

## Antioxidant Properties of Cocoa Pod Husk Powder as Affected by Slicing and Oven-Drying

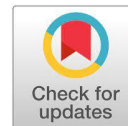
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Received: 9 November 2022 / Accepted: 2 Desember 2022



### Abstract

Cocoa pod husk (CPH) represents an underutilized by-product of the cocoa commodity. Their utilization has been hindered by their bulkiness, high processing cost and their limited use as food ingredients. A functional and effective processing method is needed to fully optimize this commodity. This study evaluated the effect of slicing and oven-drying treatment on the antioxidant properties of cocoa pod husk powder. A response surface methodology (RSM) was used to contour-plot the drying rate, and antioxidative properties of CPH as the effect of different treatments. A central compositing design consisting of three levels of drying temperature (55, 65, and 75°C) and three levels of slice thickness (1, 2, and 3 mm) was used in triplicate. The results showed that this technique could produce CPH powder with a light-brown appearance. Drying at 51-55°C with 1-3 mm thickness produced CPH powder with high antioxidative properties. A quick-drying method with a maximum temperature of 65°C and slice thickness of 0.6 mm could also produce CPH powder with high antioxidative properties. Principal component analysis showed that 65°C might be the maximum drying temperature limit to obtain CPH powder with high phenolic. Drying method used in this study enhanced the potential of CPH powder for food ingredient due to its good appearance and high phenolics characteristic.

**Keywords:** low-temperature drying, sun-drying, oven-drying, functional food

### INTRODUCTION

Cocoa pod husk (CPH) is an underutilized by-product of cocoa processing. The current processing method of cocoa beans generates around 80-90% of waste, mainly composed of CPHs and cocoa placenta. The utilization of CPH in industries is limited. Lu *et al.* (2018) reported that the CPH is currently used for fertilizer and soil organic matter, soap-making, animal feed, activated carbon, and paper-making. In terms of potential, CPH can be used as biomass resources for biofuel

and chemical industries due to their high ligno-cellulosic concentration. Furthermore, CPH contains bioactive and indigestible carbohydrates suitable for specific-purpose food ingredients. A limited number of its application in food is due to high-processing cost and lack of nutritional value (Lu *et al.*, 2018). CPH is highly perishable and could be a significant environmental issue. This is due to high moisture content and the bulky shape of the cocoa pod husk that hinder the drying process, resulting in a high proportion of unusable rotten pod husk. Unmanaged

CPH could induced the infection of cocoa pod rot in the cocoa farm (Lu *et al.*, 2018). A study on the oven-drying process (65-95°C) of sliced CPH (1 cm) to improve the drying performance has been carried out previously by Kusuma *et al.* (2019). However, a body of knowledge in this area still needs to be developed. Further study on the more effective drying method and its effect on the chemical composition is still needed.

CPH has poor nutritional value. CPH has been reported to have low protein (4.2% w w<sup>-1</sup> dry basis, DB) and fat (2.34% w w<sup>-1</sup> DB) (Martinez *et al.*, 2012). The carbohydrate content of CPH is around 29% w w<sup>-1</sup> DB, but most are indigestible carbohydrates (Daud *et al.*, 2013). Furthermore, a range of non-nutrient compounds has been detected in the CPH. They consist of phenolics, alkaloids, and pectins. This low nutritional value makes CPH not suitable for food ingredients. However, there is an increasing interest in using indigestible food ingredients as a source of dietary fiber (Delgado-Ospina *et al.*, 2021). It was associated with increased health benefits, especially in improving digestion, blood-lipid regulation, cardiovascular disease prevention, and weight management. Despite its potential in bioactive compound, CPH still have limited use for pharmaceutical purposes. Lu *et al.* (2018) showed that bioactive compound in CPH, such as pectin and phenolics, had anti-aging properties. However, the utilization is still hindered by high cost in drying process. In the current utilization of CPH for food ingredient and pharmaceutical purpose, pectins can be extracted from dried CPH (Barrios-Rodríguez *et al.*, 2022; Hennessey-Ramos *et al.*, 2021). Pectins can be used as fat and sugar replacer and to reduce blood cholesterol level and gastrointestinal disorders (Thakur *et al.*, 1997). However, information regarding commercial utilization of CPH as pectin source in industries is scarce.

Drying is a critical process for preserving food ingredients. It prolongs the shelf-life of the commodity and eases its utilization for further processing treatments. In cocoa processing, drying is related to the degradation of the bioactive component. This is due to enzymatic degradation as the result of microorganism activities. CPH has many alkaloids and phenolics in the form of theobromine, flavan-3-ols, phenolic acids, and flavonols. Belwal *et al.* (2022) reported that the total flavan-3-ols content in CPH was around 1.2% (w w<sup>-1</sup>), while theobromine content was around 6.8 mg 100 g<sup>-1</sup> of dried CPH. Considering the massive amount of CPH generated during cocoa post-harvest processing, CPH is a potential source of bioactive. However, high-moisture condition promotes the activity of oxidase enzymes such as polyphenol oxidase, decreasing the bioactive.

On the other hand, high-temperature treatment affects the bioactive negatively due to their low-thermal stability. The concentration of these bioactive compounds in CPH is important. These compounds have been reported to provide various beneficial health benefits. Considering the importance of dietary fiber and bioactivity in food systems, CPH can potentially be used as a food ingredient. The processing of agricultural products into powder or flour has been reported to enhance their usability in food formulations. This will improve the usability of CPH. Currently, the study about the production and utilization of CPH powder is still limited.

In this study, optimization of the drying process on CPH is carried out. The drying rate and antioxidant properties were analyzed to study their relationship. The results can help the cocoa processor better utilize CPH not as waste but as a potential ingredient for food and feed.

## MATERIALS AND METHODS

CPH was obtained from Kaliwining experimental station, Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember. The CPH was obtained from healthy mature fruit and only fresh CPH was used in this study. Around 5 kg of CPH was used per batch. The CPH was stored for up to 2 days during experiments. The genotypes of fruit in this study were mixed, but only green colored pod was used for this study. The CPH was obtained during the period of June-July 2022. Sample preparation and analysis were done in the post-harvest laboratory of ICCRI. The chemicals for extraction and analysis were of technical and analytical grades.

The study followed the response surface methodology (RSM) optimization design. A central composite design with  $\pm\alpha$  was used in this study. The RSM design employed three temperature levels for drying temperature (55, 65, and 75°C) and 3 levels of slice thickness (1, 2, and 3 mm). The experiments were done in triplicate. The  $\alpha$  for the temperature levels were 51 and 79°C, and 0.6 and 3.4 mm for slice thickness. The level of factor based on RSM design is presented in Table 1.

The sample was prepared by washing the CPH prior to cutting with different slice thicknesses, followed by drying in an oven (Heraeus B5050, Heraeus Hanau, Germany) until the moisture content of around 13%. The dried CPH slice was then ground and sieved in 40 mesh. CPH powder was then placed in a sealed container and stored in dry and cool condition until analyzed. The experiments were done using the same equipment for all the treatments and to ensure their reproducibility. The drying rate of the CPH slice was measured based on the moisture content differences in the sample before and after drying divided by the drying duration. The unit was  $\text{g hour}^{-1}$ .

The extraction of antioxidative compounds was done using ethanol 96% as solvent based on the method of Ananta *et al.* (2021). The extraction was done in a ratio of 1:10 ( $\text{w v}^{-1}$ ) between CPH powder to that of ethanol. The mixture was then macerated for 48 hours, followed by centrifugation to obtain the supernatant. The supernatant was then referred to as crude CPH extract (CPHE).

Antioxidative properties were analyzed using total phenolic content and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging (RSA). Total phenolic content (TPC) was analyzed based on the method of Misnawi *et al.* (2014). A known volume (0.2 mL) of CPHE was transferred into a test tube and added with ethanol (1 mL), distilled water (4.8 mL), and 0.5 mL of Follin-Ciocalteu reagent (50%). The mixture was then vortexed and rested for 5 mins. The solution was added with 1 mL of saturated sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) and topped with distilled water until 10 mL of volume. The mixture was then placed in a dark condition for 1 hour. The absorbance of 725 nm was measured by spectrophotometer against blank. The calculation used gallic acid as an external standard for preparing the calibration curve.

DPPH RSA was carried out based on the method of Gadow *et al.* (1997) with slight modifications. A known volume of CPHE was placed in a test tube and mixed with 0.5 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent. The mixture was then shaken vigorously. The solution was then placed in a dark condition for 20 minutes. The solution was added with ethanol until 5 mL of volume and then analyzed using a spectrophotometer at 517 nm. A blank mixture was used as a comparison. The antioxidant activity was measured as  $\text{IC}_{50}$ , which represents the amount of extract needed to reduce the absorbance of the blank solution by 50%.

RSM analysis was carried out using Design Expert version 10 (Statease, Minneapolis, MN, USA). Pearson correlation and principal component analysis were done using SPSS version 22 (IBM Corp., Armonk, NY, USA). All the analysis was done in triplicate.

## RESULTS AND DISCUSSION

### Drying Rate and Antioxidative Properties of CPHE

In this study, light-brown-colored CPH powder was obtained. CPH is susceptible to enzymatic hydrolysis and often produces black-colored CPH in slow drying rate treatment. The black-colored appearance of CPH is often associated with the microbial or enzymatic degradation of nutritional properties (Hinne *et al.*, 2018). This condition limits

the use of CPH in feed and food formulation. In this study, we found that the treatments prevented discoloration of CPH, improving its potential to be used in feed and food products. The result of the analysis for drying rate, TPC, and IC<sub>50</sub> based on the RSM design is presented in Table 1. Summarized ANOVA regarding the model fitting of the RSM is shown in Table 2. The responses showed that the drying rate of CPH in this study followed the quadratic trend, and the TPC and IC<sub>50</sub> followed the linear model. These models showed high accuracy of prediction for each response. The R<sup>2</sup>s ranged from 0.88 to 0.95, with adj-R<sup>2</sup> in the range of 0.86 to 0.92. This showed that the most insignificant terms/factor was excluded from the equation.

The ANOVA result showed that drying temperature was a significant factor affecting the responses. In this study, the slice thickness

Table 1. List of treatments based on RSM design and the result of analysis on dried CPH and CPH extract

No	Factor		Drying rate (g hour <sup>-1</sup> )	Total phenolic content (mg GAE g <sup>-1</sup> )	Antioxidant activity-IC <sub>50</sub> (ppm)
	(A) Drying temperature (°C)	(B) Slice thickness (mm)			
1	55	1	15.757	0.966	52.052
2	75	1	28.777	0.693	59.839
3	55	3	14.446	0.949	50.234
4	75	3	24.649	0.665	60.108
5	51	2	13.341	0.971	49.743
6	79	2	34.452	0.646	63.252
7	65	0.6	21.643	0.849	53.732
8	65	3.4	15.699	0.717	58.631
9	65	2	19.183	0.735	57.637
10	65	2	17.281	0.786	56.300
11	65	2	19.178	0.730	56.238
12	65	2	17.314	0.788	54.265
13	65	2	19.234	0.729	54.109

Table 2. ANOVA results based on central composite design of response surface methodology

Parameter	Drying rate	Total phenolic content	IC <sub>50</sub>
Model	Quadratic	Linear	Linear
Sequential p-value	0.0209*	0.0001*	0.0001*
Lack of fit	0.0763 <sup>ns</sup>	0.1947 <sup>ns</sup>	0.5790 <sup>ns</sup>
R <sup>2</sup>	0.9519	0.8836	0.8923
Adjusted R <sup>2</sup>	0.9176	0.8603	0.8708
Equation (actual levels)	Y = 67.843 – 2.143A – 0.0227A <sup>2</sup>	Y = 1.67038 – 0.012708A	Y = 24.640 + 0.460A
Significant factor	A, A <sup>2</sup>	A	A
PRESS	119.80	0.030	36.55

\*: significant (p < 0.05); ns: not significant (p > 0.05).

factor did not significantly affect the responses ( $p > 0.05$ ). This indicated that no statistical differences were found between the treatment of 1, 2, and 3 mm thickness. In this thickness range, the responses can be predicted only from the drying temperature (Table 2). In industrial applications, larger slice thickness may be preferred due to less energy and time needed than the processing of thinner slices of CPH.

RSM analysis on the drying rate of CPH showed an increase in drying rate with the increase in the temperature used for drying. No optimum point was found in RSM's contour plot and surface graph (Figure 1). In the drying process, the drying rate is often linear to temperature. It is highly related to the factor of mass transfer as the result of heating treatment (Afolabi & Agarry, 2014). However, in drying agricultural products, high temperature is often associated with the degradation of structure and nutritional properties.

Drying temperature should be decided appropriately depending on the commodity. Extreme loss of weight due to excessive drying will incur significant economic loss (Jayas & Singh, 2011). A temperature of 65-95°C was used in the previous report on the drying of CPH. Those studies showed that the drying treatment at the highest temperature (95°C) shortened the drying period to only 3 hours (Kusuma *et al.*, 2019). The use of artificial drying is convenient. It could ensure uniform drying performance and shorten the drying period due to its constant temperature. The use of artificial drying in high-value products of cocoa has been reported. The drying temperature of 60°C has been reported to be viable for cocoa beans (Dzelagha *et al.*, 2020). However, in drying bulky and low-value agricultural products, such as by-products, the use of lower temperatures with no interference from artificial dryings, such as sun-drying, is preferred due to its low cost.

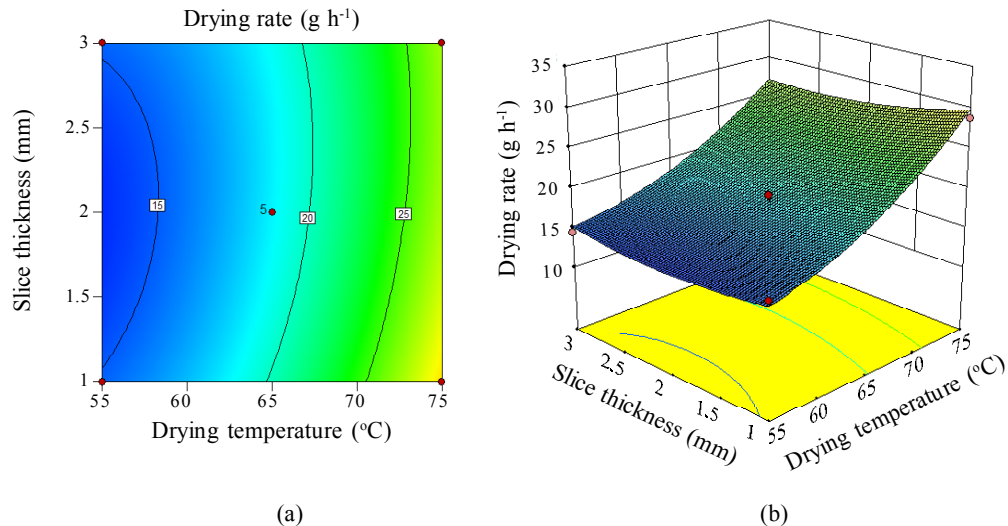


Figure 1. RSM plot of drying rate responses: contour plot (a), 3D surface (b).

In terms of the antioxidative properties of the CPHE, the RSM analysis on the TPC and IC<sub>50</sub> were presented in Figures 2 and 3. The TPC value was significantly higher in lower temperature drying, shown in the orange area in RSM's contour and surface plot (Figure 1). The highest TPC value was 0.971 mg GAE g<sup>-1</sup>. Higher temperature drying promoted the reduction of TPC. This could be associated with the reduction of flavan-3-ol due to heat-induced degradation. The cocoa plant's parts are rich in flavan-3-ols, such as epicatechin and catechin. Phenolics are thermally-unstable. A similar result has been reported in the study of Ghafoor *et al.* (2019). In cocoa products, there were several factors affecting the composition of phenolics. High moisture content promotes the activity of polyphenol oxidase.

On the other hand, high-temperature drying promotes the degradation and polymerization of phenolics. The drying treatment should be done carefully to inhibit PPO's activity while also preventing phenolics' degradation. In this study, we found that drying at 51 and 55°C effectively produce CPH with high phenolics compound.

Pearson correlation analysis showed that the value of TPC and IC<sub>50</sub> of the CPHE were significantly related ( $r = 0.81, p < 0.05$ ). This indicated that their phenolic compounds contributed to the antioxidant properties of CPHE. Phenolic compounds are antioxidants. They can react with free radicals. Phenolic compounds such as flavan-3-ols were reported to exhibit health benefits (Febrianto *et al.*, 2021). This study obtained high antioxidant properties in drying with temperature of 51–55°C (Figure 3). The slice thickness of the CPH may also contribute to this. The previous report showed that sun-drying promoted the reduction of antioxidant activity, mainly due to enzymatic activity during a long drying duration (Sartini *et al.*, 2017). In this study, the thickness used was considered smaller than the previous study by Kusuma *et al.* (2019), which used 1 cm of thickness. The thinner slice resulted in a higher surface area, resulting in a better drying rate and inactivation of PPO enzymes. Furthermore, any temperature of drying in the range of thickness used in this study produced the dried product with a nice brown appearance.

Principal component analysis of the antioxidant properties of the CPHE showed

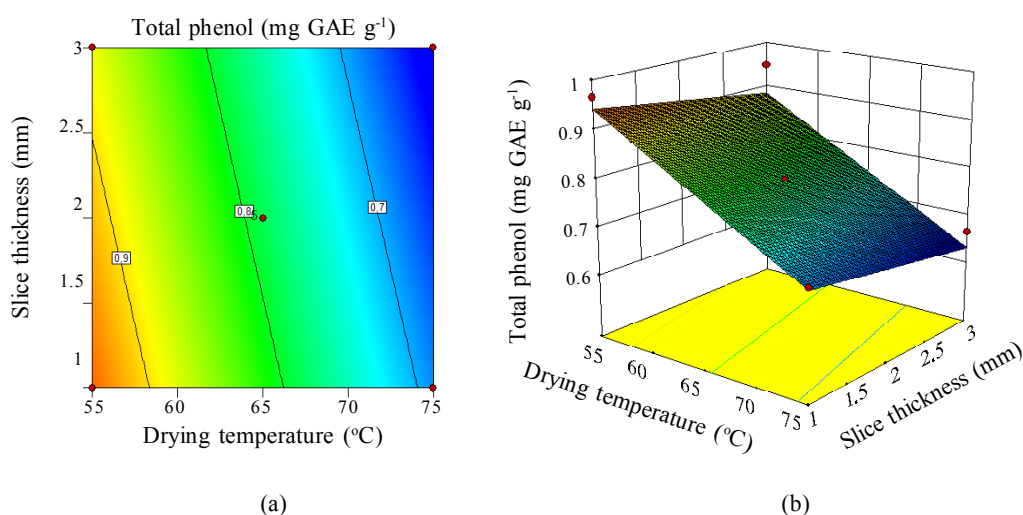


Figure 2. RSM plot of total phenolic content responses: contour plot (a), 3D surface (b).

that the drying rate had a negative correlation to that of TPC while positively correlated to that of IC<sub>50</sub>. Low drying rate samples were located in quadrants II and III (QII and QIII). Medium drying rates were located in quadrant IV (QIV). Surprisingly, sample 7 (65°C, 0.6 mm) had high TPC content despite its relatively high drying rate. This showed that thinner slice promotes more efficient drying of CPH. This is reasonable since thinner slice provided more surface area, resulting in the improvement of the drying process. Clear group separation between drying temperature of 51-55 and that of 65-79°C was evaluated from the PCA

biplot (Figure 4). A distance between samples from 55°C (QII) and 65°C (QIV) showed that a 10°C difference resulted in significant differences in phenolic concentration. A temperature of 55°C could be the maximum temperature for drying without triggering heat-induced degradation of phenolic when using the slice thickness of 1 to 3 mm. On the other hand, thinner slices (0.6 cm) may be combined with higher temperature drying (65°C) to perform quick drying, effectively preventing PPO activity while limiting the time of heat-induced degradation of phenolics.

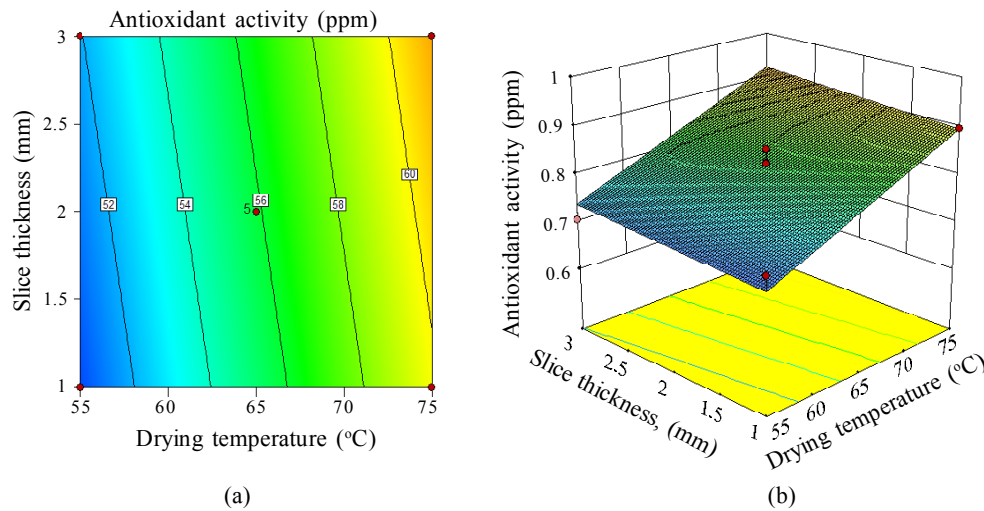


Figure 3. RSM plot of antioxidant activity responses: contour plot (a), 3D surface (b).

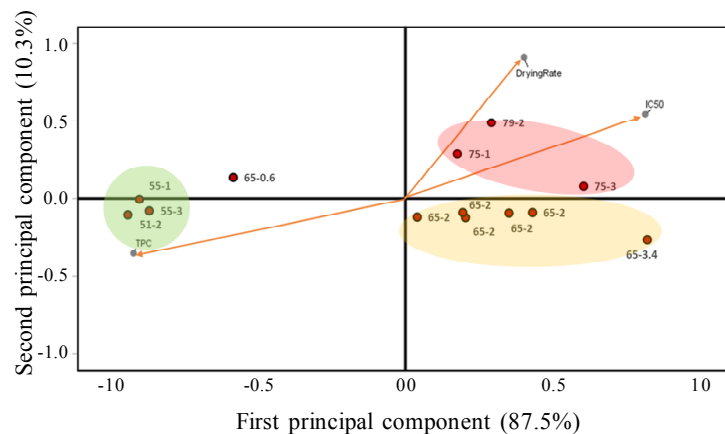


Figure 4. PCA biplot for antioxidant properties of CPHE on different level of drying treatment

## Importance of Drying Process of CPH in Cocoa Industry

The drying process is an important preservation step in agricultural products. In industries, the drying method holds significant importance in the cost increase due to its high energy requirement. In the cocoa industry, cocoa plantations are managed mainly by smallholders. Drying is usually performed by sun-drying due to its low cost. In government-managed plantations, drying can be done artificially. However, sun-drying is the most economical drying method for cocoa and cocoa-related products, if possible.

The choice of drying method is also highly related to the climate condition. In Indonesia, the harvest seasons of cocoa mainly occur in July-August and November-December. July to August is a transition period from a dry to a rainy season in Sumatra and Java (BMKG, 2022). This resulted in an uncertainty of sunshine and a rainy period. In this period, sun-drying can still be done to dry CPH efficiently for a limited time.

On the other hand, November-December is a rainy season in most areas of Indonesia. In some parts of Sumatera and mainly in Java, this period is the peak of the rainy season. High moisture conditions are preferred for microbial and enzymatic activities. In the cocoa industry, the drying of cocoa beans will most likely become the main priority. Hence, the CPH may be ignored and left untreated. Some of the untreated CPH will be used as organic fertilizer. This is a waste of potential agricultural by-products.

The drying of CPH should be done effectively, considering the weather condition of the cocoa harvest season. This includes the cutting of CPH into thin slices and the use of relatively high temperatures. In this study, we found that temperatures of 51 to 55°C were sufficient to obtain high-quality CPH. The drying time varied from 5 to 13 hours.

This duration is feasible even for the thickest slice and lowest temperature used in this study (3–3.4 mm). However, this temperature is not achievable by direct sun-drying (Dharma *et al.*, 2020). Modified solar drying techniques such as drying houses and closed solar drying have been reported to achieve a higher temperature. A previous report by Widyotomo (2014) showed that drying house temperature can rise to 52°C in a full sunshine period. Effective drying can be achieved by doing size reduction treatment (slicing) before drying. In the industrial scale of cocoa processing, the cutting of CPH can be done using a commercial slicer. Commercial slicers for various agricultural products, such as cassava, can slice with a thickness of 0.8 to 1.5 mm. These slices can then be dried directly under the sun, preferably in a facility optimized for solar drying. This method will surely improve the drying process of the CPH, thus enhancing its potential for other industrial use.

The relation between the antioxidant activity of CPH and the genotype factor has yet to be studied. Three major cocoa groups are currently cultivated in Indonesia, namely Criollo, Trinitario, and Forastero. A previous study by Febrianto & Zhu (2019) showed that cocoa beans of various genotypes had diverse phenolic compositions, affecting their antioxidant properties. Furthermore, the safety aspect of CPH utilization should be studied more. Cocoa cultivation has been known to be chemical-intensive due to various pests and diseases. The use of the chemical may leave residues on CPH, thus affecting the safety of its consumption. The study on these topics is important to strengthen the body of knowledge on CPH utilization. Using CPH from an organic-based farm would be the best option for current utilization.

The finding of this study is essential to open the possibility of CPH utilization as a food ingredient. Further study on the utilization



of CPH powder should be carried out. Another study on the nutrients and non-nutrient aspects of CPH is essential to optimize its utilization as a food ingredient further. Considering the relatively high content of phenolics in the CPH ( $>0.85$  mg GAE  $g^{-1}$ ) obtained by drying up to  $65^{\circ}C$ , CPH powder may be suitable as the ingredient in the formulation of a functional food product.

## CONCLUSIONS

The study showed that drying on the temperature up to  $65^{\circ}C$  combined with proper size reduction prior to drying could result in efficient drying of CPH. This drying technique could shorten the drying of CPH to less than 13 hours. This method produced CPH powder with a light-brown appearance and high concentration of phenolic compounds ( $>0.85$  mg GAE  $g^{-1}$ ). The use of temperature of more than  $65^{\circ}C$  was not preferable since it induced the reduction of phenolics. The use of slice thickness less than 3 mm is not recommended due to its insignificant effect on the drying rate. This method could be used as an alternative of CPH drying to produce dried CPH powder with high anti-oxidant activity.

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