Effects of cocoa clones and fermentation times on physical and chemical characteristics of cocoa beans (*Theobroma cacao* L.)

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Effects of cocoa clones and fermentation times on physical and chemical characteristics of cocoa beans *(Theobroma cacao* L.*)*

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Abstract. The effects of cocoa clones and fermentation time on the physical and chemical characteristics of cocoa beans has been examined. This study used cocoa pods of superior clones, namely Sulawesi 1, Sulawesi 2, and MCC 02 clones cultivated in East *Luwu*, South Sulawesi, Indonesia. Prior to the fermentation, the mature cocoa pods were stored for six days. Fermentation took place for 24, 48, 72, 96, and 120 hours using fermentation boxes made of *styrofoam* with the capacity of box is 7 kg of wet beans. The drying of the cocoa beans used sun drying method. The results showed that the interaction between fermentation time and type of clones have significantly affected moisture content, proportion of cocoa nibs and shells, and fat content; but the interaction of the both had no significant effect on pH of cocoa nibs and FFA content. In general, the Sulawesi 2 clone had better physical and chemical characteristics than those of the Sulawesi 1 and MCC 02 clones.
Keywords: fermentation, physical, chemical, cocoa beans, clones

1. Introduction

Cocoa (*Theobroma cacao* L.) is cultivated mainly in West Africa, Central and South America and Asia. Based on the ICCO 2017 data, eight largest cocoa producing countries today are Ivory Coast, Ghana, Indonesia, Nigeria, Brazil, Equador, and Malaysia. The total world cocoa production in 2015/2016, is estimated 3.972 thousand tonnes, in which Indonesia contributed for about 8.0 % of the total world production [1].

Cocoa bean is the main raw material for chocolate manufacture, and therefore it is very important to maintain cocoa bean quality and apply strick requirement, so the chocolates produced safe for consumption. The government of Indonesia has established SNI 2323-2008 standards for cocoa beans, in which the bean quality is classified according to physical, chemical, and organoleptic performances of the cocoa beans [2].

One of the efforts to increase productivity and quality of the cocoa beans is by using excellent cocoa clones. Beside having larger bean sizes and higher fat contents, the cocoa also relatively resistant towards fungi, insect and other plant diseases. In South Sulawesi in primarily, some of such the cocoa clones have been developed and cultivated in this area since several years ago, namely Sulawesi 1 and Sulawesi 2 clones [3] and local clones namely MCC 01 and MCC 02 clones [5]. One of the cocoa production centers of this types of the cocoa clones is East *Luwu* Regency, South Sulawesi Province, Indonesia with production around 480 ton a year [6].

The low quality of the Indonesia cocoa beans, especially cocoa beans originating from South Sulawesi relates to the ŕelatively high acidity along with weak in flavor [3], [7]. In addition, it relates to inconsistency in quality and mostly unfermented or partially fermented. As we know, the aims of fermentation is to form flavor precursor (combination of aroma and taste), to reduce bitterness and astringency, and to improve physical performance, such as color of the cocoa beans [8]. During fermentation process, the cocoa beans experience physical and chemical changes which will influence the cocoa bean quality.

Since fermentation time is a key factor in the cocoa bean fermentation, it is required to apply the appropriate fermentation times. One of the factors affects this fermentation time is the variety of the cocoa [9]. The other factors are the pulp content and the layer height of the cocoa beans in the fermentation boxes [3].

The aims of this study was to examine the effects of cocoa clones and fermentation times on the physical and chemical characteristics of the cocoa beans. The study used cocoa pods of the superior clones, namely Sulawesi 1, Sulawesi 2, and MCC 02 clones, cultivated in East *Luwu*, South Sulawesi, Indonesia.

2. Materials and Methods

2.1. Preparation of the cocoa beans

In this study the cocoa pods of the Sulawesi 1, Sulawesi 2, and MCC 02 clones were procured from Sahar Cocoa Village (SCV) farm, a plasma cocoa farm of Mars Symbioscience Indonesia Ltd., located in *Maliowowo* Village, District of Angkona, East *Luwu*, South Sulawesi, Indonesia.

The mature cocoa pods were first storaged for six days before the beans were removed from the pod husk. The cocoa beans were then separated from the placenta. Fermentation time of 24, 48, 72, 96, and 120 hours used fermentation boxes made of s*tyrofoam* with the capacity of 7 kg wet beans, each. After fermentation, the cocoa beans were sun-dried for 5 days, until reaching 6 to 7% moisture contents.

2.2. Preparation of the samples and analysis

Preparation and analysis of the cocoa bean samples, as dried fermented beans, were conducted at Laboratory of Chemical and Microbiologycal Testing, Center for Plantation Based Industry, in Makassar, South Sulawesi, Indonesia.

Assessment for the physical and chemical characteristics of the cocoa beans referred to *SNI Biji Kakao*: 2323-2008 for moisture, pH of nibs, proportion of cocoa nibs, proportion of shell, fat contents, and FFA contents.

3. Result and Discussion

The ANOVA summary of the effects of the clone types and fermentation times on the physical and chemical characteristics of the cocoa beans are presented in Table 1.

Table 1. ANOVA summary of the effect of clones and fermentation times on the physical and chemical characteristics of the cocoa beans (Sulawesi 1, Sulawesi 2, and MCC 02 clones)

Variable	Characteristic of Physical and Chemical Quality in Cocoa Beans					
	Moisture	**pH of nibs**	**Proportion of cocoa nibs**	**Proportion of shells**	**Fat content**	**FFA content**
Clone (C)	0.44^{*}	0.14^{*}	71.25^{*}	73.89^{*}	27.61^{*}	0.20^{*}
Fermentation (FT)	0.26^{*}	4.39^{*}	53.65^{*}	52.87^{*}	4.77^{*}	0.44^{*}
Interaction (C x FT)	1.13^{*}	0.20^{ns}	16.57^{*}	17.39^{*}	0.39^{*}	0.04^{ns}

* = significant at $p < 0.05$ ns = not significant

The ANOVA analysis showed that the clone types and fermentation times and their interaction significantly affected the physical and chemical characteristics of the cocoa beans at $p < 0.05$, except for the pH of nibs and free fatty acid levels.

Further analysis using Duncan's test to examine the effects on the physical and chemical characteristics of the cocoa beans is presented in Table 2.

Table 2. Duncan summary test for the effects of the clones and fermentation times on the physical and chemical characteristics of the cocoa beans (Sulawesi 1, Sulawesi 2, and MCC 02 clones)

Variables	Characteristic of Physical and Chemical Quality in Cocoa Beans					
	Moisture	**pH of nibs**	**Proportion of cocoa nibs**	**Proportion of shells**	**Fat content**	**FFA content**
Clones						
Sulawesi 1	6.40^{b}	5.41^{a}	81.08^{d}	18.90^{a}	49.88^{d}	0.73^{d}
Sulawesi 2	6.62^{a}	5.36^{a}	83.50^{b}	16.48^{b}	50.28^{c}	0.78^{c}
MCC 02	6.45^{b}	5.25^{b}	84.84^{a}	15.08^{c}	51.93^{b}	0.85^{b}
Fermentation time (hours)						
24	6.40^{c}	6.05^{a}	80.58^{e}	1.44^{a}	50.02^{e}	0.63^{e}
48	6.47^{b}	5.19^{c}	82.63^{c}	17.28^{c}	50.54^{d}	0.68^{d}
72	6.50^{b}	5.10^{d}	81.59^{d}	18.38^{b}	50.95^{c}	0.82^{c}
96	6.63^{a}	4.95^{e}	83.86^{b}	16.14^{d}	51.03^{b}	0.91^{b}
120	6.33^{d}	5.39^{b}	85.22^{a}	14.75^{e}	51.17^{a}	0.96^{a}

The values followed by different notation (a, b, c, d) are significantly different at $p < 0.05$

a. Moisture

The result of analysis showed that he highest moisture content in Sulawesi clones 2 (6.62) followed by MCC 02 (6.45) and Sulawesi 1 (6.40). The highest moisture obtained at 96 hours fermentation is 6.63 and is significantly different from other fermentation time treatments. The fermentation time of 48 hours (6.47) and 72 hours (6.50) shows the same moisture or not significantly different. The lowest moisture was obtained at 120 hours fermentation time of 6.33.

The moisture content of dried cocoa beans is full fermented which is less than 7.5%. Table 2 shows that the moisture content of dry cocoa beans obtained ranged from 6.33 to 6.62 for the three clones. This means that the moisture of the three clones has met the requirements of *SNI 2323 Biji Kakao: 2008* which is a maximum of 7.5% [2]. The moisture of more than 8% causes the cocoa beans to be easily attacked by fungi and insects, thus increasing the risk of cocoa beans damage, but if the moisture of less than 5% will cause the cocoa beans to break easily [10], [11]. In addition to the fermentation process, the moisture is also affected by the way of drying. The drying process can cause the moisture of cocoa beans to decrease from 60% to 7% [12], [13].

b. pH of cocoa nibs

The highest pH of cocoa nibs obtained from Sulawesi 1 (5.41) clones followed by Sulawesi 2 (5.36) and MCC 02 (5.25). The highest pH of cocoa nibs at 24 hours fermentation was 6.05 and the lowest was at 96 hours fermentation time of 4.95.

The pH of the cocoa nibs is an important parameter to determine the acidity of fermented cocoa beans. The acidity of the cocoa nibs greatly determines the flavor of chocolate because it is not only related to the taste of the acid, but also determines reaction of flavor compounds during roasting [14], [3]. Biehl *et al.* [15] reported that flavor formation is more likely to occur in seeds at pH of 5.0 - 5.5 than at pH of 4.0 - 4.5. The pH of cocoa nibs that are too high (near neutral pH) will produce a low flavor. This is because cocoa beans which have high pH are likely to have mouldy beans. Whereas if the pH is too low (very acid < 4.5) caused by excessive production of acetic acid it will also produce a low flavor. This occurs because of a strong acidification process so that the exopeptidase enzyme conducts proteolysis and produces a low flavor [17], [18].

Yusianto *et al.* [19] and Biehl *et al.* [16] states that fruit ripening affects the pH value or acidity of nibs. The process of fruit ripening causes some pulp to be used for the respiration process and some pulp content evaporates. This results in a reduction in alcohol formation during fermentation because the substrate is reduced and the fermentation conditions are not aerobic, resulting in the formation of acetic acid only slightly so that the acid content of the dried cocoa beans becomes low.

In this study it was found that the interaction between differences in clones and fermentation time had no significant effect on the pH of cocoa nibs. This is because the chemical changes that occur during fermentation will still take place during drying and it is suspected that the type of acid diffused into the cocoa nibs has a pH that is relatively the same at each fermentation time.

The cocoa nib of the Sulawesi 1 clone has higher pH than that of the Sulawesi 2 and the MCC 02 clones. This is because the bean size of the Sulawesi 1 clone is relatively smaller compared to the bean sizes of the two other clones causing better oxidation process of the acid compounds in fermentation. This was parallel to the Duncan *et al.* finding [16]. However, the three clones had quite low acidity levels of the beans, indicated by pH values above 5.0.

c. Proportion of cocoa nibs

Duncan's test results showed the effect of clones and fermentation time were significantly different on the proportion of cocoa nibs. The highest proportion of cocoa nibs was obtained at MCC 02 clone (84.84%) followed by Sulawesi 2 (83.50%) and Sulawesi 1 (81.08%). The highest proportion of cocoa nibs at 120 hours fermentation was 85.22% and the lowest at 24 hours fermentation was 80.58%.

According to Wood & Lass [12] and Afoakwa *et al.,* [20], important factors that influence bean size are temperature and rainfall distribution during the fruit development process. High temperatures and low rainfall can cause lower bean levels. The number of nib contained in cocoa beans is a major concern for the cocoa industry or processor because the high content of the nibs will produce more cocoa fat yield.

d. Proportion of shells

Duncan's test results showed the effect of clones and fermentation time were significantly different on the proportion of cocoa shells. The highest proportion of cocoa shells in Sulawesi 1 clone (18.90%) followed by Sulawesi 2 (16.48%) and MCC 02 (15.08%). The highest proportion of cocoa shells obtained at 24 hours fermentation is 19.44% and is significantly different from other fermentation time treatments. The lowest proportion of cocoa shells level at 120 hours fermentation is 14.75%.

The proportion of cocoa shells content is influenced by the type of clone, fermentation time and drying. The slime layer of unfermented cocoa beans will have a slippery mucus layer and the structure of nibs tends to be solid and hard [19]. High of proportion of nibs content cause the energy and time needed to evaporate water to get out through thicker layers of cocoa shells for relatively longer.

e. Fat content

Duncan's test results showed that the effect of clones and fermentation time were significantly different on the fat content of dry cocoa beans. The highest fat content in the MCC 02 clone (51.93%) was followed by Sulawesi 2 (50.28%) and MCC 02 (49.88%). The range of fat content in Indonesian cocoa beans is 49 - 52% [4].

The highest fat content obtained at 120 hours fermentation is 51.17% and is significantly different from other treatments of fermentation time. The lowest fat content at 24 hours fermentation is 50.02%. In general, the fat content of the three clone types increased with the increase of fermentation time. This is because the fermentation process can cause a decrease in the content of non-fat ingredients such as proteins, polyphenols and decomposed carbohydrates so that relatively fat levels will increase [21], [13].

The cocoa beans of the MCC 02 clone contained higher fat contents (5,39%) compared with the two other cocoa beans of the Sulawesi 1 clone (49.88%) and of the Sulawesi 2 clone (50.28%). The higher cocoa bean size of the MCC 02 clone most probably caused this high fat content. As stated by

[3] the cocoa bean size contributes to the fat content. The higher the bean size, the higher its fat content is, and vice versa.

Cocoa butter from Indonesia is famous for having a higher level of hardness than cocoa butter from Africa. If used in making chocolate, it will produce chocolate that is not easy to melt because of the higher level of hardness of fat [4].

f. FFA content

The presence of free fatty acids (FFA) in cocoa butter must be avoided because this is one indicator of quality damage. The results of FFA levels analysis of the three clones ranged from 0.63 to 0.96%. The highest FFA level was in MCC 02 clone (0.85%) followed by Sulawesi 2 (0.78%) and MCC 02 (0.73%). The highest FFA levels were obtained at 120 hours of fermentation, which was 0.96% and the lowest at 24 hours fermentation was 0.63%.

The interaction between clone differences and fermentation time had no significant effect on FFA levels. The same thing was reported by Ramlah [22] that fermentation time did not give a significant effect on FFA levels. Free fatty acids generally appear when dried cocoa beans are stored in a warehouse that is less clean and moist.

Free fatty acids are free fatty acids, not bound as triglycerides. FFA levels that can be received according to the Codex Allimentarius are <1.75% and the best is <1.3% ([23], [4]. Cocoa beans are considered to begin to suffer damage if FFA levels are greater than 1.3%.

Free fatty acids can be formed through oxidation reactions or hydrolized enzymes that come from broken seeds or poor storage so that they break down. Free fatty acids that are too high will cause undesirable taste and are not good for heart and blood vessel health because they cause high cholesterol triggered by the buildup of FFA in the blood. Cocoa beans with moldy interior will produce fat with high FFA levels [4].

4. Conclusion

The types of the cocoa clones and the fermentation times and their interaction affected on moisture content, proportion of cocoa nibs, proportion of shells, and fat content, but the interaction of the both did not significantly affected on the pH of cocoa nibs and the FFA content.

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5. References

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