The potential of dry fermented cocoa (*Theobroma cacao* L.) variety Lindak bean shell treated at different degrees of roasting as a functional food

E Lembong, M Djali, Zaida and G L Utama

Dept. Food Technology Industry, Faculty Technology Industry of Agriculture, Universitas Padjadjaran, Bandung, Indonesia

E-mail: elazmanawati.lembong@unpad.ac.id

**Abstract.** Cocoa bean shell is a waste from the chocolate processing industry that has not been used optimally and still contains 5.78% polyphenols which has potential as a source of natural antioxidant compounds. The chocolate is processed in the industry with light, medium and high roasting, based on the desired product. The purpose of this study was to determine the polyphenol content and antioxidant activity of the dry fermented cocoa shells as a result of the best degree of roasting. The research method used was an experimental method using a Randomized Block Design (RBD) with 4 treatments roasting dried fermented cocoa beans of Lindak varieties by using different degrees of roasting (Without roasting; low roasting (110°C for 35 minutes), medium roasting (140°C for 30 minutes), and high roasting (190°C for 15 minutes)) and repeated 3 times. The results showed the dry fermented cocoa shells of Lindak varieties by high roasting treatment has the best phenolic total (5.24%) and antioxidant activity (37.92 ppm).

1. Introduction

Indonesia state has abundant plantation products, including cacao (*Theobroma cacao* L.). Cacao plants can grow in the lowlands at an altitude of fewer than 700 m above sea level with tree heights reaching 12 m-15 m [1]. Cacao plants can be found in most parts of Sulawesi, Java, and Sumatra [2].

Cocoa has a relatively high price. According to [3], the price of cocoa on the world market in 2015 reached 3.14 USD / kg unit. Besides, cocoa is very easy to market and has economic significance, which is as a source of foreign exchange [4].

Indonesia is the third-largest cacao producing country in the world after Ivory Coast and Ghana [2]. Total cocoa production in Indonesia in 2016 reached 656,817 tons with a total of 240,569 tons exported. Cocoa beans can be processed into various kinds of preparations such as cocoa liquor, cocoa butter, and cocoa powder which can be used further as a variety of processed ingredients [3].

Cocoa beans consist of 86% -90% of the weight of the beans (nibs) and 10% -14% of the cocoa shells. In some chocolate industries, the cocoa beans used are dry fermented cocoa beans. Fermentation represents an important step towards better quality and taste of chocolate. Fermented cocoa beans will have a good physical appearance, have a distinctive taste of cocoa, and have a little bitterness and tasting taste [5]. Fermentation is one of the solutions for cocoa farmers to produce beans
with good appearance, especially in the follow-up cocoa beans. It is known that the problems with Lindak’s cocoa beans are low quality, high bean acidity, low flavor, presence of poorly fermented cocoa beans, mixed with a spicy taste, and non-uniform bean size [6].

An important step in cocoa beans processing is roasting. According to [7], roasting aims to develop flavor and aroma, reduce water content, and facilitate the separation of the cocoa bean shells from the cotyledons.

In the processing of dry cocoa beans into chocolate products, only the cocoa beans are used, while the cocoa bean shells are not used thus become waste. Cocoa bean shells are thin, soft, and slightly slimy skins that have undergone a separation process using a separator machine from cocoa beans [8]. In 2012, the waste of cocoa bean shells produced by the chocolate processing industry was estimated at 52,500 tons per year and increased to 60,000 tons in 2014 [9]. Cocoa bean shells waste has not been used optimally, generally, cocoa bean shells are only used as animal feed and compost [10].

Cocoa bean shells still contain functional components that can be utilized. The functional components contained in the cocoa bean shells are polyphenols, caffeine, fiber, and alkaloid compounds such as theobromine [11]. Cocoa bean shells have the potential to be used as a source of natural antioxidant compounds because they contain polyphenols of 5.78% [12].

The fermentation and roasting processes can affect the polyphenol content in the cocoa bean shell. According to [13], during the fermentation process anthocyanin compounds which are antioxidants can change. Besides, there is also a change in pH which affects antioxidant activity. During the roasting process, the higher the temperature and roasting time used, the greater the loss of polyphenols. Roasting temperatures above 100°C can cause 10% of flavanols to disappear [14].

The natural antioxidant content in the cocoa bean shells is the component that has the potential to be applied further as a food processing ingredient. Based on the description above, a study is needed to determine the effect of roasting degrees (light, medium and high roasting) on the polyphenol content and antioxidant activity of fermented dry cocoa bean shells.

2. Materials and Methods

2.1. Stages of roasting process for dry fermented cocoa beans with different roasting degrees

The raw material used in this experiment was cocoa bean shells from dry cocoa beans fermented by the bulk cocoa variety. Cocoa beans were obtained from the Ngudi Raharjo II Farmer Group Bunder Patuk Gunungkidul.

The degree of roasting of cocoa beans is divided into three groups, that is low roasting around 110°C-115°C for 60 minutes to produce fat and chocolate candy, medium roasting around 140°C for 40 minutes to produce chocolate bars, powder, and chocolate paste, and high roasting (about 190°C-200°C) for 15-20 minutes to produce a dark chocolate paste [13].

Preliminary experiments were carried out to see the suitability of the temperature and time used. The experimental results showed that light roasting (110°C, 60 minutes) and medium roasting (140°C, 40 minutes) resulted in burnt roasted cocoa beans, this is due to the use of different roasting tools. So that the roasting time used for light roasting (110°C, 35 minutes) and medium roasting (140°C, 30 minutes) is reduced. This determination is based on the moisture content in the cocoa beans has reached 0% and to produce a good final product (cocoa butter, cocoa powder, or dark chocolate).

The main research will begin with roasting dry fermented cocoa beans using light roasting (110°C, 35 minutes), medium roasting (140°C, 30 minutes), and high roasting (190°C, 15 minutes). The comparison sample used fermented dry cocoa beans without roasting. This process aims to produce a good final product (processed chocolate), this process will affect the components contained in the cocoa bean shells, including polyphenols and antioxidants.
2.2. Extraction of cocoa bean shell powder by maceration method
The purpose of the extraction process is to obtain cocoa bean shell powder extract, pull out active compounds contained in the cocoa bean shells to facilitate its utilization and to test antioxidant activity and total phenolic activity.

Following the procedure for the extraction process of cocoa bean shells. Cocoa bean bark powder was macerated using 70% ethanol solvent with a ratio of powder and solvent 1: 5 (w/v) 24 hours at room temperature. Filtering uses a vacuum filter to separate the filtrate (extract of 70% ethanol-soluble cocoa beans) with pulp (cocoa bean shells powder), then stored in a closed container and airtight. The filtrate obtained was concentrated using a rotary evaporator at a temperature of ± 45 °C to remove the solvent (ethanol) to form a concentrated extract of cocoa shells soluble in ethanol 70%.

2.3. The antioxidant activity of the DPPH method (1,1-diphenyl-2-picrylhydrazine) using a spectrophotometer
The antioxidant activity of cocoa bean shell extract was measured using the DPPH (2,2-diphenyl-1-picrihydrazil) method with a UV-Vis spectrophotometer [15]. The ability to capture radical DPPH by an antioxidant is expressed as a percent capture of radicals. The higher value indicates that the sample of the compound used is indeed a potential antioxidant [16].

The addition of the DPPH solution to the sample is marked by changing from purple to yellow, which means the process of capturing free radicals. The amount of antioxidant activity is indicated by the IC50 value (50% inhibition concentration), which is the concentration of the sample solution needed to inhibit 50% of DPPH free radicals. The 50% inhibition is obtained from the curve between the percent inhibition of the sample concentration of the equation.

IC50 values are calculated from the linear regression curve between% absorption inhibition with various concentrations of the test solution using the formula:

\[
\% \text{ Inhibition} = \frac{A_{\text{blanko}} - A_{\text{Sample}}}{A_{\text{blanko}}} \times 100\% \tag{1}
\]

IC50 value is calculated based on the linear line equation that has been obtained by replacing the variable y with the number 50 so that the value of variable x can be obtained which is the value of IC50. Numbers 50 indicate the concentration of inhibition of test solutions that can ward off 50% of DPPH free radicals.

2.4. Total phenolic Folin-Ciocalteu test method
The total phenolic test was used by using the Folin-Ciocalteu reagent [17], the use of the Folin-Ciocalteu reagent was based on the ability of the reagent to reduce hydroxyl groups from phenolic compounds.

The exact mechanism of the Folin-Ciocalteu reagent is unknown, but the presence of aromatic nuclei in phenol compounds (phenolic hydroxyl groups) can reduce phosphotolstate phosphotungstate in the reagent into blue. As much as 0.2 g of the extract was weighed and put into a beaker glass and 0.8 mL of distilled water was added, then homogenized; 0.2 mL from the dilution results was pipetted and put into a 25 mL volumetric flask; Folin-Ciocalteu 50% reagent was added as much as 0.5 mL, into a measuring flask and shaken for 5 minutes; After 5 minutes, added 2.5 mL, 7% Na 2 CO 3 and adjusted with distilled water to the 25 mL limit mark; The solution is homogenized for about 40 minutes in dark conditions; Measurements were taken with a UV-Vis spectrophotometer at a wavelength of 765 nm using gallic acid (0, 2, 4, 6, 8, 10 ppm) as standard.
3. Results and Discussion

3.1. Total phenolic

Polyphenols are compounds composed of many phenolic compounds. Polyphenols are included in the flavonoid class that acts as antioxidants [24]. Total phenolics of fermented dry cocoa bean shells are expressed in% (g/100 g). The total phenolic value of the dry fermented cocoa bean shells of the Lindak variety can be seen in Table 1.

Table 1. Effect degree of roasting on total phenolic.

| Treatment                              | Total Phenolic (%) |
|----------------------------------------|--------------------|
| A : Control (Without roasting)         | 6.35 ± 1.05         |
| B : Light roasting (T:110°C, t : 35 minutes) | 5.03 ± 0.56         |
| C : Medium roasting (T:140°C, t : 30 minutes) | 4.46 ± 0.15         |
| D : High roasting (T:190°C, t : 15 minutes) | 5.24 ± 1.07         |

Note: The average treatment marked with the same letter states no significant difference at the 5% test level based on the Duncan test.

Based on the ANOVA results, the degree of roasting had no significant effect on the total phenolic content of the dry fermented cocoa bean shells of the Lindak variety. These results indicate that the phenol properties are more resistant to temperature and heating time of the cocoa bean shells at various degrees of roasting. The results of the Duncan test at the 5% level shown in Table 1 show that the light roasting cocoa bean shells is not significantly different from the medium and high roasting cocoa bean shells.

Without roasting (control) cocoa bean shells were not significantly different from the light and high roasting but were significantly different from medium roasting cacao bean shells. The total phenolic values obtained ranged from 4.46% - 6.35%, whereas according to [12] stated that the cocoa bean shells contain polyphenol compounds with a total phenolic content of 5.78%, the results obtained in the study were not much different from this value.

The results showed that the highest total phenolic value of cocoa bean shells was found in the treatment without roasting, which is 6.35%, followed by high roasting, which is 5.24%, light roasting, which is 5.03. %, and finally the lowest total phenolic value was found in the cocoa bean shells with medium roasting, which is 4.46%. This is consistent with research conducted by [19], who showed that roasted cocoa beans had a lower total phenolic content than unroasted cocoa beans.

Total phenolic decreased with increasing degree of roasting to 140°C and increased at 190°C. The decrease in total phenolic content occurs due to the non-enzymatic oxidation of polyphenol compounds. The oxidation of polyphenols is followed by polymerization and the formation of pigment compounds. The decrease in total phenolics was also caused by the reaction of phenolic compounds with protein [19].

The increase in total phenolic content at the degree of roasting in high roasting at 190°C for 15 minutes was due to the increased release of phytochemical components from the cell matrix such as phenolic acids. High temperatures can damage cell walls and cell membranes and release dissolved phenolic components from insoluble ester bonds [25]. This statement is supported by [26], the use of high temperatures will cause the total phenolic content to be higher because high temperatures can increase the release of phenolic compounds on the cell walls. One of the factors that influence the polyphenol content is the polyphenol oxidase enzyme. Heating can inactivate the enzyme polyphenol oxidase. The higher the temperature used, the higher the inactivation of the polyphenol oxidase enzyme so that the enzyme activity will be lower and the polyphenol damage will be smaller. This causes the detected polyphenol content to be even higher [27]. Research by [28] showed the same results, where of the three levels of steeping water temperature of avocado leaf herbal tea (70°C, 85°C, and 100°C) which had the highest total phenolic, which is avocado leaf tea brewed at a temperature of 100°C amounting to 291.63 mg/100g.
3.2. **Antioxidant activity**

The shells of the fermented dry cacao beans of the Lindak variety are made into a powder then extracted and tested for their antioxidant activity based on IC$_{50}$. The value of the antioxidant activity can be seen from the IC$_{50}$ value of an extract. The IC$_{50}$ (Inhibitory Concentration) value shows the ability of antioxidants to reduce 50% of the DPPH free radical concentration. The IC$_{50}$ value is inversely proportional to the antioxidant activity, the smaller the IC$_{50}$ value obtained, the greater the antioxidant activity [15].

The value of the antioxidant activity of the dry fermented cocoa bean shells of the Lindak variety can be seen in Table 2.

**Table 2.** Effect degree of roasting on antioxidant activity.

| Treatment                | Antioxidant IC$_{50}$ (ppm)  |
|--------------------------|-------------------------------|
| A : Without roasting     | 26.71 ± 2.71$^b$             |
| B : Light roasting (T:110°C, t : 35 minutes) | 41.66 ± 3.61$^a$           |
| C : Medium roasting (T:140°C, t : 30 minutes) | 44.66 ± 5.42$^a$           |
| D : High roasting (T:190°C, t : 15 minutes) | 37.92 ± 7.08$^a$           |

Note: The average treatment marked with the same letter states no significant difference at the 5% test level based on the Duncan test.

Based on the ANOVA results, the degree of roasting affected the antioxidant activity of the dry fermented cocoa bean shells of the Lindak variety. When seen from Table 2, the fermented dry cocoa bean shells of the Lindak variety have very strong antioxidant activity, ranging from 26.71 ppm - 44.66 ppm. According to [15], if the IC$_{50}$ value is less than 50 ppm, it is included in the very strong antioxidant group.

The results of the Duncan test at the 5% level shown in Table 2 show that the antioxidant activity value of light roasting is not significantly different from the medium and high roasting cocoa bean shells. Meanwhile, the shells of cocoa beans without roasting showed significantly different results with light, medium, and high roasting treatments. The results showed that the highest antioxidant activity value was found in cocoa bean shells without roasting, which is 26.71 ppm, followed by cocoa bean shells with high roasting at 37.92 ppm, cocoa bean shells with light roasting, which is 41.66 ppm, and finally the lowest antioxidant activity value was found in the extract of the cocoa bean shell with medium roasting, which is 44.66 ppm.

The antioxidant activity obtained is related to the phenolic compounds in Table 1. The higher the total phenolic value obtained, the higher the antioxidant activity. According to [18] stated that the antioxidant activity is related to the phenolic compounds contained in each extract of the cocoa bean shells, this shows that the higher the phenolic compounds contained in the extract, the higher the antioxidant activity.

The roasting process can cause changes in the antioxidant compounds in the shells of the cocoa beans. The antioxidant activity decreased with increasing degree of roasting up to 140°C and increasing at 190°C. The decrease in antioxidant activity is caused by decreased levels of polyphenols in the shells of the cocoa beans. In the roasting process, polyphenol compounds do the oxidation process which is accelerated by the influence of temperature and roasting time. In polyphenol oxidation, the H atom in the OH group is taken up by the oxidizing compound to become another compound. The more H atoms are taken, the smaller the polyphenol content in the material [19], thus the antioxidant activity will decrease.

When viewed from the degree of roasting, high roasting at 190°C for 15 minutes had the highest antioxidant activity compared to light and medium roasting, but not significantly different. This is because during roasting, proteins and polyphenols will experience a Maillard reaction which produces melanoidin. This melanoidin has potential as an antioxidant and can form a reduktion structure, which is enaminol [20]. The high antioxidant activity at 190°C is thought to be because at that temperature there are flavonoid components. The flavonoid group polyphenols contain more OH groups than the
Theobroma cacao) has the potential as a functional food as seen from its very strong antioxidant activity.

The increase in antioxidant activity during roasting may be due to the formation of new products that have the potential to act as antioxidants, such as the reaction product between carbonyl compounds and amino acids, the reaction between lipid oxidation products with proteins and carbohydrates, or maybe the caramelization reaction of sugars [23]. The main components of polyphenols in the cocoa bean shell which function as antioxidants are the flavonoids, especially catechins and epicatechins [23].

4. Conclusions
The higher degree of roasting used, the total phenolic content and the resulting antioxidant activity decreased and experienced an increase in the degree of high roasting. The dry fermented cocoa bean shells of the Lindak variety with high roasting treatment (T: 190 °C, t: 15 min) have the best total phenolic (5.24%) and antioxidant activity (37.92 ppm). Cocoa bean shells in a degree of roasting can be said to be potential as a functional food as seen from its very strong antioxidant activity.

References
[1] Beckett S 2008 The science of chocolate edition 2 (Cambridge: The Royal Society of Chemistry Publishing)
[2] Fowler M S 2008 Cocoa beans: from tree to factory (Industrial chocolate manufacture and use 4th edition) ed. Beckett S T (York, UK: Wiley-Blackwell) chapter 2
[3] Direktorat Jenderal Perkebunan. 2016 Statistik perkebunan Indonesia kakao 2015-2017 (The crop estate statistics of Indonesia cacao 2015-2017) (Jakarta: Direktorat Jenderal Perkebunan) [In Indonesian]
[4] Kuswinanti T 2005 Pengaruh lama penyimpanan terhadap keberadaan cendawan dan bakteri pasca panen pada biji kakao (The effect of storage time on the presence of post-harvest fungi and bacteria on cocoa beans) J. Sains & Technology 5 3 154-158 [In Indonesian]
[5] Clapperton J F 2007 A review of research to identify the origins of cocoa flavor characteristics Cocoa Grower’s Bull 48 (2007) 7-16
[6] Wahyudi T., Misnawi 2007 Fasilitas perbaikan mutu dan produktivitas kakao Indonesia (Facilitating the improvement of Indonesian cocoa quality and productivity)Warta Pusat Penelitian Kopi dan Kakao Indonesia 23 (1) 32-43 [In Indonesian]
[7] Minifie B W 1999 Chocolate, cacao and confectionary (Gaithersburg, Maryland : Aspen Publisher Inc)
[8] Djali M, Sumanti D M, Kayaputri L I,Indarto R 2012 Pengujian keamanan pangan biji kakao sebagai bahan baku produk pangan (Food safety testing of cocoa bean shells for possible use as raw material for food products). Laporan Akhir Kegiatan Ilmiah Universitas Padjadjaran Jatinangor [In Indonesian]
[9] Utami R R, Supriyanto S, Rahardjo S, Armunanto R 2017 Aktivitas antioksidan kulit biji kakao dari hasil penyangraian biji kakao kering pada derajat ringan, sedang dan berat (Antioxidant activity from shell of roasted cocoa bean with low, medium and high roasting degree) AGRITECH 37 88-94 [In Indonesian]
[10] Mariani L 2011 Ekstraksi dan identifikasi senyawa polifenol dalam kulit biji kakao dan potensinya sebagai antioksidan (Extraction and Identification of Polyphenol Compounds in Cocoa Bean Shells and their Potential as Antioxidants). Thesis Gadjah Mada UniversityYogyakarta[In Indonesian]
[11] Kayaputri L I, Sumanti D M, Djali M, Indiarto R, Dewi D 2014 Kajian fitokimia ekstrak kulit biji kakao (Theobroma cacao L.) (Phytochemical study of cocoa bean shell extract (Theobroma cacao L.)) Chimica et Natura Acta 2 1 83-90 [In Indonesian]
[12] Lecumberri E, Mateosa R, Pulido M I, Ruperez P, Goya L, Bravo L 2007 Dietary fibre
composition, antioxidant capacity and physico-chemical properties of a fibre-rich product from cocoa (*Theobroma cacao* L.) *Food Chemistry* **104** 948-954

[13] Haryadi, Supriyanto 2012 Teknologi cokelat (Chocolate technology) (Yogyakarta: Gadjah Mada University Press) pp 238

[14] Cruz J F, M, Leite P B, Soares S E, Bispo E S 2013 Assessment of the fermentative process from different cocoa cultivars produced in Southern Bahia, Brazil *African Journal of Biotechnology* **12** 33 5218-5225

[15] Molyneux P 2004 The use of stable free radical diphenylpicrylhidrazyl (DPPH) for estimating antioxidant activity *Songklanakarin Journal Science Technology* **26** 2 211-219

[16] Prakash F R, E. Miller 2001 Antioxidant activity (Minnesota: Medallion Laboratories Analytical Progress)

[17] Tambe V D, Bhambar R S 2014 Estimation of total phenol, tannin, alkaloid and flavonoids in *Hibiscus tilasseus* Linn. woos extracts *Journal of Pharmacognosy and Phytochemistry* **2** 4

[18] Kiessoun K, Souza A, Meda N T R, Coulibaly A Y, Kiendrebeogo M, Lamien M A, Lamidi M, Míllogo-Rasolodimby J, Nacoulma O G 2010 Polyphenol contents, antioxidant and anti-inflammatory activities of six malvaceae species traditionally used to treat hepatitis b in Burkina Faso *European Journal of Scientific Research* **44** 4 570-580

[19] Wibowo, T Y, Lamhot P M, Astuti Jusuf J 2017 Pengaruh penyangraian dengan teknologi vibro-fluidized terhadap aktivitas antioksidan biji kakao (Effect of roasting with vibro-fluidized technology on antioxidant activity of cocoa beans) *Jurnal Teknologi Pertanian* **18** 1 55-57 [In Indonesian]

[20] Rosida D F, Fardiaz D, Apriyanto A, Andarwulan N 2006 Isolasi dan karakteristik melanoidin kecap manis dan perannya sebagai antioksidan (Isolation and characterization of sweet soy sauce melanoidin and its role as antioxidant) *Jurnal Teknologi dan Industri Pangan* **17** 3 204-213 [In Indonesian]

[21] Cao G, Sofic E, Prior R L 1997 Antioxidant and prooxidant behavior of flavonoid: structure-activity relationships *Free Rad. Biol. Med.* **22** 5 749-760

[22] Rahayu W M, Sulistiawati E 2018 Evaluasi komposisi gizi dan sifat antioksidatif kedelai hitam Malika (*Glycine max*) akibat penyangraian (Nutritional and antioxidative properties of roasted black soybean var, Malika (*Glycine max*) Agroindustrial Technology Journal **2** 1 86-87 [In Indonesian]

[23] Supriyanto, Haryiadi, Rahardjo B, Marseno D. W 2007 Perubahan suhu, kadar air, warna, kadar polifenol dan aktivitas antioksidatif kakao selama penyangraian dengan energi gelombang mikro (Changes in temperature, moisture content, color, polyphenol content, and antioxidative activity of cocoa during microwave roasting) *AGRITECH* **27** 1 18-25 [In Indonesian]

[24] Lingga L 2012 The healing power of antioxidant (Jakarta: Elex Media Komputindo) [In Indonesian]

[25] Dewanto V, Wu X, Liu R H 2002 Processed sweet corn has higher antioxidant activity *Journal of Agricultural and Food Chemistry* **50** 17 4959-4964

[26] Wazir, Ahmad S, Muse R, Mahmood M, Shukor M.Y. 2011 Antioxidant activities of different parts of *Gnetum gnemon* L. *Journal Plant Biochemistry and Biotechnology* **20** 2 234-240

[27] Kusnandar F 2010 Kimia pangan komponen makro (Food chemistry: macro’s components) (Jakarta: PT. Dian Rakyat) [In Indonesian]

[28] Dewata I P, Wipradnyadewi P A S, Widarta I W R 2017 Pengaruh suhu dan lama penyeduhan terhadap aktivitas antioksidan dan sifat sensoris the herbal daun alpukat (*Persea americana* Mill.) (Effect of temperature and duration of brewing on antioxidant activity and sensory properties of avocado leaf herbal tea (*Persea americana* Mill.)) *Jurnal Ilmu dan Teknologi Pangan* **6** 2 30-39 [In Indonesian]