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Protein and Mineral Contents in Fermented Cocoa Beans Originating from East Luwu, South Sulawesi

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Abstract

Cocoa beans, as a key raw material in the cocoa derived products are rich in proteins and minerals. The availability of sufficient quantities greatly determines the nutritional quality and flavor of cocoa products. One of the post-harvest processes affecting the protein and minerals contents in cocoa beans is fermentation. The purpose of this study was to determine the protein, macro and micro minerals contents in fermented cocoa beans of PBC 123, BR 25, and MCC 02 clones with fermentation time for 24, 48, 72, 96, and 120 hours. Methods of analysis used Kjeldahl and atomic absorption spectrophotometer. The results showed that type of clones, fermentation time, and their interaction affect the protein, macro and micro minerals contents in cocoa beans. The interaction between BR 25 clone and fermentation time of 48 hour showed the highest protein content (13.34%). The highest macrominerals contents were (1) Ca in PBC 123 (72 hours), (2) Mg in MCC 02 (24 hours), (3) Na in BR 25 (72 hours), and (4) K in BR 25 (24 hours) of fermentation. For microminerals, the highest contents were (1) Fe in PBC 123 (96 hours), (2) Mn in MCC 02 (20 hours), and (3) Zn in BR 25 (96 hours) of fermentation. Fermentation time and types of clone showed variable effects on the studied protein, macro and micro minerals contents in cocoa beans.

Keywords: Cocoa beans, cocoa clone, flavour, fermentation, protein, minerals

INTRODUCTION

Indonesia is the third largest cocoa producer in the world after Ivory Coast and Ghana. Indonesia contributed 8.0% of the total 3.972 thousand tonnes of world cocoa production in 2016 (ICCO, 2017). About 70% of the total national cocoa production comes from Sulawesi (Ditjenbun, 2015).

The most important and most used part of the cocoa pods is the beans. Cocoa beans are needed in the food-beverage industry, pharmaceutical industry, cosmetic industry, and are often used as functional food and supplements. Some studies reported that cocoa bean contains bioactive compounds such as flavonoid, alkaloids, mineral, protein, and organic acids (Yunus *et al*., 2013). Aikpopodion (2010), Pinto (2013), Araujo *et al*. (2014), and Loureiro (2016) stated that the macrominerals contents of Ca, Mg, N, P, K, S and micro mineral Fe, Mn, Zn,

Cu, B, Ni are related to the quality of cocoa beans.

The chemical compounds of cocoa beans are strongly influenced by types of clones, climates, growing soils, fruit maturity levels, and post-harvest processing (Smit, 2011; Wahyudi *et al*., 2013). Cocoa beans are an essential source of minerals and chocolate as one of the cocoa processed products has potential as human diet (Paoletti *et al*., 2012; Colombo *et al*., 2013).

Fermentation are primary step of cocoa bean processing, which are very important to promote the occurrence of chemical and biochemical reactions to produce precursor compounds. One of the factors affecting successful of fermentation process is fermentation time. Rapid fermentation will produce low quality and slaty of cocoa beans, while overtime fermentation causes the seed shell to become brittle and thinner, causing the reduced weight of cocoa beans, and sometime produce hammy flavour. The presence of weight loss is closely related to chemical changes occurred during fermentation such as changes in protein, fat, polyphenol compounds and alkaloids contents (Amin, 2005). Polyphenols are subjected to biochemical reaction through polymerization, and complex with proteins during fermentation, thereby decreasing their solubility and astringency (Bonvehi & Coll, 2000; Misnawi, 2003).

High-yielding and quality productivity of clone are the criteria the consumers demand. In South Sulawesi particularly, there are a number of superior clones that have been cultivated, i.e. PBC 123 (Sulawesi 1) and BR 25 (Sulawesi 2) (Direktorat Jenderal Perkebunan, 2009). In addition, local superior clones of Forestero variety have been developed, i.e. MCC 01 (M01) and MCC 02 (M 45) clones with yield productivity comparable to PBC 123 and BR 25 clones, and have larger seed size (Dinas Perkebunan Provinsi Sulawesi Selatan, 2014) These three clones have been widespread among farmers, but information on protein, macro and micro minerals contents in fermented cocoa beans are still limited, especially in PBC 123, BR 25, and MCC 02 clones. This study aimed to determine the essential proteins, macro and micro minerals contents in fermented cocoa beans in the PBC 123, BR 25 and MCC 02 clones originating from East Luwu, South Sulawesi.

MATERIALS AND METHODS

The cocoa beans for assessment were prepared as dried fermented cocoa beans. They consisted of three cocoa clones, namely PBC 123 (Sulawesi 1), BR 25 (Sulawesi 2), and MCC 02 (M 45). Cocoa pods were procured from Sahar Cocoa Village, a plasma cocoa farm of Mars Symbioscience Indonesia Ltd., located in Maliowowo village, Angkona District, East Luwu, South Sulawesi. The mature cocoa pods were stored for six days before the beans were removed from the pods. The cocoa beans were then separated from its placenta. Fermentation took place for 24, 48, 72, 96, and 120 hours using fermentation boxes made of styrofoam. The capacity of each box is 7 kg of wet beans. After fermentation, the cocoa beans were sun-dried for 5 days, until reaching 6% moisture content.

Preparation and analysis of the cocoa bean samples were conducted at Laboratory of Chemical and Microbiologycal Testing, Center for Plantation Based Industry, in Makassar. The cocoa nibs were separated from their bean shells and then ground with ceramic mortar.

Determination of Total Protein

Determination of total protein using the Kjeldahl method. About 2 g of the sample was taken and heated in 25 mL concentrated H_2SO_4 in the presence of a selenium catalyst and an anti bumping agent. The mixture was heated until all the carbon and hydrogen were oxidized (indicated by clear solution). The sample solution was transferred into 100 mL volumetric flask and topped up to the mark. Twenty five (25) mL of 2% boric acid was put in a 250 mL conical flask and two drops of mixed indicator added, and then placed under a condenser in such a way that the tip of the condenser is completely immersed in the solution. Ten (10) mL of the digested sample solution was poured with the aid of a funnel in the steam jacket. Eighteen (18) mL of 40% NaOH was added to the sample solution in the steam jacket. The stopcock of the funnel was closed to drive liberated ammonia into the collection flask. Steam was forced through the decomposition chamber by shutting the stopcock on the steam trap outlet. The boric acid changed to bluish-green on contact with ammonia. This continued for about 5 minutes. The flask was removed and the content was titrated against 0.1M HCl to the end point (colourless) in duplicate.

Mineral Analysis

Mineral analyses were determined using AOAC (2005) methods with slight modifications. About 0.5 g of the sample was weighed into a 250 mL beaker. Twenty five (25) mL of concentrated nitric acid was added and the beaker covered with a watch glass. The sample was digested with great care on a hot plate in a fume chamber until the solution was pale yellow. The solution was cooled and 1 mL perchloric acid (70% HCLO4) added. The digestion was continued until the solution was colourless nearly so (the evaluation of dense white fumes was regarded to be indicative of the removal of nitric acid). When the digestion was completed, the solution was cooled slightly and 30 mL of distilled water added. The mixture was brought to boil for about 10 min and filtered hot into a 100 mL volumetric flask using a Whatman No. 4 filter paper. The solution was then made to the mark with distilled water.

The concentrations of macronutrients calcium (Ca), magnesium (Mg), sodium (Na), potassium (K); and micronutrients iron (Fe), manganese (Mn), and zinc (Zn) were determined using Atomic Absorption Spectrophotometer (Shimadzu, AA-7000) with an acetylene flame. One (1) mL aliquots the digest was used to determine the Ca, Mg, Na, K, Fe, Mn, and Zn of the samples. Data analysis used ANOVA followed by Duncan Multiple Range Test used STAR program if there was a significant difference.

RESULTS AND DISCUSSION

Protein content in cocoa beans with PBC 123, BR 25, and MCC 02 clones in some variations of fermentation time is presented in Table 1. The ANOVA analysis indicated that both types of clone and fermentation time significantly ($p<0.05$) affected the protein content in cocoa beans (Table 1).

Tables 1. ANOVA summary for the effect of fermentation and cocoa clone on protein content in cocoa beans

Variable	Protein
Clone (C)	3.36 *
Fermentation (FT)	4.69 *
Interaction (C x FT)	10.63 *

*Significant at $p<0.05$.

Further analysis using the Duncan test showed that the highest protein content is

in PBC 123 followed by MCC 02 and BR 25 clones. The highest protein content at 48 hour and it was significantly higher than other fermentation time. Fermentation time for 24 and 72 hours showed that protein content were not significantly different with 96-hour fermentation. The lowest protein content occurred at 120-hour (Table 2).

Table 2. Effects of fermentation period and clone on protein content in cocoa beans

Variables	Protein, %
Clones	
PBC 123	12.35 a
BR 25	11.72 c
MCC 02	11.58 b
Fermentation time (hours)	
24	11.99 b
48	12.42 a
72	11.95 b
96	11.86 b
120	11.19 c

Protein content also decreased with the increasing of fermentation time. This was due to the loss of dissolved protein and breakdown during the fermentation process. According to Afoakwa *et al*. (2008), the decrease in protein occurred partly due to hydrolysis to amino acids and peptides and partly by conversion to insoluble forms by the action of polyphenols as well as losses through diffusion. Polyphenols are subjected to biochemical reaction through polymerization, and complex with proteins during fermentation, thereby decreasing their solubility and astringency (Bonvehi & Coll, 2000; Misnawi, 2003).

The highest protein content of BR 25 clone found in fermentation time of 48 hour (13.34%) and the lowest was in 72 hour (10.29%). Meanwhile, PBC 123 and MCC 02 clones showed the highest protein content in 72 hour (12.53%) and 24 hour (12.67%) of fermentation, respectively. Degradation of protein occurred during pod storage might initiate the release of peptide and free amino acids which could influence the processes for the formation of flavor precursors in the bean during fermentation and drying (Afoakwa & Paterson, 2010).

In addition to the protein content, the macro and micronutrients contents in the cocoa beans were significantly influenced by clone differences, fermentation time, and their interaction. However, Mg content was not affected by clone differences (Table 3). Minerals in cocoa beans are categorized into two groups, namely macro and micronutrients. Macronutrients in cocoa beans are Ca, Mg, Na, and K, while micronutrients are Fe, Mn, and Zn.

Table 3. Protein content in cocoa beans as affected by clone and fermentation period

Clones	Fermentation time, Hours	Protein, %
PBC 123	24	11.49±0.05
	48	11.68±0.02
	72	12.53±0.06
	96	11.79±0.12
	120	11.08±0.04
BR 25	24	11.81±0.13
	48	13.34±0.01
	72	10.29±0.63
	96	11.41±0.09
	120	11.07±0.03
MCC 02	24	12.67±0.16
	48	12.23±0.15
	72	11.43±0.05
	96	12.38±0.19
	120	11.43±0.04

Tables 4. ANOVA summary of the effect of fermentation and clone on macro and micronutrients content in cocoa beans

Variables	Ca	Mg	Na	K	Fe	Mn	Zn
Clone (C)	13369.02 *	7.67 ns	76.59 *	10019.81 *	62.41 *	0.13 *	0.11 *
Fermentation (FT)	6631.06 *	1082.47 *	156.68 *	66039.61 *	68.26 *	0.41 *	0.55 *
Interaction (C x FT)	4585.39 *	3076.55 *	621.69 *	7029.89 *	143.90 *	0.74 *	1.22 *

*Significant at p<0.05 ns = not significat.

Table 5. Macro and micronutrients in cocoa beans as affected by clone and fermentation period

Variables	Ca	Mg	Na	K	Fe	Mn	Zn
	mg/100g						
Clones							
PBC 123	129.01 a	106.23 a	12.78 a	329.8 b	8.01 a	0.62 b	1.58 a
BR 25	77.31 c	107.13 a	8.89 c	357.27 a	5.52 b	0.61 b	1.53 b
MCC 02	102.20 b	107.98 a	10.89 b	312.94 c	4.59 c	0.75 a	1.48 c
Fermentation time (hours)							
24	85.77 d	114.24 a	12.09 b	390.18 a	4.22 d	0.56 d	11.99 c
48	86.37 d	110.72 a	11.64 b	373.85 b	5.33 c	0.58 cd	12.42 b
96	104.60 c	109.04 a	13.55 a	258.51 e	5.13 c	0.61 c	11.96 b
72	114.53 b	104.83 ab	7.11 d	310.19 d	7.10 b	0.68 b	11.86 a
120	122.91 a	96.74 b	9.19 c	334.04 c	8.40 a	0.88 a	11.19 b

In addition to the protein content, the macro and micro minerals contents in the cocoa beans were significantly influenced by types of clone, fermentation time, and their interaction. However, Mg content was not significant affected by clone differences (Table 3). Minerals in cocoa beans are categorized into two groups, namely macro and micro minerals. Macrominerals contents in cocoa beans are Ca, Mg, Na, and K, while microminerals contents are Fe, Mn, and Zn.

In general, PBC 123 clone had the highest mineral content in Ca, Mg, Na, Fe, Mn, and Zn. BR 25 clone had the highest mineral content in Mg and K, while MCC 02 clone had the highest content in Mg and Mn. The lowest mineral contents found in BR 25 was Ca, while the lowest mineral contents found in MCC 02 were K, Fe, and Zn.

The result of the analysis showed that variation of macro and micro minerals contents were influenced by fermentation time. The highest Ca, Fe and Mn mineral contents were obtained at fermentation time 120 hour. The highest Mg mineral content was obtained at fermentation time for 24, 48, 96, and 72 hours and decreased after 120 hours. The highest Na, K, and Zn minerals were obtained at the fermentation time for 96, 24, and 72 hours, respectively.

Interactions between different clones and fermentation times occurred in the formation of mineral deposits in cocoa beans. The highest Ca content in PBC 123 clone of 138.44 mg/100 g occurred at fermentation time of 72 hour, while in BR 25 (116.48 mg/100 g) and MCC 02 (113.95 mg/100 g) clones occurred at 120 hour. In all variation of fermentation time, Ca mineral content tended to increase with increased fermentation times. It was similar for the presence of Mg mineral. PBC 123 (121.79 mg/100 g) and BR 25 (121.33 mg/100 g) clones showed the highest Mg content at the same fermentation time for 96 hours, whereas in MCC 02 clone (127.99 mg/100 g) occurred at 24 hour.

The fermentation time significantly affects for the formation of Na mineral in the three clones. The highest Na content in PBC 123 (17.35 mg/100 g), BR 25 (19.05 mg/100 g), and MCC 02 (16.83 mg/100 g) were at fermentation of 20 hour, 72 hour, and

Table 6. Effects of fermentation period and clone in the content of macro and micronutriens in cocoa beans

Clones	Fermentation time (hours)	Ca	Mg	Na	K	Fe	Mn	Zn
		mg/100g						
PBC 123	24	117.16±0.81	110.19±0.18	13.18±0.29	443.40±28.47	3.59±0.11	0.49±0.01	1.27±0.14
	48	121.22±0.74	99.23±1.46	14.87±1.43	312.61±15.59	7.23±0.39	0.78±0.01	1.81±0.04
	72	138.44±3.18	92.90±4.41	8.68±0.61	244.86±4.70	3.60±0.32	0.57±0.03	1.56±0.92
	96	129.93±0.66	121.79±5.93	9.82±0.29	288.98±30.24	13.06±0.55	0.48±0.05	1.45±0.12
	120	138.29±0.35	111.52±0.53	17.35±0.93	359.55±7.57	12.54±0.58	0.75±0.01	1.56±0.04
BR 25	24	40.15±0.13	104.53±2.71	6.28±0.19	446.04±28.45	5.41±0.28	0.72±0.01	1.64±0.06
	48	42.58±0.62	101.23±1.87	3.41±0.04	359.01±15.59	3.10±0.42	0.58±0.01	1.36±0.01
	72	82.24±1.86	87.78±4.93	19.05±1.07	254.15±4.70	6.69±0.73	0.45±0.02	1.43±0.01
	96	105.09±0.83	121.33±26.69	7.95±0.67	290.77±30.24	6.20±0.14	0.57±0.02	2.29±0.02
	120	116.48±0.93	116.27±7.63	7.73±0.25	400.38±7.57	6.20±0.35	0.73±0.01	1.17±0.02
MCC 02	24	100.00±0.14	127.99±19.30	16.83±0.64	281.08±8.58	3.65±0.99	0.46±0.04	1.55±0.14
	48	95.33±0.87	114.04±5.95	16.64±0.92	413.94±11.81	5.67±0.03	0.38±0.01	1.51±0.01
	72	93.13±2.33	109.52±0.65	12.93±0.49	276.52±0.79	5.10±1.04	0.79±0.01	1.43±0.56
	96	108.60±1.57	89.03±3.49	3.56±0.01	350.83±7.15	2.10±0.09	0.97±0.02	1.57±0.01
	120	113.95±1.02	99.35±1.43	2.9±0.02	242.19±10.90	6.47±0.49	1.16±0.01	1.35±0.01

Mean values ± standard deviation.

24 hour, respectively. Meanwhile, the highest K content of PBC 123 (443.40 mg/100 g) and BR 25 (446.4 mg/100 g) clones occurred at the same fermentation time i.e. 24 hours and MCC 02 (413.94 mg/100 g) of 48 hours of fermentation.

For the formation of microminerals, the highest Fe content for the three clones occurred at different of fermentation. PBC 123 (13.06 mg/100 g), BR 25 (6.69 mg/100 g), and MCC 02 (6.47 mg/100 g) clones had the highest Fe content with 96 hour, 72 hour, and 120 hour, respectively; while the highest Mn and Zn on BR 25 (1.58 mg/100 g) and MCC 02 (1.48 mg/100 g) clones occurred at the same fermentation time, i.e. 24 hours.

The presence of macro and micro minerals in cocoa is strongly influenced by the availability of minerals in the growing soil, as well as the potential to be supplied from the use of various fungicides during the growth process (Borchers *et al*., 2000). In general, the soil minerals content from the sampling sites at 30 cm depth were Ca, Mg, K, and Na of 42.57, 1.70, 0.14, and 0.97 mEq/100 g respectively, whereas at 50 cm depth were 23.08, 0.83, 0.11, and 0.64 mEq/100 g (BPPP, 2016).

Potassium was the most abundant mineral in cocoa beans from each clone compared to other minerals. This is consistent with the results reported by Afoakwa *et al*. (2011) that Ghanaian cocoa beans have the high value K mineral content and this might have originated from the soil on which the cocoa were planted. Potassium plays a role in the process of photosynthesis, namely the formation of chlorophyll and the formation of tissues. Potassium is easily soluble in water so that its level is very high. In contrast to Mg, the absorption process in plant tissue is lower than that of K (ICCRI, 2004).

Macro and micro minerals contents in cocoa beans are important to be analyzed because they are not only useful to improve the quality of health, but also have the detrimental effect to health. The existence of certain minerals causes health problems if the concentration exceeds the required tolerance threshold in foods. For example, Ca and Mg in sufficient quantities are very important for the body. Calcium is required for blood vessel contraction, muscle function, nerve transmission, intracellular signaling, and hormonal secretion (Borchers *et al*., 2000). Ca consumption should not exceed 2500 mg

daily, and when consumed in excess can cause kidney disorders. Mg minerals play a role in catalyzing biological reactions, including protein synthesis, transmission of nerve impulses, muscle relaxation, energy production, and bone and tooth adsorption. According to Spiegel & Sager (2008), Mg content in cocoa beans is 4-5 times higher than peas, white wheat, corn, and rice.

CONCLUSIONS

Different types of clones, fermentation times, and their interactions affect protein, macro and micro minerals content in the cocoa beans. The interaction between BR 25 clone and 48 hour fermentation time showed the highest protein content (13.34%). The highest macrominerals contents were (1) Ca in PBC 123 (72 hour), (2) Mg in MCC 02 clone (24 hour), (3) Na in BR 25 clone (72 hour), and (4) K in BR 25 clone (24 hour) of fermentation. The highest microminerals contents were (1) Fe in PBC 123 clone (96 hour), (2) Mn in MCC 02 clone (120 hour), and (3) Zn in BR 25 (96 hour) of fermentation.

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