Effects of Postharvest Storage Temperature on Physical Characteristic, Phenolic Compounds, and Antioxidant Activity of Cocoa Pod Husk

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ABSTRACT

Cocoa pod husk (CPH) is a good source of bioactive components such as phenolic compounds, which are known to have potential as antioxidants. Research on the effects of postharvest storage conditions on bioactive compounds of CPH is still limited. Therefore, this study aimed to evaluate the impact of postharvest storage temperature (20, 30, and 40 °C) and time on the physical characteristics, phenolic compounds, and antioxidants of CPH. The results indicated that storage for nine days reduced the color and moisture content of CPH while the texture hardened (especially at 20 and 40 °C). Total phenolic compounds (TPC) increased to 97.9% when CPH was stored at 20 °C for nine days, which was associated with an increase in catechin content and antioxidant activity. The first-order kinetic model was suitable for predicting the kinetics of changes in color, moisture content, hardness, TPC, and DPPH. The calculated Ea values indicated that the storage temperature caused the color of CPH to change easily to brown and the catechin and caffeic acid compounds to form easily. Overall, it is better to store CPH at 20 °C for nine days to increase the quality.

Keywords: Antioxidant activity; Cocoa pod husk; Phenolic compounds; Physical characteristic; Postharvest storage

INTRODUCTION

Cocoa, *Theobroma cacao* L., is the most widely cultivated plantation commodity for commercial purposes compared to 22 other Theobroma genera (World Agriculture, 2011). In 2020/2021, the total world cocoa production is estimated at 5.024 million tons (International Cocoa Organization (ICCO), 2020). The area for cocoa production in Indonesia in 2019 reached 1,560,944 Ha, and 98% of it was smallholder plantations (Directorate General of Estate Crops, 2020). The amount of cocoa processed for commercial needs in the agro-industrial process will also impact the amount of cocoa pod husk (CPH) waste produced. CPH must be utilized because they are a source of phytochemical components that can provide health benefits (from now on referred to as nutraceutical components) (Campos-Vega et al., 2018). In addition, if the husk of the cocoa pod is not used, it will trigger the growth of diseases, such as black pot rot, which can interfere with the production of cocoa plants due to the unpleasant odor released. Generally, the husk of a ripe cocoa pod will be yellow. Then, the CPH can change

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color to brown due to drying during the postharvest process (Kamelia & Fathurohman, 2017).

CPH is known to have nutraceutical components, such as minerals, pectin, dietary fiber, and phenolic compounds (Yapo et al., 2013). Dietary fiber and phenolic compounds are the most commonly found among the four nutraceutical components (Rojo-Poveda et al., 2020). The high total dietary fiber contained in CPH can be useful for preventing colon disorders and reducing the risk of coronary heart disease and type 2 diabetes (Elleuch et al., 2011). In addition, phenolic compounds, especially in CPH, are compounds known to have antioxidant properties (Lateef et al., 2016). The total dietary fiber in the CPH can be used as a source to make antioxidant-rich fiber-rich foods so that it can compensate for today's modern foods that are vulnerable to free radical interference (Yapo et al., 2013). Some phenolic compounds in fresh CPH include catechins, quercetin, epicatechin, gallic, coumaric, and protocatechuic acid (Valadez-Carmona et al., 2017). The presence of these phenolic compounds also makes CPH have added value for health compared to other dietary fiber sources (Perez et al., 2015). However, the content of phenolic compounds in CPH can be influenced by several factors, such as postharvest processing and storage.

Postharvest processes such as drying methods have been carried out on CPH, and the results can affect the bioactivity of their phytochemicals (including phenolic compounds and dietary fiber) (Nieto-Figueroa et al., 2020; Valadez-Carmona et al., 2017). Meanwhile, research related to storage temperature conditions of fresh CPH has not been carried out, especially on the phenolic content and their antioxidant activity. The proper storage condition greatly determined the success of suppressing damage to phenolic compounds and antioxidant properties in dried *Piper betle* extract (Ali et al., 2018). Other research (Sun et al., 2022) stated that storage at low temperatures could successfully prevent quality loss and increase the amount of a bioactive component (γ-aminobutyric acid) in coffee leaves. In addition, the content of phenolic compounds (anthocyanins) in red oranges can also increase if the fruit is stored at low temperatures (Piero et al., 2005).

The antioxidant potential of phenolic compounds can decrease partly because of the heat factor. Storage at temperatures that are too high can negatively affect the fruit's physical characteristics (weight, size, color, and shape) (Balasooriya et al., 2020). Besides, storage at high temperatures can degrade the phenolic compounds contained in the material, thereby decreasing the antioxidant activity and shelf life (Kim et al., 2018). Meanwhile, storage at low temperatures may effectively inhibit microbial growth and browning reactions, preventing the quality deterioration of fresh fruits and vegetables (Wong et al., 2021; Zhong et al., 2023). Changes in food quality, such as color, enzyme activity, and bioactive substances during storage and processing, can be assessed kinetically (Ling et al., 2021). In this case, kinetic parameters can effectively predict changes in CPH quality during postharvest storage. Since there is no information regarding the maximum postharvest storage temperature of CPH, which can reduce the damage to its bioactive components, this study aimed to evaluate the effect of different storage temperatures during storage time on physical characteristics, levels of phenolic compounds, and antioxidant activity of CPH. Additionally, the kinetics of changes in these quality parameters were investigated. This study will give valuable information for choosing the ideal postharvest storage conditions for CPH.

MATERIALS AND METHOD

Materials

This research used cocoa pod husk (CPH) of the MCC-02 cocoa variety as a sample obtained from the Sido Dadi Farmer Group in Gunung Kidul Regency, D.I. Yogyakarta, Indonesia, in September 2022. The chemicals used in this study included HPLC grade methanol, glacial acetic acid, Folin–Ciocalteu reagent, methanol, and Na_2CO_3, which were from Merck (Darmstadt, Jerman), and catechin, caffeic acid, gallic acid and DPPH, which were from Sigma-Aldrich (St. Louis, MO, AS).

Preparation of Cocoa Pod Husk

The Cocoa pod was cut into two halves to remove the beans and pulp. The CPH obtained was then washed with water three times to remove the remaining mucus. The CPH were stored at different temperatures of 20, 30, and 40 °C for 1, 3, 5, 7, and 9 days in a storage box designed to use lights to regulate the temperature at 30 and 40 °C, respectively, while the storage area at 20 °C used air-conditioned room. Then, CPH were tested for their physical characteristics before drying at 40 °C for 24 hours. The dried CPH was then ground and sieved using an 80 mesh sieve (Miranda et al., 2020) and stored under moisture-free conditions using a desiccator until chemical analysis.

Physical Characteristics of Cocoa Pod Husk

Physical characteristics measured on the stored CPH include color, texture (hardness), and moisture content. The surface color was measured by a chromameter (Konica Minolta, Japan) performed in the CIE L*a*b* system, and the total color difference ΔE was calculated according to Eq. (*1*).

$$\Delta E = \sqrt{(L_1^* - L_0^*)^2 + (a_1^* - a_0^*)^2 + (b_1^* - b_0^*)^2} \quad (1)$$

L_1^*, a_1^*, and b_1^* are the color indexes of samples at different storage temperatures; L_0^*, a_0^*, and b_0^* are the color indexes of fresh CPH. The hardness test was performed by placing CPH on the universal testing machine Zwick/Z0.5 (Zwick Roell, German) and then giving a compression pressure of 10 mm/minute until it deformed. The moisture content was determined gravimetrically according to AOAC (2005), in which one gram of stored CPH was dried at 105 °C for 24 hours to determine the moisture content. All the physical characteristics measurements were performed in three replicates.

Cocoa Pod Husk Chemical Analysis

Firstly, the dried CPH was extracted with 96% ethanol in a 1:20 ratio (w/v) at room temperature for 48 hours in the dark (Dwipayanti et al., 2020). Subsequently, the solvent was evaporated at 40 °C at a pressure of 175 bar until a thick extract was obtained. Then, chemical analysis was carried out in three replicates, including total phenolic compounds (TPC) measurements, DPPH scavenging assay, and identification of phenolic compounds by high-performance liquid chromatography-diode array detector (HPLC-DAD).

TPC of CPH extract was determined by following the Folin-Ciocalteu reagent assay described by Sakanaka et al. (2003). A total of ± 0.01 g of sample was dissolved in 85% methanol using a 5 mL

volumetric flask, homogenized, and centrifuged at 3000 rpm for 15 minutes until the supernatant was obtained. The supernatant was filtered to obtain a filtrate. 50 µL of the filtrate was pipetted, then 350 µL of 85% methanol and 400 µL of Folin–Ciocalteu reagent, vortexed until homogeneous and incubated for 6 minutes before 4.2 mL of 5% Na_2CO_3 solution was added. The samples were incubated for 30 minutes at room temperature before reading the absorbance value at a wavelength of λ 760 nm. A standard curve was prepared using gallic acid (0.01 g/100 mL) with concentrations in series 10, 20, 40, 60, 80, and 100 mg/L. Results were expressed as mg gallic acid equivalent (GAE)/ g sample on dry weight.

The antioxidant capacity was evaluated using the DPPH method described by Blois (1958). 50 µL of extract diluted in 99.9% MeOH was added with 450 µL MeOH and 3.5 mL DPPH (0.0039 g in 99.9% methanol solvent 100 mL), then mixed and stored in dark condition at room temperature for 30 min. The absorbance was measured at 517 nm by a spectrophotometer UV-Vis 10S (genesis). A calibration curve was prepared using a standard of gallic acid (0.01 g/100 mL) with concentrations series of 0, 2.5, 5, 10, and 20 ppm. Results obtained from triplicate analysis were expressed as mg gallic acid equivalent (GAE)/ g sample on dry weight.

Phenolic compounds contained in CPH extract were identified using HPLC-DAD (Shimadzu Corp., Kyoto, Japan), as Thorvaldsson et al. (2022) described. A sample of 1.5 mL was taken and filtered with a 0.45 µm nylon filter and then injected into the HPLC-DAD (Shimadzu Corp., Kyoto, Japan). Phenolic compounds were separated on a C18 reverse phase column (5 µm, 4.6 × 150 mm). The mobile phase consists of two solvents: A: 2% acetic acid and 5% methanol in water and B: 2% acetic acid and 88% methanol in water. The gradient profile was as follows (time, % solvent B): 0 min, 20%; 15 minutes, 100%; and 18 minutes, 100% at a flow rate of 1 mL/minute. The column temperature was set at 30 °C. DAD (diode array detector) performs a comprehensive spectrum scan (200-400 nm) to identify phenolic compounds. Compounds found in the samples were identified by comparing their retention times and spectra with the standard compounds catechin and caffeic acid. Specific wavelengths were used at maximum absorbance for the corresponding compounds for quantification: 280 nm for catechin and 320 nm for caffeic acid. A standard compound concentration of 100 ppm was used to measure compound concentrations.

Kinetics Analysis

The zero-order and first-order kinetic models (Eqs. (2) and (3)) were used to describe the reaction rate of quality changes in physical, phenolic components, and antioxidants of CPH during storage (Deng et al., 2022; Sun et al., 2022).

$$\text{Zero - order: } f(x) = f(x_0) - kx \quad (2)$$

$$\text{First - order: } f(x) = f(x_0)\exp(-kx) \quad (3)$$

Where x is the storage time; f (x_0) is the initial quality value of the sample; f (x) is the quality value of the sample at time x; k is the reaction rate constant. The Arrhenius equation (Eq. (4)) was used to calculate the activation energy (Ea) of the sample quality.

$$k = A \times \exp(-\frac{Ea}{RT}) \quad (4)$$

Where A is the pre-exponential factor; R is the gas constant, 8.314 J/(mol·K); Ea is the activation energy, J/mol; T is the absolute temperature, K.

Statistical Analysis

The results were expressed as the mean of triplicate analysis and standard deviation. One-way analysis of variance was performed to determine whether there was a significant difference ($p < 0.05$) using Duncan's test by SPSS software (version 25.0, IBM, SPSS Inc.).

RESULTS AND DISCUSSION

Effects of Postharvest Storage on Cocoa Pod Husk Physical Characteristics

Color of Cocoa Pod Husk

Storage time had a significant effect ($p<0.05$) on cocoa pod husk (CPH) color, while differences in storage temperature did not change significantly ($p>0.05$) (Table 1). All color parameters (L*, a*, b*) decreased during storage. The L* (Lightness) value decreased by 54.5%, 55.3%, and 55.5% at 20, 30, and 40 °C, respectively, until the end of the storage time, indicating that the skin color of CPH became darker. The a* values decreased by 43.0%, 43.7%, and 44.1% at 20, 30, and 40 °C, respectively, after 9 days, indicating a change in a red component of the chromatic diagram (L*, a*, b*) in the red-green color scale. Meanwhile, a decrease in the value of b* (yellowness) by 83.4%, 88.6%, and 84.1% at 20, 30, and 40 °C, respectively, after 9 days, originated a change in the yellow component. Overall, the husk color of the cocoa pods, which was initially yellow, gradually changed to brown until the end of storage time with an average total color change (ΔE) of 50.77. The discol-

Table 1. Effects of postharvest storage on color parameters of CPH

Temperature	Storage (d)	L*	a*	b*	ΔE
-	FCPH*	56.42 ± 0.54^{a}	22.47 ± 2.50^{a}	45.03 ± 0.60^{a}	0.00 ± 0.00^{a}
20 °C	1	53.14 ± 2.70^{ab}	20.72 ± 3.69^{a}	43.83 ± 0.51^{a}	5.68 ± 1.02^{b}
	3	40.95 ± 2.21^{c}	17.54 ± 2.65^{b}	31.14 ± 1.50^{c}	21.47 ± 1.27^{c}
	5	41.08 ± 2.65^{c}	17.00 ± 1.47^{b}	23.47 ± 0.16^{e}	27.08 ± 2.75^{d}
	7	29.83 ± 0.38^{de}	13.29 ± 0.45^{bc}	12.07 ± 0.59^{fg}	43.34 ± 0.62^{f}
	9	25.67 ± 0.45^{ef}	11.38 ± 0.39^{d}	7.46 ± 0.10^{hi}	49.36 ± 0.46^{g}
30 °C	1	51.17 ± 0.40^{b}	20.50 ± 2.84^{a}	44.07 ± 0.83^{a}	5.79 ± 0.02^{b}
	3	39.26 ± 0.72^{c}	16.36 ± 1.29^{b}	27.75 ± 2.07^{cd}	25.18 ± 1.60^{cd}
	5	37.69 ± 1.20^{c}	15.43 ± 0.98^{b}	15.75 ± 0.54^{f}	35.48 ± 1.38^{e}
	7	29.15 ± 0.41^{def}	13.22 ± 5.21^{bc}	10.26 ± 2.03^{gh}	45.43 ± 2.37^{f}
	9	25.23 ± 1.16^{f}	11.24 ± 1.24^{d}	5.15 ± 0.16^{i}	51.58 ± 0.31^{g}
40 °C	1	51.50 ± 0.38^{b}	18.72 ± 0.59^{a}	39.33 ± 1.96^{b}	8.50 ± 1.57^{b}
	3	31.52 ± 0.78^{d}	15.45 ± 0.29^{b}	24.66 ± 1.27^{de}	32.93 ± 1.43^{e}
	5	28.92 ± 0.72^{def}	14.52 ± 1.16^{b}	14.18 ± 0.58^{fg}	42.10 ± 0.68^{f}
	7	27.79 ± 0.92^{def}	12.17 ± 0.30^{bc}	11.71 ± 0.61^{fg}	45.12 ± 1.10^{f}
	9	25.11 ± 0.29^{f}	11.16 ± 0.22^{d}	7.14 ± 0.12^{hi}	50.44 ± 0.14^{g}

Values are means ± standard deviation. The values followed by different letters indicate a significant statistical difference ($p < 0.05$). *FCPH represents fresh husks without storage, L* represents the brightness level, +a* represents redness, and +b* represents yellowness.

oration of the CPH was caused by cutting the cocoa pod tissue, leading to an enzymatic browning reaction. Enzymatic browning may occur due to polyphenol oxidases (Sun et al., 2022). Similar research was also reported by Guo et al. (2020), stating that oxidation of phenolic compounds was observed on areca nut seeds during storage.

Meanwhile, there were no significant differences in the L*, a*, and b* values of CPH between storage temperatures of 20, 30, and 40 °C at the same storage time (Table 1). The results obtained in this study were similar to research on storage conditions of strawberries conducted by Ayala-Zavala et al. (2004), reporting that storage temperature did not affect the color of the fruit. Still, there were differences in the total phenolic content of the fruit.

Hardness and Moisture content of Cocoa Pod Husk

The hardness of CPH tended to increase significantly ($p<0.05$) at 20 and 40 °C but not at 30 °C (Figure 1A). At 30 °C, the CPH hardness was relatively stable or even tends to decrease. This phenomenon was consistent with the moisture content at each storage temperature (Figure 1B). The moisture content at temperatures of 20 and 40 °C decreased by 84.8% and 94.4%, respectively, while at a temperature of 30 °C, it decreased by 55.4% during 9 days of storage. In line with the findings of Miranda et al. (2014), this study showed that the hardness of CPH increased while moisture content decreased during storage. The hardness behavior is consistent with that observed in beans after storage and linked to texture changes caused by a variety of chemical processes, including lipid oxidation, insoluble pectate production, protein-starch interaction, phytate loss, and changes to the cotyledon cells connections and cell wall constituents (Alves et al., 2021; Demito et al., 2019). However, those compounds were not identified in this experiment.

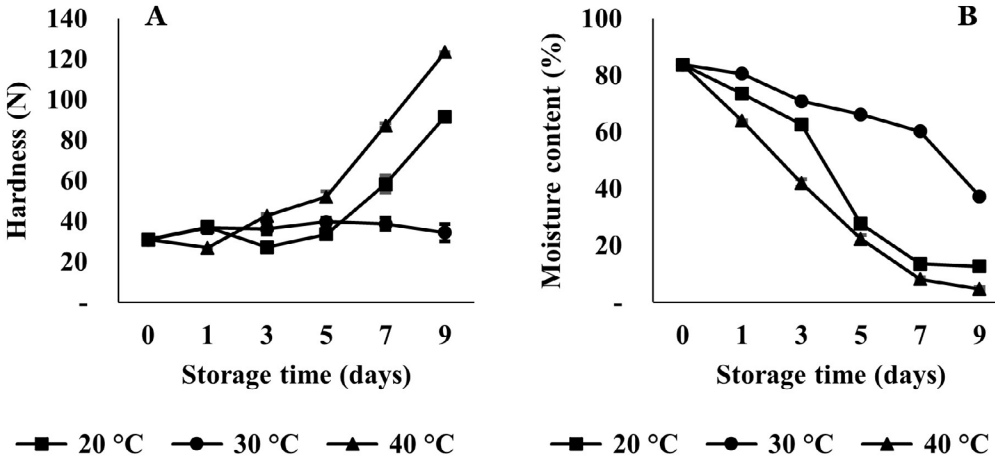

Figure 1. Changes in physical characteristics of CPH during storage. A) Pod husk hardness, N, Newton; B) moisture content.

Based on visual observations, during storage at 30 °C, fungal growth occurred after the third day of storage, which was marked by the appearance of small dark lesions and white layers (Figure 2). However, at both 20 and 40 °C storage, no fungal growth was found until the ninth day. It was strongly suspected that the small hardness value at 30 °C was caused by fungal growth converting the components of cocoa into simpler molecules. Pathogenic fungi can secrete pectinase enzymes

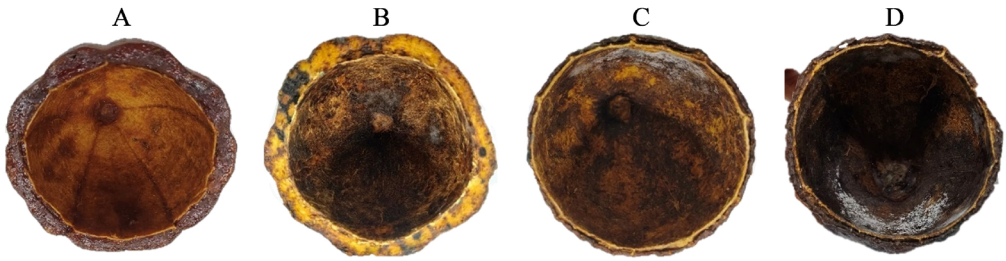

Figure 2. Fungal contamination of CPH at 30 °C. A) Fresh CPH; B) fifth day of storage; C) seventh day of storage; D) ninth day of storage.

responsible for breaking down intercellular tissue, with the highest activity obtained at 30 °C (Zhang et al., 2018). Unfortunately, in this study, identification of the type of fungus that grew was not carried out. According to Guest (2007), *Phytophthora palmivora* is a common decomposing fungus found on CPH with symptoms beginning with small, hard, dark spots on the pod, and the optimum temperature is between 29 and 30 °C (Brasier & Griffin, 1979). This fungus is a type of microbe that reforms components by producing water (Wang et al., 1979), thus affecting the increase in moisture content in CPH to be higher than other storage temperatures.

The increase in hardness value may be caused by lignification of CPH during storage, as reported by Liu et al. (2023). The study revealed that the hardness of pomegranate seeds increased with longer storage time due to lignification. Meanwhile, the moisture content decreased at temperatures above 20 °C because higher temperatures accelerated the rate of gas exchange and caused materials to be more susceptible to water loss.

In addition, storage at 30 °C for 9 days had no significant effect ($p>0.05$) on the hardness of the CPH. This showed that the cocoa pod tissue did not undergo significant morphological changes at 30 °C. The results obtained were similar to studies on the storage of areca nuts (Pan et al., 2021), reporting that between temperature variations of 5, 10, and 25 °C, the storage temperature of 10 °C did not cause significant morphological changes in areca nuts, while at 25 and 5 °C, lignification occurred in the areca bean mesocarp tissue. An increase in lignin content was caused by natural aging and cold stress, respectively. Besides, the high moisture content of CPH at 30 °C affected the growth of fungi on the skin surface, which was observed from the fifth day of storage. Other research also stated that the water available in the sample contributed to mold growth on the surface and caused damage so that the shelf life was short (Guimarães et al., 2021). Thus, it can be said that CPH stored at 30 °C had a short shelf life due to physical damage caused by microbial growth.

TPC and DPPH Scavenging Activity

CPH was stored at 20 to 40°C for 9 days to investigate the impact of storage temperature on TPC and antioxidant activity (Table 2). The total phenolic content (TPC) of fresh CPH in this study was 55.96 mg GAE/g. This value was lower than that reported by Yapo et al. (2013), which was around 60 mg GAE/g, and Dwipayanti et al. (2020) of 146.67 mg GAE/g, but higher than that reported by Valadez-Carmona et al. (2017), which was 3.24 mg GAE/g. Those TPCs were due to differences in growing conditions, climate, varieties (Dragovic-Uzelac et al., 2007), storage, and drying methods (Deng et al., 2019).

Table 2. Total phenolic compounds and antioxidant activity of CPH phenolic extracts during storage period

Temperature	Sample	TPC (mg GAE/g)	DPPH (mg GAE/g)
-	FCPH*	55.96 ± 4.59^{ab}	107.66 ± 0.41^{c}
20 °C	1	58.26 ± 2.29^{ab}	169.52 ± 2.85^{e}
	3	182.11 ± 2.29^{bc}	187.43 ± 3.66^{f}
	5	351.83 ± 2.29^{de}	364.86 ± 0.41^{j}
	7	1278.44 ± 20.64^{h}	594.80 ± 7.33^{m}
	9	2773.85 ± 144.50^{j}	815.37 ± 0.81^{o}
30 °C	1	108.72 ± 2.29^{abc}	139.81 ± 0.81^{d}
	3	237.16 ± 6.88^{cd}	303.41 ± 2.44^{i}
	5	459.63 ± 41.28^{e}	225.28 ± 5.70^{g}
	7	168.35 ± 2.29^{bc}	68.59 ± 1.22^{b}
	9	19.27 ± 9.17^{a}	4.70 ± 0.81^{a}
40 °C	1	163.76 ± 2.29^{bc}	174.40 ± 4.48^{e}
	3	434.40 ± 2.29^{e}	292.02 ± 4.88^{h}
	5	656.88 ± 18.35^{f}	385.21 ± 0.41^{k}
	7	863.30 ± 50.46^{g}	423.87 ± 0.81^{l}
	9	1434.40 ± 11.47^{i}	608.63 ± 1.63^{n}

Values are means ± standard deviation. The values followed by different letters indicate a significant statistical difference ($p < 0.05$).
*FCPH represents fresh husks without storage.

Table 3. Catechin and caffeic acid (ppm) content under different storage temperatures for 9 days

Storage Time (Days)	Storage Temperature					
	20 °C		30 °C		40 °C	
	Catechin	Caffeic acid	Catechin	Caffeic acid	Catechin	Caffeic acid
FCPH*	0.10 ± 0.01^{a}	9.12 ± 0,11^{a}	0.10 ± 0.01^{a}	9.12 ± 0.11^{a}	0.10 ± 0.01^{a}	9.12 ± 0,11^{a}
1	13.02 ± 0.13^{b}	16.64 ± 0,80^{b}	0.76 ± 0.67^{ab}	9.82 ± 0,05^{ab}	20.21 ± 0.62^{b}	73.33 ± 1,72^{b}
3	26.36 ± 0.39^{c}	69.44 ± 0,54^{e}	4.47 ± 0.69^{c}	37.87 ± 1,16^{c}	21.68 ± 1.87^{b}	73.97 ± 2.06^{b}
5	30.78 ± 3.55^{c}	63.40 ± 2,58^{d}	27.67 ± 0.49^{d}	43.00 ± 0,13^{d}	52.06 ± 1.41^{c}	76.59 ± 0,69^{b}
7	56.93 ± 2.58^{d}	60.21 ± 1,23^{d}	1.97 ± 0.04^{a}	11.91 ± 0,12^{b}	51.19 ± 1.16^{c}	74.51 ± 5,53^{b}
9	67.95 ± 0.83^{e}	36.13 ± 0,41^{c}	0.62 ± 0.42^{ab}	11.93 ± 1.18^{b}	60.99 ± 0.15^{d}	75.80 ± 6.63^{b}

Values are means ± standard deviation. The values followed by different letters indicate a significant statistical difference ($p < 0.05$).
*FCPH represents fresh cocoa pod husks without storage.

During storage at 20 and 40 °C, TPC increased significantly ($p<0.05$) by 97.9% and 96.1%, respectively, while at 30 °C it showed fluctuating value. This increase was thought to be due to the activity of indigenous enzymes during storage. These enzymes may be able to release soluble phenolics from bound phenolic compounds. This enzymatic reaction was likely to form intermediates during phenolic oxidation, contributing to high antioxidant activity (Ihns et al., 2011). The same thing was reported in a study on the storage of white rice (Walter & Marchesan, 2011), that the concentration of free phenolic acids in white rice increased significantly during storage due to the enzymatic release of bound phenolic acids.

Based on HPLC analysis, there was an increase in the content of catechin by 99.84% and 99.82% at 20 and 40 °C, respectively, while caffeic acid showed fluctuating values (Table 3). The change in phenolic compounds was due to polyphenol oxidase activity. Polyphenol oxidase produced catechins and lignin via the phenylpropanoid pathway (Campos-Vega et al., 2018), so the levels in Cof increased. Meanwhile, lignin formation involved the formation of intermediate substances such as

caffeic acid (Ihns et al., 2011), so levels of CPH fluctuated.

In addition, the TPC obtained on days 7 to 9 at 40 °C was less than that obtained at 20 °C. This was because phenolic compounds were more sensitive to high temperatures due to their unstable structure (Ali et al., 2018). However, in this study, a storage temperature of 30 °C showed a much lower TPC than a temperature of 40 °C because the growth of fungus on the surface of the CPH, which was observed from the 5th day of storage, was thought to have contributed to the decrease in phenolic compounds as a result of physical damage caused.

The change in antioxidant activity is in line with the change in TPC. The higher the concentration of phenolic compounds contained in CPH, the more electrons can be donated to neutralize free radicals to increase the antioxidant activity. Kohartono et al. (2014) also found that the ability of black rice to capture DPPH free radicals tended to increase during storage. Thus, storage at 20 °C for 9 days was the best condition to produce the highest TPC of 2773.85 mg GAE/g and to maximize the antioxidant potential of CPH.

Kinetics of Changes in The Quality Parameters of Cocoa Pod Husks

All of the observed parameters in this study were subjected to zero-order and first-order kinetic simulations (Table 4) to describe the reaction rate of quality changes in CPH during storage. The

Table 4. Zero-order kinetic, first-order kinetic, and activation energy (Ea) of the changes in color, moisture content, hardness, TPC, and antioxidant activity of CPH stored under different temperatures and times

Parameter	T (°C)	Zero-order kinetics			First-order kinetics			Ea (kJ/mol)
		k	R^2	ΣR^2	k	R^2	ΣR^2	
L*	20	3.42	0.96		0.09	0.96		
	30	3.92	0.91	2.67	0.09	0.92	2.73	1.38
	40	3.46	0.80		0.09	0.85		
a*	20	1.20	0.98		0.07	0.97		
	30	1.46	0.93	2.82	0.08	0.94	2.87	0.11
	40	1.16	0.91		0.07	0.96		
b*	20	4.49	0.99		0.20	0.96		
	30	6.26	0.97	2.89	0.22	0.96	2.92	0.37
	40	4.30	0.93		0.21	0.99		
ΔE	20	-5.64	0.98		-0.38	0.79		
	30	-7.40	0.98	2.85	-0.68	0.86	2.32	0.04
	40	-5.61	0.89		-0.36	0.67		
MC	20	8.75	0.94		0.24	0.94		
	30	3.66	0.98	2.86	0.05	0.98	2.91	10.93
	40	8.82	0.94		0.33	0.98		
Hardness	20	-5.88	0.69		-0.11	0.69		
	30	-1.42	0.73	2.32	-0.04	0.71	2.36	14.80
	40	-10.24	0.90		-0.17	0.96		
TPC	20	-272.55	0.79		-0.45	0.97		-12.34
	30	-80.00	0.97	2.73	-0.41	0.98	2.85	
	40	-142.48	0.96		-0.32	0.89		
Catechin	20	-7.28	0.97		-0.53	0.58		
	30	-5.31	0.81	2.67	-1.07	0.97	2.06	-4.73
	40	-6.47	0.90		-0.51	0.51		
Caffeic acid	20	-3.72	0.26		-0.15	0.41		
	30	-7.72	0.90	1.53	-0.36	0.99	1.76	-1.06
	40	-4.68	0.37		-0.15	0.35		
DPPH	20	-77.69	0.94		-0.22	0.98		
	30	-29.16	0.54	2.45	-0.18	0.64	2.54	-9.36
	40	-51.32	0.97		-0.17	0.92		

Note: k, rate constant; R^2, coefficient of determination; ΣR^2, the sum of R^2

higher the value of the reaction rate constant k indicated a faster rate of changes in quality. By looking at the higher value of ΣR^2, it can be determined that all parameters were fitted better with first-order kinetics, except ΔE. The activation energy (Ea) values of L*, a*, b*, ΔE, moisture content, hardness, TPC, catechin, caffeic acid, and DPPH were 1.38, 0.11, 0.37, 0.04, 10.93, 14.80, -13.03, -4.73, -1.06, and -9.36 kJ/mol, respectively. Zhou et al. (2009) explained that the reaction rate was quick if the predicted Ea was less than 40 kJ/mol.

Subsequently, the negative value of Ea represents an exothermic reaction due to the formation of phenolic compounds. It can be said that catechin and caffeic acid compounds tend to form while CPH is stored because the Ea value was small. The total color change (ΔE) parameter of CPH also showed the smallest activation energy value, meaning that the color parameter was sensitive to changes. These results were comparable to those found for fruit (Deng et al., 2022) and leaves (Sun et al., 2022), indicating that the color quality of CPH also decreased rapidly during storage.

Even though the color parameter of CPH changed quickly, other parameters, including hardness, moisture content, bioactive components, and their antioxidant properties, were relatively good and stable because the Ea value was large. As it is known, activation energy is the minimum energy needed to result in a chemical reaction (Kumar et al., 2023). Thus, a large Ea value indicated that the component was difficult to change. Therefore, the right storage conditions (temperature and time) should be chosen to preserve the quality of CPH prior to processing. As a source of functional food ingredients, it is preferable to keep CPH at 20 °C for up to 9 days, and it is not recommended to store CPH for more than 5 days at 30 °C due to mold contamination. This study is still limited to the storage time (9 days). It is hoped that future research can use a longer storage time.

CONCLUSIONS

Storing CPH at temperatures of 20, 30 and 40 °C for 9 days could reduce the color and moisture content of CPH while storing CPH at temperatures of 20 and 40 °C for 9 days could increase the hardness and the total phenolic content of CPH by 97.9% and 96.1%, respectively, which was related to increasing catechin levels, thereby increasing their antioxidant properties, while storage at 30 °C for 9 days could reduce both phenolic compound levels and antioxidant activity of CPH caused by fungal contamination. In this study, the physical characteristics, phenolic compounds, and antioxidant activity of CPH after postharvest storage were investigated for the first time. The results also showed that changes in color index (L*, a*, b*), moisture content, hardness, TPC, caffeic acid, and DPPH followed a first-order kinetic model. In contrast, the changes in catechin and ΔE followed a zero-order kinetic model. The smallest Ea value for the color, caffeic acid, and catechin parameters of 0.04, -4.73, and -1.06 indicated that the storage temperature caused the color of CPH to easily change to brown and the catechin and caffeic acid compounds to be easily formed. CPH as a source of quality functional food, and it is preferable to store CPH at 20 °C for 9 days.

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