Antioxidant activities of cocoa bean shell from North Luwu and Gunungkidul as an active compound of packaging material

R R Utami, Jamilah, R Wavyudi, W P Tangkin, I Thamrin, A N Amalia, D Indriana, Rosniati, M Yumas, A Assa, and M Ariyanti
Balai Besar Industri Hasil Perkebunan, Kementerian Perindustrian, Jl. Prof. Abdurahman Basalamah No. 28, Makassar, South Sulawesi, Indonesia, 90231
ratri.retno.u@gmail.com

Abstract. The development of the cocoa processing industry must balance with the handling of cocoa bean shell waste. Cocoa bean shell is by product from cocoa processing industry. Active compounds of cocoa bean shell show antioxidant activity as antimicrobial. Based on this information, the cocoa bean shell has the potential to be used as an active compound in the packaging. The objective of this study was to evaluate the antioxidant activity of cocoa bean shell extract from North Luwu and Gunungkidul. Both regions had differences in cocoa bean processing and it may affect the antioxidant activity. The extraction of an active compounds was carried out by preparation of cocoa bean shell powder, defatted with hexane, then extracting active compounds with 70% ethanol. The analysis of cocoa bean shell powder including proximate and color analysis. The cocoa bean shell extract analysis including antioxidant activity of Radical Scavenging Activity (RSA) DPPH, total phenolic, total flavonoid, FTIR, and total dissolved solids. The result show that North Luwu cocoa bean shell extract has a higher active compound content than Gunungkidul, and this is reflected in RSA DPPH.

1. Introduction
The packaging industry is currently innovating by adding antioxidants into the packaging. Antioxidants developed are natural antioxidants that can replace synthetic antioxidants because consumers have aware to reduce the use of artificial additives [1]. Inside the packaging, the antioxidant acts as a barrier for the diffusion of O2 and transfer it’s to the packaged product to prevent oxidation. Previous research on the addition of antioxidants and antimicrobial from natural ingredients for the packaging are from grape seed extract [2], green tea extract [3], orange extract [4] and essential oils [5].

In the cocoa industry, during processing, nib (deshelled cocoa beans) used as a raw material of chocolate, while the cocoa bean shell (about 15% of the total cocoa beans weight) is waste [6]. Cocoa bean shell contains total polyphenol of 41.82 mg GAE/g and total flavonoid of 5.49 mg CE/g [7]. Polyphenol compounds of cocoa bean shell show antioxidant activity as antimicrobial [7] and can inhibit linoleic acid oxidation [8]. The development of cocoa processing industry must balance with the handling and utilization of cocoa bean shell waste. One of the efforts is to utilize the cocoa bean shell polyphenols as a natural antioxidant compound. Based on this information, the cocoa bean shell has the potential to be used as an active compound in the packaging.
The active packaging made by adding an antioxidant compound to the polymer matrix or packaging material. This compound will diffuse into food products so that it can maintain or extend the shelf life of a food product [9]. Therefore, it is necessary to develop an active packaging given antioxidant or antimicrobial substances. But there has been no publication use of cocoa bean shells as an active ingredient in making packaging. This research is an early stage of the development of sago starch-based packaging manufacturing with the addition of cocoa bean shell active compounds. The initial phase of this research was the manufacture of an active compounds from cocoa bean shell extract. The objective of this study was to evaluate the antioxidant activity of cocoa bean shell extract as an active ingredient.

Cocoa bean shell is skin that adheres to the outside of the nib and protects the nib from environmental conditions, pests or fungi. Cocoa bean shell is a waste that has no economic value [10]. The shell is usually used as a fuel for cocoa processing (steam boiler) and compost. The main components contained in the cocoa beans shell are crude fiber and cellulose, respectively 18.6% and 13.7% [11]. Cocoa bean shell also contains carbohydrates, aldehydes, polyphenols, amino acids, and others, such as those listed in the nib [12]. One of the main polyphenol compounds in cocoa bean shells is epicatechin [13]. Utilizing waste to increase added value is a waste management effort. So far, the economic value of the cocoa beans case is low because it has not been used optimally. Consumer demand for food packaging at this time is packaging that is environmentally friendly, natural, and without preservatives. The food packaging industry innovated to accommodate their customer needs, through the active packaging. Active packaging is packaging designed to release active components into food, such as antioxidants, aroma, or color. This active packaging is made by adding a compound that has an antioxidant activity to the packaging material. This combined will then diffuse into food products so that it can extend the shelf life of a food product [14]

2. Method
The research conducted in BBIHP on March-June 2019, samples analysis conducted in BBIHP, Faculty of Agricultural Technology and LPPT UGM. This research consists of several states namely the preparation of cocoa bean shell, defatted, active compounds extraction and analysis. This research flowchart is seen in Figure 1.

2.1. Materials
Cocoa bean shell obtained from the cocoa processing industry in North Luwu, South Sulawesi, and Gunungkidul, Yogyakarta. Both regions had differences in cocoa bean treatmens and processing, where cocoa beans from North Luwu is half fermented (3 days fermentation) and Gunungkidul have full fermented cocoa beans (6 days fermentation). The process of separation (deshelled) between nib and shell is also different, North Luwu deshelled manually and Gunungkidul by machine. Ethanol, hexane, distilled water used as solvent. The equipment used in this study is oven, sieve, centrifuge, magnetic stirrer, hot plate, blender, and rotary vacuum evaporator.

2.2. Research Procedure
The first step is preparation of cocoa bean shell, samples collected and sorted. Sorting conducted to remove dirt, e.g. stones, leaves, etc. Cocoa bean shell crushed and then sifted until obtained cocoa bean shell powder with size 20 mesh. Next step is defatted with hexane with ratio shell:hexane is 1:2. The aim of this step is to remove the fat from the shell. Then the separation of filtrate and residue with centrifugation. The next step is the extraction of active compounds contained on the residue of cocoa beans shell. Extraction using 70% ethanol, where the ratio between the shell:ethanol is 1:20. Then centrifugation is conducted to separate the filtrate and residue, and the ethanol that still contained in the filtrate evaporated with a rotary evaporator. This process will produce a viscous extract of the active compounds of cocoa beans shell. Last stage is the antioxidant analysis contained in the extract.
2.3. **Statistical Analysis**

The experimental design in this study used randomized design with three replicated. The data obtained were analyzed statistically with SPSS Statistics 20 and MS Excel 2007. ANOVA was used to compare the effect of treatment if there were differences between treatments, followed by Duncan's Multiple Range Test (DMRT) with a significance of $p < 0.05$.

3. **Results and Discussion**
The proximate analysis of cocoa bean shell powder shown in Figure 2. There are differences in the condition of fermented cocoa beans, where North Luwu cocoa beans are half fermented with 3 days fermentation and Gunungkidul cocoa beans is fully fermented through 6 days fermentation. Based on Table 1, it can be seen that the water content of Gunungkidul cocoa bean shells is lower than North Luwu. Fermentation causes a decrease in water content in cocoa beans [15]. The fermentation of cocoa beans causes cocoa bean shell to become permeable [16] so that when the drying process is carried out, water easily comes out of the shell.

![Figure 2. Proximate analysis of cocoa bean shell](image)

Based on statistical analysis, only the ash content parameter that has no significant difference between cocoa bean shell from North Luwu and Gunungkidul. Ash content is related to the mineral. The fat content of cocoa beans shells because among shell waste, there possible small flakes of cocoa beans involved. The process of separating cocoa beans with cocoa bean shells is also different where North Luwu separated manually and Gunungkidul with machined so the possibility of cocoa beans become waste along with the shell is higher and cause fat content of Gunungkidul higher than North Luwu.

Protein in cocoa beans, during fermentation, is caused by the degradation of proteolysis enzymes become amino acids [17]. This does not occur in the shell of Gunungkidul cocoa beans; protein content increases with fermentation. The increase in protein content is due to the increase in theobromine, which is an alkaloid compound containing N with the molecular formula C$_7$H$_8$N$_4$O$_2$ (LCMS data not shown) [18]. Increased theobromine in the cocoa bean shell due to diffusion from cocoa beans during fermentation. Carbohydrates and crude fiber are the main components of cocoa bean shells with the content of more than 60% dry weight [7,19].

Table 1 shows the results of the analysis of active compounds of cocoa bean shells consisting of total dissolved solids, total phenols, total flavonoids, and DPPH radical scavenging activity.

| Table 1. The analysis of the active compound extract of cocoa bean shell |
|-------------------------------------------------------------------------------------------------|
| Cocoa bean shell (Extract) | North Luwu | Gunungkidul |
| Total dissolved solids (%) | 2.83±0.52$^a$ | 1.99±0.05$^a$ |
| Total phenol (Gallic Acid Equivalent (% w/v)) | 1.04±0.27$^a$ | 1.21±0.10$^a$ |
| Total flavonoids (Quercetin Equivalent (% w/v)) | 0.10±0.01$^a$ | 0.21±0.00$^a$ |
| RSA DPPH (%) | 84.96±0.30$^a$ | 78.90±2.40$^b$ |
Total dissolved solids are the number of solids contained in the extract or residual extract after the water, and the solvent has been evaporated. Based on Table 1 it can mean that North Luwu cocoa bean shell extract has a higher active compound content than Gunungkidul, and this is reflected in the Radical Scavenging Activity DPPH. Total phenol in cocoa bean shell was 23.7 mg GAE/g dry weight and the flavonoid content of cocoa bean shell extract was 1.65 mg QE/g [7]. The different results with this research due to differences in testing conditions, methods, and standards used.

![Color analysis results](image1.png)

**Figure 3.** Color analysis results

The results of this research are almost the same as those obtained by [8], namely the activity of radical scavenging activity DPPH of cocoa bean shell extract at a concentration of 200 ppm was of 78.41-91.67% [8]. But there is a difference wherein the phenol content of cocoa bean shell extract was 19.94-39.97 mg GAE/g dry weight [8], due to differences in conditions and testing methods.

![FTIR spectra](image2.png)

**Figure 4.** FTIR spectra of cocoa bean shell extract
Based on the statistical analysis, all parameters show a significant difference between the cocoa bean shell from North Luwu and Gunungkidul. The active compound extract produced has a brown color in which the North Luwu cocoa beans shell has a stronger brown color, this is probably because the roasting process, where North Luwu has longer roasting time than Gunungkidul. Roasting also can produce melanoidin which is a Maillard Reaction Product that shown antioxidant activity, that’s why cocoa bean shell from North Luwu has stronger antioxidant activity compared to cocoa bean shell from Gunungkidul. The infrared spectrum provides information about the functional groups of a molecule [20]. The infrared absorption of cocoa bean shell extract can be seen in Figure 4.

Figure 4 shows the absorption of the hydroxyl (OH) group at 3500-3300 cm -1, and absorption of the C = O group at 1820-1600 cm -1. The results show that the flavonoid group dominates the predicted compound in the cocoa bean shell extract. This assumption based on the content of aromatic functional groups (C=O) and hydroxyl groups (OH), which is one of the characteristics of flavonoid compounds. The hydroxyl group has a role in antioxidant activity. The antioxidant activity of the phenolic component depends on the position and number of hydroxyl groups that can act as reducing agents, hydrogen donors, and singlet oxygen absorbers [21]. The location of OH in the molecule determines the antioxidant activity of compounds. The OH groups attached to the C3, C4, and C5 positions in the B ring and OH in C3 in the C ring play the main role in antioxidant activity. Loss of OH groups other than in this position does not significantly affect the antioxidant activity of compounds [22].

The number of OH groups influences their antioxidant activity, and the less the amount of OH causes the lower antioxidant activity. Changes in this functional group cause differences in the antioxidant activity of the sample.

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