Mineral contents in fermented bean shells of Forastero-cocoa (Theobroma cacao L.) clones

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Abstract. The cocoa bean shell (CBS) is a valuable by-product obtained from the chocolate industry. It is rich in protein, minerals, ash and some valuable bioactive compounds. One of the post-harvest processes affecting the mineral contents in CBS is fermentation. The purpose of this study is to determine the mineral contents in CBS of Sulawesi 1, Sulawesi 2, and MCC 02 clones for 24, 48, 72, 96, and 120 h of fermentation. Method analysis was used Atomic Absorption Spectrophotometer. Research will be conducted at the Laboratory of Chemical and Microbiology Testing, Center for Plantation Based Industry. The results showed that types of clones, fermentation time, and their interaction affect mineral contents in CBS. The highest mineral contents of CBS were Ca in Sulawesi 2 clone (48 h), Mg (96 h) and Na (48 h) in Sulawesi 1 clone, while K (24 h) and Zn (120 h) in MCC 02 clone. Total mineral contents of CBS were significantly affected by fermentation and types of clones.

1. Introduction

The cocoa bean shell (CBS) is a valuable by-product obtained from the chocolate industry. The CBS production is very significant and it estimated represent 12-20% of the weight of the cocoa bean [13]. Cocoa bean contents are in the form of bioactive components such as flavonoids, alkaloids; minerals, proteins, and organic acids [17]. [1], [4], Loureiro (2016) stated that macronutrients content of Ca, Mg, N, P, K, S, and micronutrients Fe, Mn, Zn, Cu, B, Ni are related to the quality of cocoa beans. The chemical components of cocoa beans are strongly influenced by varieties or clones, climates, places of growth, fruit maturity levels, and post-harvest processing [18]. Cocoa beans are an essential source of minerals and chocolate as one of the cocoa processed products has potential as the source of human diet [6], [14].

One of the post-harvest processes affecting the mineral contents in CBS is fermentation. Fermentation lead in death and cell permeability damaged of seed, resulting diffusion of polyphenol compounds from pigment cells to all parts of the cotyledons. During fermentation, there is a change in the chemical composition such as amino acids, fats, carbohydrates, vitamins and minerals and then usually accumulate in the outer parts of plant i.e. bean shells [20].
The cocoa shell is removed from the cocoa bean after the seeds are roasted, it is considered a by-product of the cocoa industry that is usually underutilized or considered waste, and it is mainly used as fuel for boilers [2], [9] and applications in animal feed and fertilizer preparation [12]. Some studies have been developed in order to give economic value added. The beneficial effects of CBS is due to its high nutritional value owing to the presence of a variety of bioactive compounds, such as phenolic, minerals, dietary fibers, and a lipid profile similar to that of cocoa butter, besides its chocolate colour and flavour [13].

The mineral content of CBS was needed for the proper functioning of the nervous system, especially nerve impulse transmission. Ca and Mg in sufficient quantities are very important for the body. Calcium is required for blood vessel contraction, muscle function, nerve transmission, intracellular signalling, and hormonal secretion [5].

The objectives of this study were to determine the minerals content in fermented CBS of Sulawesi 1, Sulawesi 2 and MCC 02 clones as local superior clones of Forestero originating from East Luwu, South Sulawesi. These three clones have been widespread among farmers, but there is limited information on mineral contents in fermented of CBS.

2. Materials and Methods

2.1. Preparation of the CBS

The CBS samples for assessment were prepared as dried fermented cocoa bean. They consisted of three Forastero cocoa clones, namely Sulawesi 1, Sulawesi 2, and MCC 02.

The CBS were procured from 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 pod husk. Fermentation took place for 24, 48, 72, 96, and 120 hours using fermentation boxes made of styrofoam. After fermentation, the cocoa beans were sun-dried for 5 days, until reaching 6% moisture content. The cocoa beans were then separated from its bean shells.

2.2. Preparation of the samples and analysis

Preparation and analysis of the CBS samples were conducted at Laboratory of Chemical and Microbiological Testing, Center for Plantation Based Industry, in Makassar, South Sulawesi, Indonesia.

The CBS were separated from their nibs and then ground with ceramic mortar. 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. 42 filter paper. The solution was then made to the mark with distilled water.

The concentrations of minerals calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), and zinc (Zn) 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, and Zn of the samples. Data analysis used ANOVA using SPSS (version 16) and analyzed by Duncan Post-ANOVA test if there was a significant difference at 5%.
3. Result and Discussion

Mineral contents in the CBS with Sulawesi 1, Sulawesi 2, and MCC 02 clones in the some variations of fermentation time is presented in table 1. The ANOVA analysis showed that were significantly influenced by types of clone, fermentation time, and their interaction.

**Tables 1.** ANOVA summary showing F-ratio for variation minerals content in fermented CBS.

| Variables         | Ca     | Mg    | Na    | K      | Zn      |
|-------------------|--------|-------|-------|--------|---------|
| Clone (C)         | 0.54*  | 0ns   | 3.55* | 129.74*| 125.82* |
| Fermentation (FT) | 2.34*  | 0.004*| 3.07* | 974.48*| 726.89* |
| Interaction (C x FT) | 7.58*  | 0.005*| 9.4*  | 4839.29*| 1275.49* |

*significant at p<0.05                ns = not significant

Total mineral contents in the CBS were significantly influenced by types of clone, fermentation time, and their interaction. However, Mg content was not significant affected by clone differences.

In general, Sulawesi 1 clone had the highest mineral content in Mg and Na. Sulawesi clone had the highest mineral content in Ca, while MCC 02 clone had the highest content in K and Zn. The lowest mineral contents found in Sulawesi 1 were Ca, K, and Zn. Sulawesi 2 clone had the lowest mineral content was Mg, while Na mineral content found in MCC 02 clones.

**Table 2.** Minerals content of CBS as affected by clone and fermentation time

| Variables         | Ca     | Mg    | Na    | K      | Zn      |
|-------------------|--------|-------|-------|--------|---------|
| Clones            |        |       |       |        |         |
| Sulawesi 1        | 0.97 a | 0.36 a| 1.12 c| 1.98 a | 2.50 a  |
| Sulawesi 2        | 1.24 c | 0.35 a| 0.68 b| 2.23 b | 2.56 b  |
| MCC 02            | 1.09 b | 0.36 a| 0.45 a| 2.39 b | 2.88 c  |
| Fermentation time (h) |    |       |       |        |         |
| 24                | 0.87 b | 0.35 ab| 0.38 a| 2.56 e | 2.48 c  |
| 48                | 1.36 d | 0.34 b| 1.09 e| 2.44 c | 2.36 b  |
| 96                | 0.79 a | 0.35 ab| 0.98 d| 2.52 d | 2.34 a  |
| 72                | 1.25 c | 0.36 b| 0.55 b| 2.17 b | 2.62 d  |
| 120               | 1.24 c | 0.37 b| 0.75 c| 1.31 a | 3.42 e  |

Total mineral content of CBS were significantly affected by fermentation. Result in table 2 showed that the highest Ca and Na mineral contents were obtained at 48 h of fermentation. The highest K mineral content was obtained at 24 h and decreased until the end of fermentation (120 h), while the highest Zn mineral was occurred at 120 h of fermentation.

Interactions between different clones and fermentation times occurred in the formation of mineral deposits in CBS. The highest Ca content in Sulawesi 1 clone of 1.33 mg/100 g occurred at 120 h of fermentation, while in Sulawesi 2 (2.19 mg/100 g) at 96 h and MCC 02 (2.05 mg/100 g) clones occurred at 48 h of fermentation. In all variation of fermentation, Ca mineral content tended to increase with increased fermentation time. Unlike case with Mg mineral, the concentration obtained from the three clones is the same in each variation of fermentation, i.e. 0.37 mg/100 g, as shown table 3.
Table 3. Effects of fermentation and types of clone on minerals content in CBS

| Clones   | Fermentation time (Hours) | Ca (mg/100 g) | Mg (mg/100 g) | Na (mg/100 g) | K (mg/100 g) | Zn (mg/100 g) |
|----------|---------------------------|---------------|---------------|---------------|--------------|---------------|
|          |                           | 24            | 48            | 72            | 96           | 120           |
| Sulawesi 1 | 24                        | 0.54±0.01     | 0.37±0.02     | 0.39±0.02     | 15.13±0.03   | 32.30±0.00    |
|          | 48                        | 1.07±0.01     | 0.34±0.03     | 2.43±0.02     | 41.84±0.04   | 15.77±0.04    |
|          | 72                        | 0.83±0.01     | 0.35±0.01     | 1.79±0.01     | 15.45±0.04   | 26.33±0.03    |
|          | 96                        | 1.10±0.05     | 0.35±0.00     | 0.60±0.03     | 12.03±0.04   | 22.38±0.02    |
|          | 120                       | 1.33±0.03     | 0.36±0.03     | 0.41±0.01     | 14.37±0.05   | 28.16±0.06    |
| Sulawesi 2 | 24                        | 1.06±0.02     | 0.34±0.02     | 0.40±0.02     | 15.47±0.06   | 27.47±0.03    |
|          | 48                        | 0.95±0.01     | 0.32±0.02     | 0.45±0.00     | 15.68±0.04   | 22.72±0.03    |
|          | 72                        | 1.04±0.02     | 0.36±0.02     | 0.54±0.02     | 35.72±0.02   | 20.55±0.03    |
|          | 96                        | 2.19±0.08     | 0.37±0.02     | 0.64±0.02     | 36.05±0.05   | 24.05±0.05    |
| Mcc 02   | 24                        | 1.00±0.02     | 0.35±0.01     | 0.37±0.02     | 46.29±0.03   | 14.64±0.03    |
|          | 48                        | 2.05±0.00     | 0.36±0.03     | 0.38±0.03     | 15.54±0.04   | 32.25±0.06    |
|          | 72                        | 0.49±0.01     | 0.34±0.02     | 0.62±0.00     | 24.32±0.03   | 23.32±0.03    |
|          | 96                        | 0.45±0.00     | 0.36±0.03     | 0.42±0.02     | 17.12±0.03   | 32.15±0.05    |
|          | 120                       | 1.43±0.03     | 0.37±0.03     | 0.46±0.01     | 16.13±0.03   | 41.56±0.05    |

Mean values ± standard deviation

The fermentation time significantly affects for the formation of Na mineral in the three clones. The highest Na content in Sulawesi 1 (2.43 mg/100 g) clone occurred at 48 h, while in Sulawesi 2 (1.38 mg/100 g) at 120 h and MCC 02 (0.52 mg/100 g) clones occurred at 72 h of fermentation.

The highest K content for the tree clones at different clones occurred at different fermentation times. Sulawesi 1 (41.84 mg/100 g), Sulawesi 2 (36.05 mg/100 g), and MCC 02 (46.29 mg/100 g) clones had the highest K content with 48 h, 96 h, and 24 h of fermentation, respectively; while the highest Zn on Sulawesi 1 (32.30 mg/100 g) at 24 h, Sulawesi 2 (32.96 mg/100 g) and MCC 02 (41.56 mg/100 g) clones occurred at the same of fermentation time, i.e. 120-hours.

The presence of minerals in CBS 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 [5]. The availability of mineral dissolution depends on the properties of ionic species formed in soil solutions and regulated by a system of chemical variables in the soil such as electron transfer reactions, ligand exchange reactions, etc [21].

In general, potassium and zinc were the most abundant mineral in CBS from each clone compared to other mineral. The highest K and Zn content in CBS which is due to the soil nutrients at the growth site. As stated by Ellen [19], zinc is very important in maintaining the function of the immune system. Zinc participates in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids and in metabolism zinc stabilizes the molecular structure of cellular components [16].

4. Conclusion
Types of clones, fermentation time, and their interaction affect mineral contents in CBS. The highest mineral contents of CBS were Ca in Sulawesi 2 clone (48 h), Mg (96 h) and Na (48 h) in Sulawesi 1 clone, while K (24 h) and Zn (120 h) in MCC 02 clone. Total mineral contents of CBS were significantly affected by fermentation and types of clones.
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