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Fat, polyphenols, fermented and non-fermented cacao beans (*Theobroma cacao* L.) grown under three types of stand structure with the engineering of light distribution enhancement through pruning management in soppeng regency of South Sulawesi, Indonesia

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ABSTRACT

This study was aimed to figure out total fat and polyphenols in cacao beans, fermented and non-fermented ones that grow under three types of stand structure with the engineering of enhancing the sun rays through pruning management. This study was conducted in Salaonro Village, Lilirilau Subdistrict, Soppeng Regency, South Sulawesi from March to November 2018, using a two-factor factorial design. The first factor was types of stand structure consisting of Bitti (*Vitex coffasus*) timber plant (N1) Gamal (*Gliricidia sepium*) (N2) and Teak (*Tectona grandis* L.) (N3). And the second factor was the time to prune, namely (P0) no pruning, (P1) once-a-month pruning, (P2) once-two-months pruning. The study results showed that the time to prune (pruning time) had different influence on the total of fat with fermentation, treatment (P2) was significantly different with (P1), however was not significantly different with (P0). Also, total fat of non-fermented cacao beans during pruning time had significantly different influence, treatment (P2) was significantly different with (P0), yet insignificantly different with (P1). It can be seen that the highest fat value was at (P2) of total fat for non-fermented cacao beans (28.96) and the lowest was at (P1) of total fat for fermented ones (20.30). Likewise, total polyphenols for fermented cacao beans demonstrated that treatment (P2) was significantly different with (P1) and (P0), however in polyphenols for fermented cacao beans, three types of stand structure exhibited significant difference, and it can be seen that (N1) was significantly different with (N2), yet not significantly different with (N3). Furthermore, it can be seen that for each treatment, the highest and the lowest total polyphenols, both for fermented and non-fermented ones, were at (P2) total polyphenols of cacao beans with the highest fermentation (0.386) and the lowest at (N2) of total polyphenols for non-fermented cacao beans was (0.252).

Key words : *Cacao, Engineering, Type of stand, Fermentation, Fat, Polyphenols*

Introduction

Cacao plant (*Theobroma cacao* L.) constitutes one of plantation commodities in Indonesia and such a flagship commodity of South Sulawesi which plays important role in the national economy, where South Sulawesi contributes 60% nationwide (BPS, 2014). planting cacao for the community themselves is one of livelihoods that provides income, both as their own farm business or as farm workers. Indonesia is ranked as the third cacao producers in the world following after Ghana and Cote d'Ivoire (Ivory Coast). The cocoa plantation development in Indonesia is marked by some less fortunate things, such as the low quality of cacao beans since they do not meet the criteria for quality and the presence of insect contamination thus suffered from automatic detention, especially for cacao beans exported to the U.S. Those detained cocoa then will be returned to the importer's ports for fumigation and the cost is immediately calculated on the purchase price (Direktorat Jendral Bina Produksi Perkebunan, 2000). Moreover, there are also the low of cacao productivity, which is less than 1000 $kg^{-1}ha^{-1}year^{-1}$ and the disturbance of pest and disease.

Cultivation technique through plant maintenance engineering is required here. Cacao plant is a C3 plant and its growth needs a kind of shade, or it is frequently said as *shade loving tree*. However, cocoa plant still requires optimum light intensity for their normal growth and development. Cacao is classified as a C3 plants that able to photosynthesize at low leaf temperature. Maximum photosynthesis is obtained at the time of light reception at the head by 20% of the full exposure. The saturation in the photosynthesis process of each cacao leaf that has perfectly opened is in the range of 3-30% of full sunlight exposure or at 15% of full sunlight exposure. Young cacao plants require approximately 25-60% of sunlight intensity (Abdoellah and Soedarsono, 1996). Meanwhile, according to (Prawoto, 2012), 50-70% light intensity will provide the highest productivity for mature cacao trees.

The utilization of shade trees (type of stand) is an attempt to regulate the sunlight, air temperature humidity, and the rate of water loss due to both transpiration and evaporation, since the total photo energy captured the plant head is highly varied and depends on some factors like, among others, planting system, crop population, and crop growth rate. In addition to the need of shade trees, cacao plants also require pruning. Cacao pruning is aimed at regulating the proper microclimate for the flower and fruit growth or regulating the number and distribution of the leaves. According to Fatmawati *et al.* (2018), variation in types of shade provides different sunlight intensity exposed to the cacao plants. Some study results have revealed that regular pruning can give better harvest, increase production, and sustain the economic age of the crops. According to Carrillo *et al*. (2013), there are some factors that affect the quantity and quality as well as bioactive compound of cacao, i.e. genetic factor, environmental diversity (sunlight intensity, fertilization, pruning, and stress factor). Pruning is intended to: obtain balanced head growth, decrease humidity thus will be safe from pest and disease attacks, improve the air circulation around the plants, make harvesting easier, achieve high production since pruning will extend the assimilation surface, and stimulate the flowering and fertilization caused by the presence of vegetative and generative balance (Lopez *et al*., 2016). Moreover, pruning will improve the process of sun lighting that plays an important role for the continuity of the plant physiology, particularly photosynthesis, respiration, and transpiration. The rate of photosynthesis result can be approached by counting the leaf number and measuring the rate of CO2 absorption per unit leaf area. The leaf number is stated in LAI (Leaf Area Index). According to Alvim *et al.* (1972), the optimum LAI for mature cacao plant is about 3.7-5.7, namely equivalent to assimilate production of 3.5-5.0 mg dm-2day-1 or 12.8-18.2 ton of dry material per hectare-1 year-1. Angela (2011), in Bailhaqi *et al.* (2015), showed that for cacao plants with no pruning, the fruit number was decreasing from 209 to 187, whereas for plant with pruning, the fruit number was increasing from 116 to 127. Meanwhile, according to Fatmawati *et al*. (2019), twice-a-month pruning will provide the highest sunlight intensity as well as increase the wet and dry weights of the cacao beans.

Post harvest and quality are very important for cacao. Fermentation is a crucial thing in the post harvest processing of cacao beans, since fermentation is able in improving the quality of cacao beans. According to Widyotomo (2008), the fermentation of cacao beans will produce the precursor of flavor, black-brownish beans, reduce the bitter, acid, sweet tastes and flower scent, increase the flavors of cocoa (chocolate) and nut (nutty), and harden the seed skin just like a shell. While according to (Afoakwa

et.al., 2011), the formed precursor compounds will develop into aroma compounds through "stecker" degradation reaction of the non-enzymatic browning process (Maillard reaction) during the drying time. Non-fermented beans will not posses those precursor compounds thus they will have very low flavor and quality.

Cacao fat is one of the most expensive components of cacao beans, thereby this value is used by the consumers as one of benchmarks for pricing. In addition to plant materials and season, fat and polyphenol contents are influenced by the processing treatment, categories of plant materials, and seasonal factors. Puslitkoka (2008) required 50-52% of fat content for cacao beans used as chocolate raw materials. South Sulawesi cacao has its advantage of high ***hard butter*** character, namely it will not easily melt at high temperature, and another strength is high polyphenol content which is highly beneficial to cardiovascular health.

Materials and Method

Place and Time

This study was conducted in the people's cacao plantation in Salaonro Village, Lilirilau Subdistrict, Soppeng Regency, South Sulawesi, Indonesia. Geographically, it is located between 04 o 20′ 21.4″ of South Latitude and 4o 32 North Latitude, as well as 119o 57′ 47,4″ of East Longitude with average place height is 60-200 m above sea level. The research location was at 96 m above sea level. This place has type of alluvial soil. And based on Scmifdt Forgusson classification, it is categorized in Type C climate with air temperature of 24-30 °C, and average wind rate is weak to moderate. Average rainfall is 1428 mm per year.

Over the past 5 years showed the highest amount of rainfall was recorded in 2016 (2432 mm) and the lowest average rainfall was recorded in 2015 (1175 mm), (BMKG Wilayah IV, 2018).

Field research was performed from March to November 2018. Laboratory analysis was held from November to December 2018 in the Biochemistry Laboratory at the MIPA Faculty of Hasanuddin University.

Material

Materials used were Label (pressed paper), cacao trees as sample in total of 54 trees, Urea fertilizer, TSP and KCl plastic bags and labels for fruit samples, 11 x 17 cm plastic bags and label papers for cacao bean samples, and nylon cable tie and wire for binding the label on the sample trees. Plant material was cacao plants approximately 9 years old of Sulawesi Satu forestro type.

Tool

Tool used in this study was digital Lux meter SGS and LX-1010B to measure the sunlight intensity under and above the head of cacao plant, FM 7903 thermohygrometer to measure the air temperature and air humidity, ISO9001:2000 physicometer to measure soil temperature, roll meter to measure the planting distance for the shade tree and planting distance for the cacao tree, analytical balances/ scales to measure the weight of the beans, and blender to refine the cacao beans that will be tested in the Laboratory.

Method

By using the two-factor factorial design, the first factor is a plantation that has a variation of different types of stand and each has different area of land, namely location one (Group I) with cacao plantation area of 0.5 ha with type of stand of Bitti (*Vitex coffasus*)=(N1), planting distance for shade tree was 6 m x 6 m and planting distance for cacao tree was 2.80 m x 2.80 m with total of approximately 600 cacao trees. Location two (Group II) with cacao plantation area of 1 ha, type of stand was Gamal (*Gliricidia sepium*)=(N2), planting distance for shade tree was 10 m x 10 m, and planting distance for cacao tree was 4 m x 4 m with the total of approximately 1000 cacao trees. Location three (Group III) with cacao plantation area of 0.5 ha, type of stand of Teak (*Tectona grandis* L.)=(N3), planting distance for shade tree was 7 m x 7 m with total of approximately 500 cacao trees.

The second factor is treatment of pruning time which consisting of three standards, i.e. P0 = no-pruning, P1= once-a-month pruning, and P2= once per two months pruning. Therefore, there will be 9 treatment combinations, each treatment consists of two plants, so that in this study required each 18 plants for each group, 54 cacao trees.

The ripened fruits were picked and packed, the seeds were removed from the fruit bearing, where non-fermented beans were immediately dried under direct sun. Meanwhile, the fermented beans

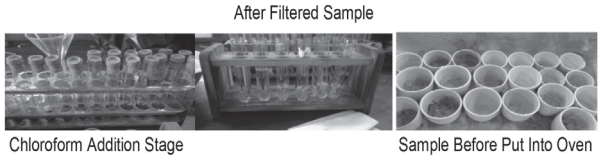
were stored and fermented for four days and then dried under the direct sun. Once dried, those beans were weighed, each 100 gram for each treatment, and then mashed by using blender in order to analyze the total fat and polyphenol contents.

Crude Fat Analysis

Weigh ± 1 gram of sample, put it into the reaction tube with 15 mL scale, add chloroform that is close to 10 mL scale, tightly close the seal and then shake and let it stay overnight, press up to 10 mL scale with chloroform, then re-shake it, strain it by using filter paper onto the reaction tube, put 5 mL of pipette into the petri dish that has been known its weight (a gram), put it into the oven at 100 °C of temperature for four hours, remove it out and put it into the exicator for ½ hour, and then weigh it (b gram)

Calculation:

$$\% \text{ Fat Level} = \frac{P \times (b - a)}{\text{Sample weight}} \times 100\% \quad P = \text{Dilution) } 10/5)$$



Procedure for Determining Total Phenol Level (Tannins)

Sample Preparation

Sample was weighed about 0.05 g, brewed with 10 mL of hot Aquades (80 °C), let it still for 10 minutes, filtered, filtrates were taken and tested, diluted if necessary (Filtrates were pipetted of 0.5 mL and then added with 4.5 mL of hot aquades (FP = 10 times))

Making Saturated Na_2CO_3

Na_2CO_3 was weighed for 3.5 g, dissolved with 10 mL of hot aquades (80 °C).

Determining Total Phenol Level (Tannins)

Sample/standardized (tannin acid)/blank was pipetted for 5 mL, added with 0.25 mL of folin reagent, added with 0.5 mL of saturated Na_2CO_3, let it settled for 30 minutes, its absorption was measured by using spectrophotometer at maximum wavelength of (680 nm).

Data Analysis

The collected data is analyzed by using analysis of variant ANOVA of total fat as well as polyphenol for fermented and non-fermented cacao beans, if there is any significant or insignificant influence thus will be followed with advanced BNJ test at levels of ˜ = 0.05 and 0.01.

Results

Total Fat (%) of Fermented Cocoa Beans, Total Fat (%) of Non-Fermented Cocoa Beans against Three Types of Stand and Pruning Time for the Cacao Trees.

The result of variance analysis revealed that interaction between three types of stand and pruning time could not be seen, however the one that had influence was single factor of pruning time which had highly significant influence on total fat of fermented and non-fermented cacao beans. However, three types of stand have not demonstrated any significant influence. Average influence of the treatment is presented in Table 1.

Table 1. Average of Total Fat for Fermented and Non-Fermented Cacao Beans against Three Types of Stand and Pruning Time for Cocoa Trees

Treatment	Averaga	
	Total Fat (%) for Fermented Cacao Beans	Total Fat (%) Non- Fermented Cacao Beans
N1	22.55 tn	25.21 tn
N2	22.33 tn	25.21 tn
N3	21.99 tn	24.87 tn
BNJ 0.01	tn	tn
0.05	tn	tn
P0	20.63 ab	22.79 b
P1	20.36 b	23.53 ab
P2	25.89 a	28.96 a
BNJ 0.01	tn	6.40
0.05	5.92	tn

Note: The numbers followed by different letters mean significant difference at the α = 0.05 and highly significant difference at α = 0.01. N1 = Type of Stand of Bitti, N2 = type of stand of Gamal, N3 = type of stand of White Teak, P0 = No Pruning, P1 = Once a month pruning, P2 = Once per two months pruning, tn = not significantly different

The result of BNJ test of α 0.05 and α 0.01 in Table 1 showed that pruning time was significantly different with total fat for fermented cacao beans, treat-

ment (P2) of once-two-months pruning was significantly different with (P1) of once-a-month pruning, however insignificantly different with (P0) no pruning. Also, total fat for non-fermented cacao beans, pruning time was significantly different with treatment (P2) of once-two-months pruning, significantly different with (P0) of no pruning, yet insignificantly different with (P1) of oncea-month pruning. It can be seen from those total fats that the highest value was at (P2) once-two-months pruning, total fat for non-fermented cacao beans was (28.96) and the lowest was at (P1) once-a-month pruning with fat total for fermented cacao beans was (20.30)

Total Polyphenols (%) of Fermented Cacao Beans, Total Polyphenols (%) of Non-Fermented Cacao Beans, against Three Types of Stand and Pruning Time for Cocoa Trees.

The results of variance analysis disclosed that interaction between three types of stand and pruning time did not happen, yet the one that gave influence was each of single factor, three types of stand had significant influence on total polyphenols for fermented cacao beans, and pruning time had significant influence on total fat for fermented cacao beans. The average for each treatment is presented in Table 2.

The result of BNJ test ˜ 0.05 and ˜ 0.01 in Table 2 exhibited that the pruning time was significantly different with total polyphenol of fermented cacao beans and it can be seen that treatment (P2) of once-two-months pruning was significantly different with (P1) once-a-month pruning and (P0) no pruning. However, total polyphenols for non-fermented cacao beans that gave significant difference was three types of stand which it can be seen that (N1) Bitti type of stand was significantly different with (N2) Gamal type of stand, yet insignificantly different with (N3) Teak type of stand. Furthermore, it can be seen from each treatment that the highest and the lowest polyphenol values, both for fermented and non-fermented ones, each at (P2) was pruned once per two months where total polyphenols for fermented was (0.386) and the lowest at (N2) for Gamal type of stand with no fermentation was (0.252).

Discussion

It can be seen from Table 1 that total fat for both fermented and non-fermented, the one that gave the highest value was at (P2) once-two-months pruning, total fat for non-fermented of (28.96) and the lowest was at (P1) once-a-month pruning, total fat for fermented cacao beans was (20.30). In general, fat level is stated in percent of dry weight of cacao nibs. In addition to plant materials and season, fat content is also influenced by processing treatment. Cacao beans derived from the fertilization during rainy season generally have higher fat levels. According to Yusianto *et al.* (1997) and Sulistyowati and Soenaryo (1988), fat level of non-fermented cacao beans is 0.07-5.69% lower than the fermented ones, depending on the fermentation time. Alleged in this study, pruning (P2) that pruned once two months gave good influence on the fat content for non-fermented cacao beans. Fat product of fermented cacao

Table 2. Average of Total Polyphenols for Fermented and Non-Fermented Cacao Beans against Three Types of Stand and Pruning Time for Cocoa Trees Average

Treatment	Total Polyphenols (%) for Fermented Cacao Beans	Total Polyphenols (%) Non Fermented Cacao Beans
N1	0.327 tn	0.288 a
N2	0.351 tn	0.252 b
N3	0.327 tn	0.274 ab
BNJ 0.01	tn	tn
0.05	tn	0.031
P0	0.301 b	0.274 tn
P1	0.319 b	0.272 tn
P2	0.386 a	0.268 tn
BNJ 0.01	0.044	tn
0.05	tn	tn

Note: The numbers followed by different letters mean significant difference at the $\alpha = 0.05$ and highly significant difference at $\alpha = 0.01$. N1 = Type of Stand of Bitti, N2 = type of stand of Gamal, N3 = type of stand of White Teak, P0 = No Pruning, P1 = Once a month pruning, P2 = Once per two months pruning, tn = not significantly different

beans gave the lowest value of (20.36 %) compared with the fat of non-fermented ones (28.96 %), with a difference of (8.66%). The difference of fat contents in this study can be seen from the following picture of the difference in the fat content between fermented and non-fermented cacao beans.

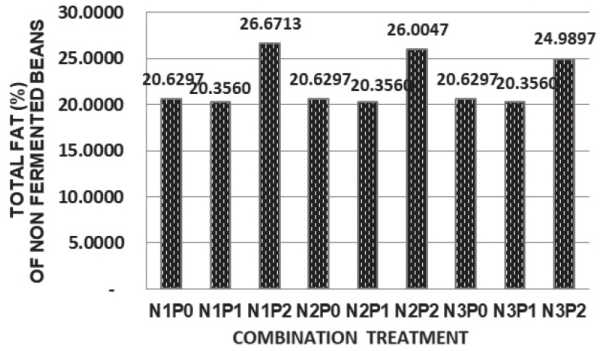


Fig. 1. Total Fat (%) of Non-Fermented Cacao Beans Against Three Types of Stand and Pruning Time for the Cacao Trees

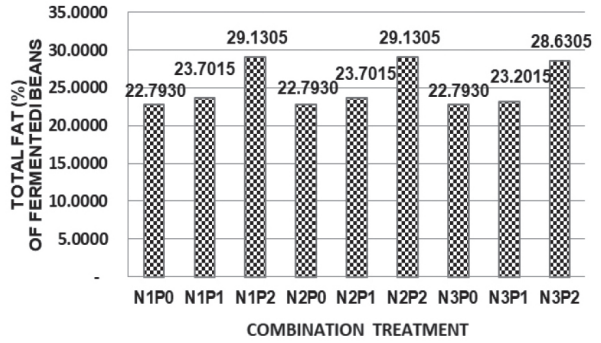


Fig. 2. Total Fat (%) of Feremented Cacao Beans Against Three Types of Stand and Pruning Time for the Cacao Trees

Fat characteristic found in cacao beans depends on its fat constituent components. These components are not relatively influenced by cocoa bean processing, rather affected by some factors, such as (1) bean's level of ripeness at the time of harvest, (2) cultivation techniques, (3) harvest season, (4) clone, and (5) the growing environment of the plant. One of those factors has been allegedly influenced the difference in the fat content between fermented and non-fermented cocoa beans, namely pruning. In tune with Carrillo *et al*. (2013), there are some factors that affect the quality, quantity, and bioactive compounds of cacao, i.e. genetic factor, environmental diversity (sunlight intensity, fertilizing, pruning, and stress factor).

Furthermore for polyphenol observation, pruning time has been significantly different with the total polyphenols for fermented cacao beans, yet for total polyphenol of non-fermented cacao beans, the one that gave significant different was three types of stand. Moreover, it can be seen from each treatment that the highest and the lowest polyphenol values, both for fermented and non-fermented cacao beans, was each at (P2) once-two-month pruning, total polyphenol for fermented was (0.386) and the lowest was at (N2) Gamal type of stand, polyphenol for non-fermented was (0.252). In the following figures (3, 4), it can be seen the different in the total polyphenol content for non-fermented and fermented cacao beans.

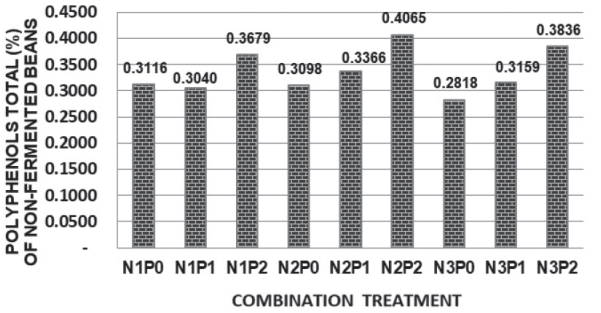


Fig. 3. Total Polyphenols (%) of Non-Fermented Cacao Beans Against Three Types of Stand and Pruning Time for Cacao Trees

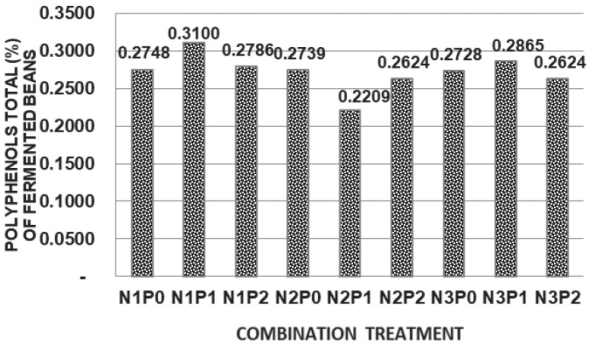


Fig. 4. Total Polyphenols (%) of Fermented Cacao Beans Against Three Types of Stand and Pruning Time for Cacao Trees

Polyphenols contained in the cacao beans are influenced by various activities and factors that play role started from pre-harvest period (*on-farm*) up tp post-harvest and processing (*off-farm*). During pre-harvest period, the one that plays significant role is

genetic factors (genotype, varieties, clones) which will interact with environmental factors and agronomically influences the polyphenol content in cacao beans. However, at the post-harvest time, there will be interaction between post-harvest components and processing including fruit storing, fermentation, and also drying. Various activities in each chain of chocolate production value will give positive contribution to the ups and downs in the polyphenol contents of cacao beans. According to Zumbe (1998), polyphenols in cacao beans reaches about 6 - 8%, whereas according to (Rusconi and Conti, 2010; Badrie *et al*., 2014) they can reach up to 10% of their dry weight. Alleged influences that are given by pruning and types of stand are basically from different growing environment, in accordance with (Yapo *et al*, 2013) that the possibility of influence from the interaction between genetic factors and their environment on polyphenol contents.

According to (Afoaka *et al*, 2012), the treatment of fruit storing has interaction with the fermentation length period and it can gradually cause the decrease in the polyphenol content of cacao beans (< 10%) due to the fermentation process conducted for six days. In tune with that, it can be seen that the difference in the polyphenol content for fermented cacao beans is lower than the non-fermented ones, as can be seen in (Figures, 3 and 4). Likewise stated by Chin *et.al.* (2013) that the contents of epicatechin, Catechin, flavanol, and antioxidant capacity of fermented cacao beans are lower than the non-fermented ones.

Conclusion

1. Once-two-months pruning (P2) gives the highest total fat value both for fermented and non-fermented cacao beans beans, namely (28.96% and 25.89%)
2. Pruning once per two months (P2) gives the highest total polyphenol value for fermented cacao beans of (0.386%).
3. Cacao plants grow under the Bitti type of stand (N1) with non-fermented beans gives the highest total polyphenol value of (0.288%).

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