ether, furan, thiazole, python, acid, ester, aldehyde, amine, oxazol, pyrazine and pyrrole [39,48,65].

Aromatic compounds was produced in local cocoa clones are almost the same as those produced in S2 clones such as : alcohol, aldehyde, esters, acids, and phenols which are precursors of cocoa aroma. Only in local cocoa clones, pyrazine compounds were not detected in non-fermented or fermented cocoa beans. In contrast, at S2 cocoa clones, pyrazine compounds were produced only during the 6 days fermentation treatment. Pyrazine derivative, namely tetramethyl pyrazine, was detected at the time retention of 9.317 minutes with an area percentage of 25.92% and the minute of 9.408 with an area percentage of 16.63%. In other treatments no pyrazine compounds were detected. Tetramethyl pyrazine gives the aroma such as roasted coffee. In addition to Tetramethyl pyrazine also produced compounds Benzeneacetaldehyde which gives specific aroma to cocoa beans . Aldehyde compounds have an important role in providing distinctive aromatic [29]. The results of chromatogram analysis of aromatic compounds on cocoa beans fermented by S2 clone on 6 days fermentation was showed in Figure 3.

For non-fermented cocoa beans in both S2 and local clones was presented the another aroma compounds such as aromadendren. Aromadendren is the one of the derivatives of hydrocarbon sesquiterpenes which are generally scented with special odor found in aloes wood (Pasaribu et al., 2015). According to Purwo (2012) state that unfermented cocoa will not have a strong aroma and taste. Cocoa beans that are not fermented or imperfectly fermented cause bad flavor because have a high concentrated bitter taste. They can not produce a distinctive chocolate flavor during the roasting process [17,42].

![Figure 3](Chromatogram-FS81-C-Q3E-Evulsion-DataProject-FS81-QGD)

**Figure 3.** Chromatograms the results of the analysis of aromatic compound profiles on the dried cocoa beans of S2 clone on 6 days fermentation.

Notes: 1. 3-(3-Oxo-3h-Benzof[Fl]Chromen-2-Yl)-2,4(1h,3h)-Quinolinedione (24.67%); 2. Benzeneacetaldehyde (14.65%); 3. Imidazole2,4-dibromo-5-nitro-(18.13%); 4. Pyrazine, Tetramethyl (25.92%); 5. Pyrazine, tetramethyl (16.63%)
The good fermented cacao contain high pyrazine (Afoakwa et al., 2008). Pyrazine-derived compounds detected in this study were small in number due to several possibilities. Commonly unfavorable sample preparation process where the distilled sample is not directly injected into the GC-MS. The another fact is possibility the aromatic compound in the sample evaporates. This is according to research by Kresnowati et al., (2017) [76] discovered that metabolites with small concentrations can be degraded if stored for long periods of time. Thus the process of sample preparation should be done thoroughly and immediately analyzed. Besides the imperfect formation of flavor components in fermented cocoa beans in this study, it is likely due to the use of styrofoam as a fermentation container. Fermentation temperature conditions in styrofoam may not be suitable in optimizing fermentation activities by the microbes involved. Related to container used in the study, Scollo et al. (2020) [77] reported that over 1000 peptides have been detected from cocoa beans in all fermentation stages used styrofoam box. Then the highest number of peptides were identified after 2 and 4 days of fermentation. The reason styrofoam box was used because cheaper than wooden box. Then Nuwiah (2010) [78] was added information when analyzing the aromatic compound profile not all aromatic components can be identified properly by GC - MS, because it is influenced by the type of column available and the way of isolation from the aromatic compound.

According to Castro-Alayo et al. (2019) [79] reported that related to the cocoa clones and their final result, the preferences based their experiment on the past about their aroma, as an attribute that determines their quality. As the highlight, Criollo cocoa is of the highest quality and used in the manufacture of fine chocolates because of its fruity aroma. The aroma of Criollo cocoa is defined by volatile compounds such as pyrazines and aldehydes, which are formed during roasting of the bean, from aroma precursors (reducing sugars and free amino acids) that are generated inside the bean via enzymatic reactions during fermentation. For this reason, fermentation is the most important process in the value chain.

Menezes et al., (2016) [24] state that properly conducted cocoa fermentation is an important step for the production of high-quality chocolate. The four cocoa varieties (CCN51, PS1030, FA13, and CEPEC 2004) inoculated with *Saccharomyces cerevisiae* CA11 on microbial communities and the profile of volatile compounds and sensory characteristics of chocolate. The *S. cerevisiae* population increased significantly (p < 0.05) during the fermentations. Sixty-one volatile compounds—including aldehydes (11), ketones (10), esters (14), acids (8), alcohols (8), pyrazines (5), furans (3), and others (caffeine and heptadecane)—were detected and quantified by GC–MS in the different chocolates. The sensory analysis showed that caramel was perceptible in the chocolate of PS1030, while CEPEC2004 was related to astringency, bitterness, and chocolate flavor attributes. The chocolates produced from FA13 and CCN51 were more similar in terms of sour and chocolate aroma. A “temporal dominance of sensation” (TDS) analysis showed that although the bitter attribute was dominant, the fruity, sweet, sour, astringent, and cocoa attributes were also perceptible, depending on the cocoa variety. These results suggest that the cocoa varieties had an influence on the chocolate’s quality, which should be considered to obtain chocolate with different sensory characteristics or for better standardization of the process, even when using yeast as a starter culture.

The aroma of chocolate products in addition to being influenced by pre-harvest conditions is also greatly influenced by post-harvest conditions including the fermentation process, how to dry and roast the seeds. The type of cocoa, genetic characteristics of the area of origin grow including climatic conditions and soil conditions are the first factors caused the type and content of chemical compounds producing aroma precursors in cocoa to be different [79].

4. Conclusion

The profiles of bioactive compounds and antioxidant activity in cocoa beans produced in this study have different values. The total content of polyphenols of cocoa beans decreased with increasing fermentation time, ranging between 6.48-22.93 mg/g. The antioxidant activity of the DPPH method has an IC$_{50}$ range of 14.39-88.67 mg/L.
The longer of the fermentation time resulted the higher of IC₅₀ value produced with the lower of the antioxidant activity. Analysis of the cocoa powder bioactive compound profile used HPLC-UV gives the retention time of each compound ranging from 3 minutes for (+) – catechin; 4.6 minutes for (-) – epicatechin; 3.2 minutes for theobromine and 3.3 minutes for caffeine. The content of catechin, epicatechin, theobromine, caffeine in S2 and local of cocoa beans are 33.67 - 104.63 mg/g; 7.37 - 41.97 mg/g; 13.98 - 36.37 mg/g; and 0.33 - 2.33 mg/g, respectively.

The results of GCMS analysis of aroma in cocoa beans during fermentation produced volatile compounds contain alcohol, aldehyde, esters, acids, purines, hydrocarbons, ketones and several other compounds. These compounds are candidate compounds forming/precursor of distinctive aroma of fermented cocoa beans. Pyrazine compounds such as tetramethyl pyrazine only detected in the 6 days fermentation treatment in S2 clones. Whereas in other treatments there was no pyrazine compound detected. Tetramethyl pyrazine gives the specific aroma like roasted coffee.

The result was showed that cocoa beans from local clones contained higher polyphenol content, antioxidant activity and bioactive compounds (catechin, epicatechin and theobromine) compared to S2 clones. While the high caffeine content and distinctive aroma of pyrazine compounds are produced from fermented cocoa beans from S2 clones.

Genetic characteristics, clones/varieties of cocoa, soil, growth conditions, weather characteristics and processing methods of cocoa beans affected the profile and concentration of chemical compounds producing aromatic precursors in cocoa beans.

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References
[1] Indonesia S P Indonesia 2014-2016. 2015 Jambu mete (Cahew Nut). Subiyantoro ME, Arianto Y, Ed. Jakarta Direktorat Jenderal Perkebunan, Kementeri. Pertan.
[2] Mazor Jolić S, Radojčić Redovniković I, Marković K, Ivanec Šipušić Đ and Delonga K 2011 Changes of phenolic compounds and antioxidant capacity in cocoa beans processing Int. J. food Sci. Technol. 46 1793–800
[3] Hurst W J, Krake S H, Bergmeier S C, Payne M J, Miller K B and Stuart D A 2011 Impact of fermentation, drying, roasting and Dutch processing on flavan-3-ol stereochemistry in cacao beans and cocoa ingredients Chem. Cent. J. 5 1–10
[4] Afoakwa E O, Paterson A, Fowler M and Ryan A 2008 Flavor formation and character in cocoa and chocolate: a critical review Crit. Rev. Food Sci. Nutr. 48 840–57
[5] Afoakwa E O 2010 Chocolate Science and Technology
[6] Ackar D, Valek Lendić K, Valek M, Šubarić D, Miličević B, Babić J and Nedić I 2013 Cocoa polyphenols: can we consider cocoa and chocolate as potential functional food? J. Chem. 2013
[7] Latif R 2013 Chocolate/cocoa and human health: a review Neth J Med 71 63–8
[8] Cooper K A, Campos-Giménez E, Jiménez Alvarez D, Nagy K, Donovan J L and Williamson G 2007 Rapid reversed phase ultra-performance liquid chromatography analysis of the major cocoa polyphenols and inter-relationships of their concentrations in chocolate J. Agric. Food Chem. 55 2841–7
[9] Di Mattia C D, Sacchetti G, Mastrocola D and Serafini M 2017 From cocoa to chocolate: The impact of processing on in vitro antioxidant activity and the effects of chocolate on antioxidant markers in vivo Front. Immunol. 8 1207
[10] Meng C C, Jalil A M M and Ismail A 2009 Phenolic and theobromine contents of commercial dark, milk and white chocolates on the Malaysian market Molecules 14 200–9
[11] Rios L Y, Gonthier M-P, Rémésy C, Mila I, Lapierre C, Lazarus S A, Williamson G and Scalbert A 2003 Chocolate intake increases urinary excretion of polyphenol-derived
phenolic acids in healthy human subjects Am. J. Clin. Nutr. 77 912–8

[12] Martínez-Pinilla E, Oñatibia-Astibia A and Franco R 2015 The relevance of theobromine for the beneficial effects of cocoa consumption Front. Pharmacol. 6 30

[13] Djauhari A, Hasibuan A M and Rubiyo R 2013 Effect Of Fermentation Technology On The Increasing Of Cocoa Beans Quality And Farmers’ income

[14] John W A, Kumari N, Böttcher N L, Koffi K J, Grimbis S, Vrancken G, D’Souza R N, Kuhnert N and Ullrich M S 2016 Aseptic artificial fermentation of cocoa beans can be fashioned to replicate the peptide profile of commercial cocoa bean fermentations Food Res. Int. 89 764–72

[15] Jespersen L, Nielsen D S, Hønholt S and Jakobsen M 2005 Occurrence and diversity of yeasts involved in fermentation of West African cocoa beans FEMS Yeast Res. 5 441–53

[16] Kadow D, Niemenak N, Rohn S and Lieberei R 2015 Fermentation-like incubation of cocoa seeds (Theobroma cacao L.)—Reconstruction and guidance of the fermentation process LWT- Food Sci. Technol. 62 357–61

[17] Biehl B and Voigt J 1996 Biochemistry of chocolate flavour precursors International Cocoa Conference, Salvador de Bahia, Brazil, November 1996

[18] Jinap S, Rosli W I W, Russly A R and Nordin L M 1998 Effect of roasting time and temperature on volatile component profiles during nib roasting of cocoa beans (Theobroma cacao) J. Sci. Food Agric. 77 441–8

[19] Paper T 2012 Kajian Fermentasi dan Suhu Pengeringan pada Mutu Kakao (Theobroma cacao L.) J. Keteknikan Pertan. 26 129–36

[20] Utami Hatmi R and Rustijarno S 2012 Teknologi pengolahan biji kakao menuju SNI biji kakao 01-2323-2008

[21] Mukhlason M, Degeng I N S and Sihkabuden 2015 Pengaruh Strategi Pembelajaran (Peta Konsep dan Rangkuman) dan Motivasi Berprestasi Terhadap Hasil Belajar Pembelajaran Sejarah J. Ilm. dan Teknol. pembelajaran 2 280–91

[22] Mulato and Widyotomo 2006 Pengolahan Produk Primer dan Sekunder Kopi. (Jember: Pusat Penelitian Kopi dan Kakao Indonesia)

[23] Indarti E, Widayat H P and Zuhri N 2011 Effect of fermentation container and thickness of bean mass during fermentation process of cocoa bean (Theobroma cacao L.) Proceedings of The Annual International Conference, Syiah Kuala University-Life Sciences & Engineering Chapter vol 1

[24] Menezes A G T, Batista N N, Ramos C L, e Silva A R de A, Efraim P, Pinheiro A C M and Schwan R F 2016 Investigation of chocolate produced from four different Brazilian varieties of cocoa (Theobroma cacao L.) inoculated with Saccharomyces cerevisiae Food Res. Int. 81 83–90

[25] Widianto D, Pramita A D and Wedhasstri S 2013 Perbaikan proses fermentasi biji kakao kering dengan penambahan tetes tebu, khamir, dan bakteri asam asetat J. Teknosains 3

[26] Lima L J R, Almeida M H, Nout M J R and Zwietering M H 2011 Theobroma cacao L.; “The food of the Gods”: quality determinants of commercial cocoa beans, with particular reference to the impact of fermentation Crit. Rev. Food Sci. Nutr. 51 731–61

[27] Widyotomo S and Mulato S 2008 Teknologi fermentasi dan diversifikasi pulpa kakao menjadi produk yang bermutu dan bernilai tambah War. Rev. Penelit. Kopi dan Kakao 24 65–82

[28] Towaha J and Anggraini D A Rubiyo. 2012 Keragaan mutu biji kakao dan Prod. turunannya pada berbagai tingkat Ferment. Stud. kausi di Tabanan, Bali. Pelita Perkeb. 28 166–83

[29] Tarigan E B 2018 Beberapa komponen fisikokimia kakao fermentasi dan non fermentasi J. Agroindustri Halal 3

[30] Siregar K 2018 Analisis Pindah Panas Pada Ruang Fermentasi Biji Kakao ( Theobroma cacao L.) Analysis Of Transfer Heat Room Of Cocoa Seed Fermentation Theobroma cacao L.) Dengan Menggunakan Kotak Kayu dan Styrofoam Alumni Mahasiswa S1 Program Studi Teknik Pertanian, Fakultas Pertanian, Universitas Program Studi Teknik Pertanian,
Sukendar N K, Tawali A B, Salengke, Syarifuddin A, Mochtar A H and Fakhiruddin A 2019 Changes in Physical-Chemical Properties During The Fresh Cocoa Fermentation Process

Canaresa J. Food Technology, Nutr. Culin. J. 02 98–105

Septianti E, Langkong J, Sukendar N K and Hanifa A P 2020 Characteristic Quality of Pinrang’s Cocoa Beans During Fermentation Used Styrofoam Containers

Canaresa J. Food Technol. Nutr. Culin. J. 3 10–25

Marinova D, Ribarova F and Atanassova M 2005 Total phenolics and total flavonoids in Bulgarian fruits and vegetables

J. Univ. Chem. Technol. Metall. 40 255–60

Isrul, Cyio M, Basir, Syamsuddin L, Nurdin R, Rustam R, Abdul, Catur A A and Darma H M 2019 Analysis of Household Food Security and Food Insecurity in Donggala Regency

Indian J. Public Heal. Res. Dev. 10

Brčanović J M, Pavlović A N, Mitić S S, Stojanović G S, Manojlović D D, Kaličanin B M and Veljković J N 2013 Cyclic voltammetric determination of antioxidant capacity of cocoa powder, dark chocolate and milk chocolate samples: correlation with spectrophotometric assays and individual phenolic compounds

Food Technol. Biotechnol. 51 460–70

Jalil A M M and Ismail A 2008 Polyphenols in cocoa and cocoa products: is there a link between antioxidant properties and health?

Molecules 13 2190–219

Crozier S J, Preston A G, Hurst J W, Payne M J, Mann J, Hainly L and Miller D L 2011 Cacao seeds are a “Super Fruit”: A comparative analysis of various fruit powders and products

Chem. Cent. J. 5 1–6

Cruz J F M, Leite P B, Soares S E and da Silva Bispo E 2013 Assessment of the fermentative process from different cocoa cultivars produced in Southern Bahia, Brazil

African J. Biotecnol. 12 5218–25

Misnawi J S 2003 Effect of cocoa bean polyphenols on sensory properties and their changes during fermentation

Pelita Perkebunan 19 90–103

Caligiani A, Acquotti D, Cirilini M and Palla G 2010 1H NMR study of fermented cocoa (Theobroma cacao L.) beans

J. Agric. Food Chem. 58 12105–11

Emmanuel O A, Jennifer Q, Agnes S B, Jemmy S T and Firibu K S 2012 Influence of pulp-preconditioning and fermentation on fermentative quality and appearance of Ghanaian cocoa (Theobroma cacao) beans

Int. Food Res. J. 19 127

Afoakwa E O, Kongor J E, Takrama J F, Budu A S and Mensah-Brown H 2013 Effects of pulp preconditioning on total polyphenols, o-diphenols and anthocyanin concentrations during fermentation and drying of cocoa (Theobroma cacao) beans

J. Food Sci. Eng. 3 235

Di Mattia C, Martuscelli M, Sacchetti G, Scheirlinck I, Beheydt B, Mastrocola D and Pittia P 2013 Effect of fermentation and drying on procyanidins, antiradical activity and reducing properties of cocoa beans

Food Bioprocess Technol. 6 3420–32

Wahyudi T 1988 Periksa kakao dan komponen-komponennya (Pelita Perkebunan)

Owusu M 2010 Influence of raw material and processing on aroma in chocolate (University of Copenhagen, Faculty of Life Sciences, Department of Food Science)

Unfermented O F 2003 Influences Of Cocoa Polyphenols And Enzyme Reactivation On The Flavor Development Of Unfermented And Under-Fermented Cocoa Beans

Puziah H S 2005 Cocoa fermentation

Ps. Penelit. Kopi dan Kakao Indones. Jember

Kowalska J and Sidorczuk A 2007 Analysis of the effect of technological processing on changes antioxidant properties of cocoa processed products

Polish J. Food Nutr. Sci. 57 95–9

Bruna C, Eichholz I, Rohn S, Kroh L W and Huyskens-Keil S 2009 Bioactive compounds and antioxidant activity of cocoa hulls (Theobroma cacao L.) from different origins.

J. Appl. Bot.
Food Qual. 83 9–13

[51] Hasanah M 2016 Analisis Golongan Senyawa Kimia dan Uji Potensi Antioksidan dari Ekstrak Daun Cokelat (Theobroma cacao L.) Hasil Ekstraksi Maserasi J. Ilm. Bakti Farm. 1 43–8

[52] Aikpokpodion P E and Dongo L N 2010 Effects of fermentation intensity on polyphenols and antioxidant capacity of cocoa beans Int. J. Sustain. Crop Prod 5 66–70

[53] Chin E, Miller K B, Payne M J, Hurst W J and Stuart D A 2013 Comparison of antioxidant activity and flavanol content of cacao beans processed by modern and traditional Mesoamerican methods Herit. Sci. 1 1–7

[54] Steinberg M K 2002 The globalization of a ceremonial tree: The case of cacao (Theobroma cacao) among the Mopan Maya Econ. Bot. 56 58–65

[55] Majewska M, Skrzyczy M and Podsiad, M. 2011 Evalution of antioxidant potential of flavonoids : an in vitro study Acta Pol. Pharm 68 611–5

[56] Belščak A, Komes D, Horžić D, Ganić K K and Karlović D 2009 Comparative study of commercially available cocoa products in terms of their bioactive composition Food Res. Int. 42 707–16

[57] Efraim P, Alves A B and Jardim D C P 2011 Polyphenols in cocoa and derivatives: Factors of variation and health effects Brazilian J. Food Technol. 14 181–201

[58] Kim H and Keeney P G 1984 (-)-Epicatechin content in fermented and unfermented cocoa beans J. Food Sci. 49 1090–2

[59] Kayaputri I L, Sumanti D M, Djali M, Indiarto R and Dewi D L 2014 Kajian Fitokimia Ekstrak Kulit Biji Kakao (Theobroma cacao L.) Chim. Nat. Acta 2 83–90

[60] Subagia S 2020 Analisis Katekin Dan Epikatekin Dalam Biji Kakao Serta Produk Olahannya menggunakan Kromatografi Cair Spektrometri Massa

[61] Cruz J F M, Leite P B, Soares S E and Bispo E da S 2015 Bioactive compounds in different cocoa (Theobroma cacao, L) cultivars during fermentation Food Sci. Technol. 35 279–84

[62] Franco R, Onatibia-Astibia A and Martinez-Pinilla E 2013 Health benefits of methylxanthines in cacao and chocolate Nutrients 5 4159–73

[63] Lee S K and Kader A a. 2000 Preharvest and postharvest factors influencing vitamin C content of horticultural crops Postharvest Biol. Technol. 20 207–20

[64] Khan N, Monagas M, Andres-Lacueva C, Casas R, Urpi-Sarda M, Lamuela-Raventos R M and Estruch R 2012 Regular consumption of cocoa powder with milk increases HDL cholesterol and reduces oxidized LDL levels in subjects at high-risk of cardiovascular disease Nutr. Metab. Cardiovasc. Dis. 22 1046–53

[65] St Sabahanunnur M, Asrul L and Bilang M 2015 Use of High Performance Liquid Chromatography (HPLC) for the Analysis of Amino Acid of Sulawesi and Local Clone Cocoa Bean Fermentation J. Food Res. 4 120–6

[66] Reges de Sena A, de Assis S A and Branco A 2011 Analysis of Theobromine and Related Compounds by Reversed Phase High-Performance Liquid Chromatography with Ultraviolet Detection: An Update (1992-2011). Food Technol. Biotechnol. 49

[67] Muflikhah M, Rusdiarso B, Putra E G R and Nuryono N 2017 Modification of Silica Coated on Iron Sand Magnetic Material with Chitosan for Adsorption of Au(III) Indones. J. Chem.

[68] Zaidi F, Nigam A, Anjum R and Agarwal R 2017 Postpartum depression in women: a risk factor analysis J. Clin. diagnostic Res. JCDR 11 QC13

[69] Elwers S, Zambrano A, Rohsius C and Lieberei R 2009 Differences between the content of phenolic compounds in Criollo, Forastero and Trinitario cocoa seed (Theobroma cacao L.) Eur. Food Res. Technol. 229 937–48

[70] Leite P B, Maciel L F, Opretzka L C F, Soares S E and Bispo E da S 2013 Phenolic compounds, methylxanthines and antioxidant activity in cocoa mass and chocolates produced from "witch broom disease" resistant and non resistant cocoa cultivars Ciência e agrotecnologia 37 244–50

[71] Kongor J E, Hinneh M, Van de Walle D, Afoakwa E O, Boeckx P and Dewettinck K 2016
Factors influencing quality variation in cocoa (Theobroma cacao) bean flavour profile—A review Food Res. Int. 82 44–52

[72] Rodriguez-Campos J, Escalona-Buendía H B, Contreras-Ramos S M, Orozco-Avila I, Jaramillo-Flores E and Lugo-Cervantes E 2012 Effect of fermentation time and drying temperature on volatile compounds in cocoa Food Chem. 132 277–88

[73] Afoakwa E O, Paterson A and Fowler M 2007 Factors influencing rheological and textural qualities in chocolate - a review Trends Food Sci. Technol.

[74] Wahyudi 2008 Panduan Lengkap Kakao (Jakarta: Penebar swadaya)

[75] Aji A A, Satria A and Hariono B 2014 Strategi pengembangan agribisnis komoditas padi dalam meningkatkan ketahanan pangan Kabupaten Jember J. Manaj. Agribisnis 11 60–7

[76] Kresnowati M T A P, Fitriana H N and Purwadi R Pemetaan Pengaruh Proses Pengolahan pada Kualitas Biji Kakao Menggunakan Metode Metabolik Profiling dengan GC/MS Reaktor 17 132–9

[77] Scollo E, Neville D C A, Oruna-Concha M J, Trotin M, Umaharan P, Sukha D, Kalloo R and Cramer R 2020 Proteomic and peptidomic UHPLC-ESI MS/MS analysis of cocoa beans fermented using the Styrofoam-box method Food Chem. 316 126350

[78] Nuwiah N 2010 Uji Senyawa aroma khas coklat hasil roasting asam amino hidrofobik dan fruktosa dalam lemak kakao J. Agriplus 20 128–854

[79] Castro-Alayo E M, Idrogo-Vásquez G, Siche R and Cardenas-Toro F P 2019 Formation of aromatic compounds precursors during fermentation of Criollo and Forastero cocoa Helixyon 5 e01157

[80] Yudi W 2015 Sistem Sosial Ekonomi dan Budaya Masyarakat Pesisir (Institut Pertanian Bogor)

[81] Aprotosoae A C, Luca S V and Miron A 2016 Flavor chemistry of cocoa and cocoa products—an overview Compr. Rev. Food Sci. Food Saf. 15 73–91