Pelita Perkebunan 40(3) 2024, 264–274
DOI: 10.22302/iccri.jur.pelitaperkebunan.v40i3.630
ISSN: 0215-0212 / e-ISSN: 2406-9574

Acidification of Cocoa Nibs using Malic Acid to Modify the Color While Preserving the Bioactive Compounds

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Received: November 9, 2024 / Accepted: December 20, 2024

Abstract

The use of unfermented cocoa beans for food products for direct-consumption is limited, even though it contains various health-promoting bioactive compounds. This is due to their excessive bitterness and astringency, and their unattractive appearance. Modifying the color of cocoa powder can be done by alkalization to improve customer preferences. However, this treatment highly reduced the number of bioactive compounds in cocoa powder. An alternative method to modify the appearance of cocoa powder is through an acidification process. In this study, malic acid was used for acidification at various concentrations (0.01, 1, 2.5, and 5%). This acid solution was used to incubate the cocoa nibs for 1, 3, and 5 hours. Physicochemical characteristics such as color changes, anthocyanin content, total phenolic content, and antioxidant activity of acidified cocoa nibs were analyzed. Fourier transform infrared spectroscopy analysis was also utilized to evaluate the changes in the functional groups. The results showed that the acidification of cocoa nibs using >1% malic acid significantly altered the color of cocoa nibs from brownish-purple to reddish color. Anthocyanin and phenolic content of cocoa nibs could be preserved to more than 61 and 65%, resulting in preserved antioxidant activity (>66%). The use of 2.5% malic acid followed by incubation for 3 hours resulted in cocoa nibs with bright red color and highly-preserved bioactive compounds.

Keywords: Ruby, polyphenol, anthocyanin, maillard non-enzymatic browning, unfermented

INTRODUCTION

The quality of cocoa beans is highly related to its post-harvest treatment. A series of processes such as sortation, fermentation, and drying should be carried out appropriately to obtain high-quality cocoa beans. However, in some areas of Indonesia, the cocoa beans produced are unfermented. This is due to several factors such as local practices, limited knowledge transfer, and the lack of price incentives (Febrianto & Zhu, 2020). The unfermented cocoa bean is considered a low-quality product due to its inferior flavor and taste quality, and unattractive appearance (slaty) (Kongor *et al*., 2016). This limits the use of unfermented cocoa beans in high-value-added products such as couverture chocolate. In industries, the modification of the color of cocoa powder is done to improve customer preferences. The resulting cocoa powders are varied in color such as reddish-brown, dark-brown, and black. This is usually carried out by means of alkalization process, by adding alkaline chemicals. However, this process significantly reduced the amount of

bioactive in cocoa powder. Alternative methods such as acidification can be done to modify the color of cocoa powder while maintaining the amount of bioactive compounds (Beccera *et al.,* 2023).

The unfermented cocoa bean is high in bioactive compounds. Fang *et al*. (2020) reported that cocoa beans contain high amounts of flavan-3-ols (epicatechin, catechin, and proanthocyanidins). Febrianto & Zhu (2019; 2020) emphasized that unfermented beans had particularly higher concentrations of monomer to oligomeric polyphenols such as anthocyanin, flavan-3-ols, flavonol, and phenolic acids than that of fermented beans. For comparison, flavan-3-ols concentration in unfermented beans was reported to be around 15 g epicatechin equiv./kg dried beans and reduced to only 2 g epicatechin equiv/kg db in six days fermented cocoa beans. In further study, it was reported that those compounds provide beneficial effects on human health. The consumption of a polyphenol-high diet was associated with reduced risk of cardiovascular disease, diabetes, and cancer and improved physical performance (Kim & Brothers, 2020; Pandurangan *et al*., 2015; Ahmed *et al*., 2020; Alvarez-Cilleros *et al*., 2020; Febrianto *et al*., 2021). However, direct consumption of food derived from unfermented cocoa beans is limited. This is due to their excessive bitterness and astringency, and their unattractive appearance. Beccera *et al*. (2023) showed that the acidification process could enhance the physical appearance of cocoa beans. The sour taste generated from acidification may also help to mask the bitter and astringent, allowing improved flexibility for food product application.

The addition of organic acid (acidification) affects the physicochemical properties of cocoa beans through various mechanisms. Diffusion of organic acids in cocoa beans affected the structure of anthocyanin (Tuenter *et al*., 2020). Anthocyanin is a dominant color pigment of cocoa beans and is highly affected by changes in pH. Tang *et al.* (2019) reported the changes in color of anthocyanin aqueous solutions from red, purple, blue to green dependent to the pH levels. This is due to the occurrence of various forms of anthocyanin such as flavylium cation (red), quinoidal base (purple or blue), carbinol pseudobase (colorless), and chalcone (yellow) which coexist in different proportion depending on the pH (Tang *et al*., 2019). Kunnaryo & Wikandari (2021) evaluated that the addition of acid helps to stabilize the anthocyanin, resulting in a more stable color. Furthermore, the extremely low pH condition inhibits the activity of polyphenol oxidase (Beccera *et al.*, 2023). However, moderate acid conditions may lead to the increased activity of cocoa's PPO. Hansen *et al.* (1998) previously found that PPO had the optimum activity at pH 5.5. Oxidized and polymerized flavan-3-ols in the form of proan-thocyanidin produced a brown color appearance of cocoa beans (Febrianto *et al*., 2021). The use of various organic acids such as acetic acid, lactic acid, and citric acid has been previously reported (Beccera *et al*., 2023). The use of other commercially available organic acids, such as malic acid, tartaric acid, and phosphoric acid for the acidification of cocoa beans should also be carried out. The aim of the study was to evaluate the effect of malic acid concentration and incubation time during the acidification process on the physicochemical properties of cocoa nibs. Malic acid is a commercially available ingredient in the market and is often used for food product formulations. The result of this study will be beneficial for industries to adjust the production process to obtain products that meet their desired characteristics. Furthermore, acidified cocoa powder resulting from this study may be used for the development of novel chocolate-based products.

MATERIALS AND METHODS

Sample Preparation

Fresh cocoa fruits were obtained from the Indonesian Coffee and Cocoa Research Institute. The fruits were freshly harvested (on the same days) without any pod storage treatment. The cocoa fruits obtained were of mixed genotypes (Sul 1 and MCC 02) and harvested during the peak maturity stage (indicated by color changes). Cocoa fruit was opened to obtain wet cocoa beans. The beans were then dried in the drying house for 5-7 days (average temperature of 45 °C) until the moisture content of 7.5%. Resulted beans were then manually deshelled and ground with pestle and mortar to obtain coarse particles. The coarse particle was then referred to as cocoa nibs. The cocoa nibs were stored in a sealed airtight container until used (no more than two month of storage).

Acidifcation

Cocoa nibs were put in a beaker glass and the acid solution was added in the ratio of 1:3 w/v (10 g of cocoa nibs mixed with 30 mL of acid solution). The experiment was carried out by using full factorial design. The concentrations of acid solution were 0.01; 1; 2.5, and 5%. The solution was then incubated for 1, 3, and 5 hours at room temperature (25 °C). After incubation, the solution was then filtered to obtain acidified cocoa nibs. Drying was carried out in an oven at 30 °C for 30 hours (layered in <0.5 cm). Dried nibs were then manually ground (50 mess) to obtain acidified cocoa powder (ACP) for analysis. Cocoa powder from nibs without acidification was also prepared as a control.

Preparation of Cocoa Extract

Cocoa methanolic extract (CME) was prepared based on the method of Febrianto and Zhu (2019) with modification. Cocoa powder was mixed with 80% methanol with a ratio of 1:20 w/v. The extraction was carried out for 16 h in a shaker (100 rpm). The solution was then filtered with filter paper to obtain CME for total anthocyanin, total phenolic, and antioxidant analysis.

Color Analysis

Color analysis was done based on CIE L*a*b* system using AMT-501 colorimeter (AMTAST Inc., USA). L* represented lightness, a* for redness-grenness, and b* for yellowness-blueness.

Fourier Transform Infra-Red Analysis

Analysis of the functional groups of the compounds in the ACP was carried out based on the method of Nurhayati *et al.* (2018) with slight modification. One gram of samples was put in an FTIR sample holder and then analyzed on the wavelength range of 600-4000 cm^{-1} using Nicolet Apex FTIR apparatus (Thermo Fisher Scientific, Waltham, MA).

Anthocyanin Content

Anthocyanin content was analyzed following the method of Aditya *et al.* (2017) based on the pH differential method of AOAC (2005). Four mL pH 1 solution was prepared using KCl-HCl and 4 mL pH 4.5 solution was prepared using CH3COONa-CH3COOH. One mL of CME was then added to each solution followed by storing in dark condition for 15 mins. The solution was then measured at the wavelengths of 510 and 700 nm using a UV-Vis Spectrophotometer. Total anthocyanin was then calculated using the following formula:

$$Anthocyanin\ content = \frac{[(Abs510 - Abs700)pH1 - (Abs510 - Abs700)pH4.5]}{29.6} \times 449.2 \times \frac{Vd}{Wd \times 1000} \times 100\%$$

Where Vd was the volume after dilution and Wd was the weight of the sample.

Total Phenolic Content

Total phenolic content (TPC) analysis was carried out based on the method of Szydlowska-Czerniak's (2010) with some modifications. Approximately 0.1 mL of CME was diluted to 2 mL of 80% methanol. As much as 0.25 mL solution was then added with 0.5 mL folin-ciocalteu reagent and 1 mL of Na_2CO_3. The mixture was vortexed and incubated for 3 minutes. The mixture was then diluted with 8.25 mL of distilled water and stored in a dark condition for 1 h. The solution was then measured on the wavelength of 725 nm. The standard solution was prepared using gallic acid. The results were expressed as mg GAE/g cocoa powder.

Antioxidant activity analysis

Antioxidant activity analysis was done based on DPPH inhibition method previously reported by Gadow et al. (1997) with modification. Approximately 20 µL of CME was added with 4.98 mL of DPPH solution. The solution was then stored in a dark condition for 30 mins. The solution was then measured at the wavelength of 517 nm. The control was prepared by replacing CME with 80% methanol. The antioxidant activity was then calculated using the following formula:

$$\text{DPPH Inhibitation (\%)} = \frac{[Abs517blank - Abs517sample]}{Abs517blank} \times 100\%$$

Statistical Analysis

All tretament were carried out in triplicate. Analysis of variance (ANOVA) and Pearson correlation analysis were carried out using SPSS software (Ver. 24, IBM Corp, Armonk, USA).

RESULTS AND DISCUSSION

Color Changes of the Cocoa Nibs

There were significant differences in the color of cocoa nibs due to acidification treatment (Figure 1). The control (untreated/ non-acidified) sample was characterized by a dull greyish-brown color. The greyish color of untreated cocoa nibs was a specific character of slaty beans, inferring poor fermentation of the cocoa beans. Acidification using 0.01% malic acid resulted in a similar brown color to that of control. This showed that the addition of low-concentration acid was insufficient to trigger color changes. The acids might react with anthocyanin, but to some extent, could not compete with the enzymatic browning that occurred during the incubation of cocoa nibs. This was in agreement with the result of Sampebarra (2018). Misnawi *et al*. (2002) reported that incubation of cocoa beans with water (neutral pH), added/absent of additives (chemicals and enzymes), could trigger enzymatic browning, increasing its fermentation index. This was related to the activity of the polyphenol oxidase (PPO) enzyme. Thus, it was observed that a low concentration of acid could not provide suitable conditions for enzyme inactivation, resulting in progressed browning reaction as indicated by lower L* values (Table 1).

The occurrence of red color started at the acidification using >1% of malic acid. This was confirmed by CIE L*a*b* analysis results. The development of red color was represented by the increase in a* value (redness). A bright red color was observed from cocoa nibs acidified by using > 2.5% of malic acid (a* value > 15). Anthocyanin, in its natural form, possesses electron deficiency which makes it reactive. The addition of organic acid altered the quinoidal structure of anthocyanin to form of red-colored flavylium cation structure from (Kunnaryo & Wikandari,

Table 1. CIE L*a*b* color of cocoa nibs obtained from various acidification treatments

Sample		L*	a*	b*
0.01%	1 h	51.61 ^{ab}	6.89 ^{b}	4.50 ^{abc}
	3 h	52.47 ^{ab}	8.25 ^{b}	3.91 ^{ab}
	5 h	52.14 ^{ab}	7.28 ^{b}	6.18 ^{de}
1%	1 h	55.34 ^{c}	15.03 ^{d}	4.70 ^{abc}
	3 h	52.88 ^{ab}	12.79 ^{c}	5.55 ^{cde}
	5 h	52.02 ^{ab}	11.41 ^{c}	5.48 ^{cd}
2.5%	1 h	51.28 ^{ab}	18.82 ^{e}	3,61 ^{ab}
	3 h	53.71 ^{bc}	20,15 ^{e}	5.80 ^{cde}
	5 h	52.73 ^{ab}	20.03 ^{e}	3.49 ^{a}
5%	1 h	52.26 ^{ab}	25.50 ^{g}	4.82 ^{bc}
	3 h	50.41 ^{a}	22.01 ^{f}	4.84 ^{bc}
	5 h	51.42 ^{ab}	23.98 ^{g}	4.85 ^{bc}
	Control	55.52 ^{c}	3.23 ^{a}	6.79 ^{e}

Notes: *(L*= Lightness; a*= redness-blueness; b*= blueness-yellowness); *)values with different letters in the same column were significantly different ($p<0.05$) based on Duncan Mutiple Range Test analysis.

Figure 1. Appearance of cocoa nibs obtained from various acidification treatment

2021; Tang *et al*., 2019). This compound is also relatively stable, resulting in a long-lasting bright red color. Furthermore, low pH conditions inhibited the activity of PPO, preventing browning reactions (Samosir *et al*., 2022). This was indicated by the decrease of b* values, indicating the reduced intensity of the yellow color.

Functional Group of Acidified Cocoa Nibs

The infra-red spectrum of untreated and acidified cocoa nibs were similar (Figure 2). This indicated that the addition of organic acids to cocoa nibs only slightly altered the chemical characteristics of cocoa nibs. This was beneficial, since except for the color, no major changes in the chemical composition of the nibs were caused by malic acid. Noticeable changes in the IR spectrum were at OH stretching (3276 cm^{-1}), C = O ester (1734.73 cm^{-1}), and OH bending (1635.41 cm^{-1}). Montoya *et al*. (2020) previously reported that the OH stretching represents the formation of brown-colored tannin. Tannin in the form of proanthocyanidins in cocoa beans was produced by oxidation and polymerization of polyphenols (Febrianto *et al*., 2021). Considering the result of color analysis, thus the degradation of polyphenol might have been still progressing during the acidification event though the brown color evaluated was unnoticeable due to the high intensity of red color. On the other hand, the changes in C = O ester and OH bending are related to the structural changes of anthocyanin from neutral quinoid to flavylium cations (Montoya *et al*., 2020; Tang *et al*., 2019). H+ from organic acid transforms the functional groups of C = O in anthocyanin to C O H. Hence, it could be concluded that, in this range of

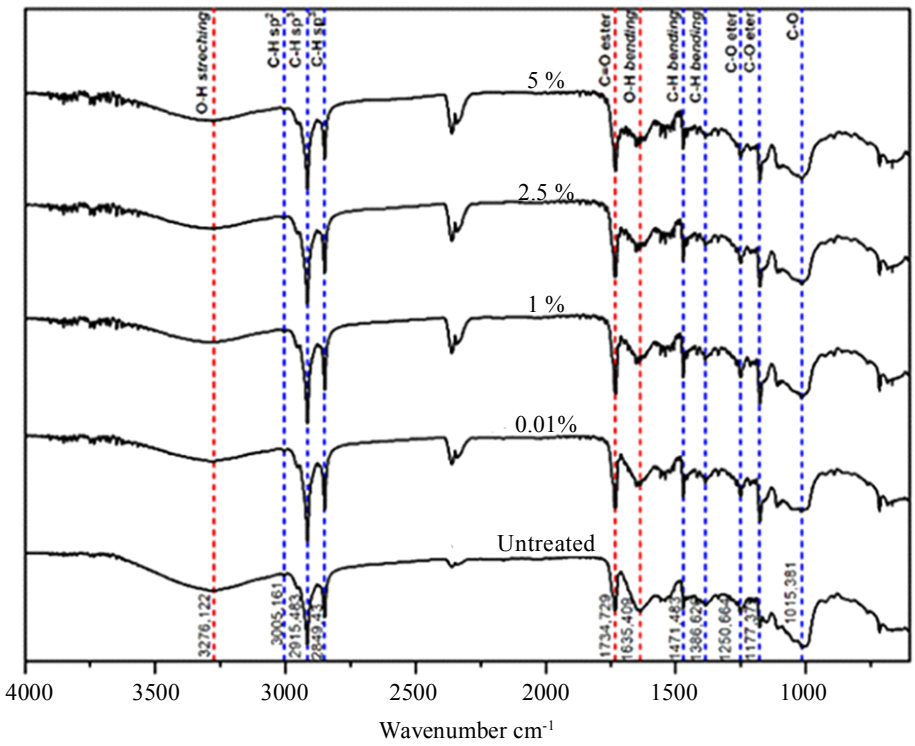

Figure 2. Infra-red spectrum of untreated and acidified cocoa nibs in various concentrations of malic acid after 5 h of incubation

concentration, the addition of organic acid in the cocoa nibs only affected the reaction related to polyphenol. However, this hypothesis might need to be confirmed through more detailed compounds-based analysis methods such as the use of compounds-targeted analysis using chromatography or spectroscopy.

Bioactive Contents of Cocoa Nibs

Polyphenol content in cocoa nibs decreased as the acidification progressed (Figure 3). The highest TPC value was evaluated on untreated cocoa powder, while the lowest was the one acidified with 5% malic acid for five hours. This result was contradictory to the previous study (Sabahannur, 2020). Polyphenol is reported to be more stable in acidic conditions. This was due to the formation of protonated and crystalline polyphenol in acidic condition. Furthermore, acidic conditions could inhibit PPO activity, resulting in less oxidation of polyphenols. However, polyphenols in cocoa beans are mostly in the form of glycosides (D'souza *et al*., 2017). Tsao (2010) reported that high acid conditions induced hydrolysis of glycosides in polyphenol structure, altering the native polyphenol profiles. These changes

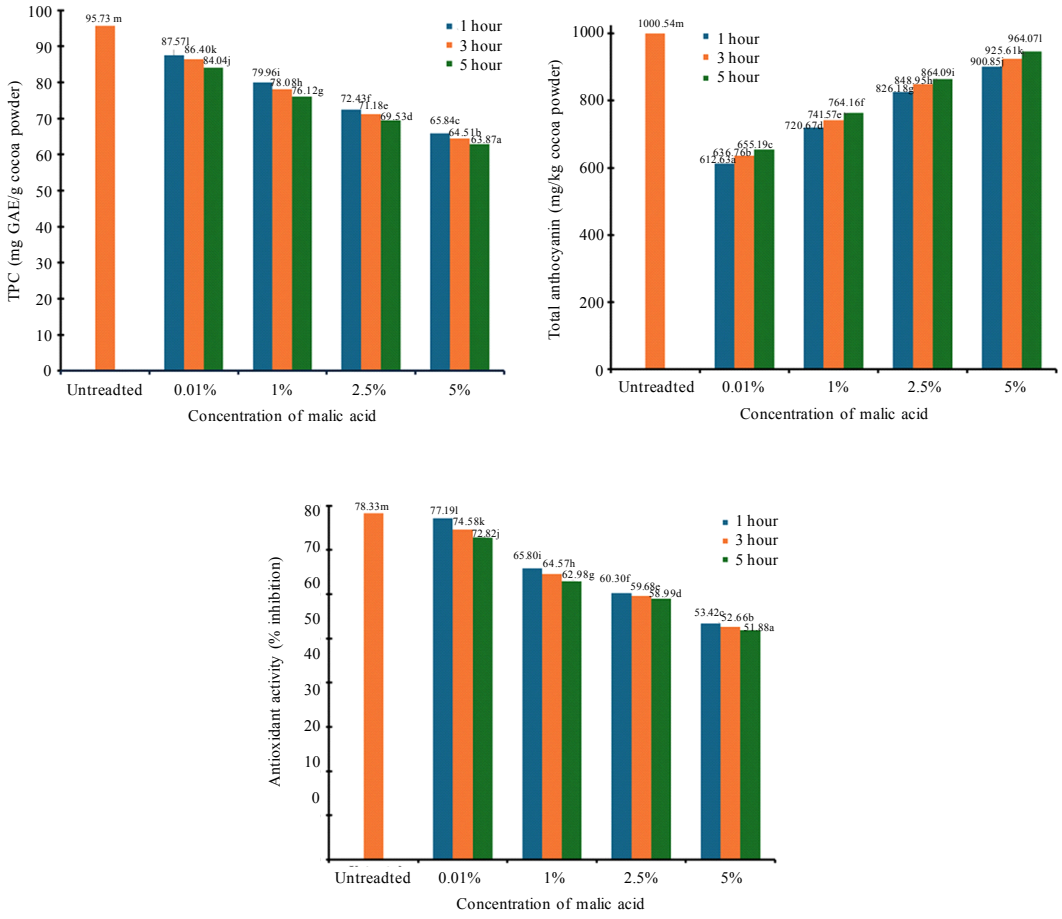

Figure 3. Total phenolic contents (top left); total anthocyanin content (top right), and antioxidant activity (bottom) of untreated and acidified cocoa nibs at various acidification treatment

might impact the amount of active polyphenols, resulting in a decrease in polyphenol content. Even though the polyphenol content decreased, the decrease was still lower than that of occurred during cocoa fermentation. Dwijatmoko *et al.* (2018) found that the TPC of fermented cocoa beans was 55.93 mg GAE/g cocoa powder, significantly lower than the TPC evaluated in this study (62.87 95.73 mg GAE/g cocoa powder). This showed that acidification could preserve the polyphenol content in cocoa nibs.

Interestingly, the decrease in TPC value was negatively correlated with the total anthocyanin content (Figure 3; r = -0.988, $p<0.05$, Figure 4). This showed that acidification affects differently towards polyphenols differently, depending on their classes. Compared to flavan-3-ols and other polyphenols that are in their glycoside form, anthocyanins in cocoa beans are naturally in their aglycone form. The addition of malic acid stabilized the anthocyanin by forming flavylium cations and prevented its oxidation by PPO (Kunnaryo & Wikandari, 2021). The addition of more malic acid resulted in more stable flavylium cations of anthocyanin, positively correlated with a high value of a* which represented the red color of the solution (r = 0.952, $p<0.05$). However, the preservation of anthocyanin could not contribute to the preservation of antioxidant activity. Antioxidant activity reduced along with the increase of malic acid concentration linearly correlated to that of TPC (r = 0.99, $p<0.05$, Figure 4). Hence, it can be concluded that the antioxidant

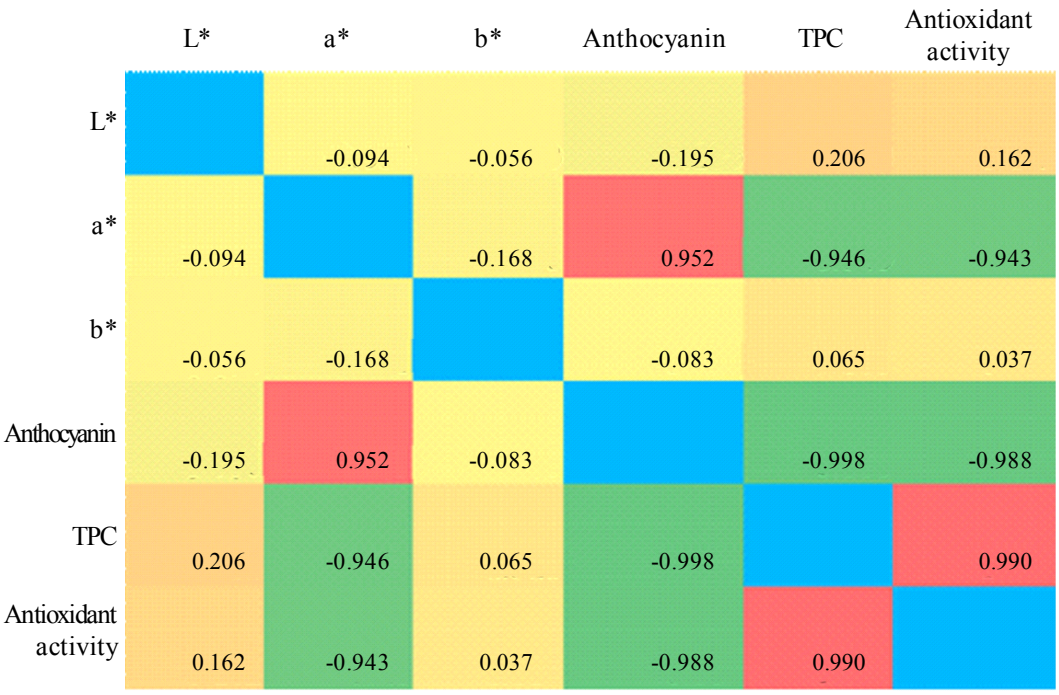

Figure 4. Correlation matrix of analyzed parameters of the acidified cocoa powder. Abbreviation: TPC, total phenolic content

activity of acidified cocoa powder was more affected by non-anthocyanin polyphenol compounds such as flavan-3-ols (epicatechin, cathecin and proanthocyanidin), phenolic acid (N-phenylpropenoyl-L-amino acids), and flavonol (quercetin glycosides).

Industrial Relevance

Acidification of cocoa nibs using malic acid successfully produced cocoa powder with a bright-red color. This opens the possibility of its usage towards innovative food products with interesting appearance. However, the stability of the color needs to be studied further. Chocolate processing is tedious, multi-step, and involves the use of heat for its treatment. Polyphenol, such as anthocyanin, is known to be heat-unstable. Thus, information on its stability during chocolate processing is important for industries to optimize the required processes. Even though the concentration of malic acid used in this study might be sufficient to maintain the red color appearance, its concentration may change during the formulation of the chocolate product. Thus, a change in color appearance, whether it is desirable or not, is expected due to the dilution of acid by the addition of another ingredient.

Sensory analysis is needed to obtain the sensory profile of the acidified cocoa powder. The addition of malic acid may also alter the taste of the cocoa powder. Excessive acidity/ sourness in fermented cocoa beans is undesirable since it masks the perception of chocolate flavor (Bangerter *et al.*, 1997). On the other hand, acidity in unfermented cocoa beans may be necessary to mask bitterness and astringency. However, less use of organic acids is preferable. Acidification may be done on cocoa cake instead of cocoa nibs. Even though the acidification in this study didn't promote any noticeable changes in the functional group (except for polyphenol), it might alter the properties of cocoa butter through hydrolysis. The addition of acid after the extraction of cocoa butter could maintain the quality of cocoa butter produced. Furthermore, the occurrence of acid taste may be beneficial for the production of fruity chocolate. This may be done by the addition of a flavoring agent (natural or synthetic) to enhance the flavor of naturally less-aromatic unfermented cocoa. Further study on chocolate processing and sensory analysis on the chocolate obtained from acidified cocoa powder could be the further direction of this study.

The preserved content of polyphenol compounds and the occurrence of a fruity taste in acidified cocoa powder could be an important added value for this product. Polyphenol-rich functional foods are often undesired by the consumer due to their excessively bitter and astringent taste, even though it is associated with improved health benefits. The occurrence of a fruity taste that masks the bitter and astringent taste offers an alternative of high-polyphenols cocoa powder with improved palatability. This can provide more enjoyable and accessible functional food for any age (especially teenagers) for improved quality of life.

CONCLUSION

Acidification of unfermented cocoa beans was successfully carried out using malic acid. The use of >2.5% of malic acid could produce a cocoa powder with a bright-red appearance, improve the stability of anthocyanin (limiting the reduction up to 17.4%), and preserve the polyphenol contents of cocoa powder (up to 65%). Acidification using 2.5% malic acid for three hours was optimal to produce a brilliant red color with high anthocyanins, TPC, and antioxidant activity. Sensory analysis on final product utilizing this red cocoa powder is

needed to improve the acidification process and obtain the desirable sensory properties.

ACKNOWLEDGEMENT

The authors would like to sincerely thank Mrs. Fitratin from ICCRI for her assistance in providing the samples and analyses.

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