

Effects of alkali concentration and conching temperature on antioxidant activity and physical properties of chocolate

Sulistyowati and *Misnawi

Indonesian Coffee and Cocoa Research Institute, Jl. PB Sudirman No. 90 Jember 68118, Indonesia

Abstract: The effects of alkalization on antioxidant activity and physical properties of chocolate were studied during conching using a chocolate recipe of the Indonesian Coffee and Cocoa Research Institute. The study was designed according to Response Surface Methodology in two variables. Alkali concentration and conching temperature were plotted in the range of 1 – 15 g kg⁻¹ and 40 – 80°C, respectively. Parameters of the study were antioxidant activity, total polyphenol, chocolate hardness and fat melting point. The results of the study showed that antioxidant activity and polyphenol concentration were reduced as alkali concentration and temperature of conching were increased. Improvement of conching temperature from 40°C to 80°C with 1 g of sodium bicarbonate reduced antioxidant activity from 84% to 67%, and with 15 g of sodium bicarbonate the activity was reduced from 78% to 55%. Reduction of the antioxidant activity due to alkali improvement from 1 g to 15 g at conching temperature of 40°C and 80°C was calculated to be 7% and 18%, respectively. Reduction of the polyphenol antioxidant activity was suggested to be triggered by heat and alkali synergistically. However, a better chocolate quality in terms of hardness and significant antioxidant activity was obtained through application of alkalization with alkali concentrations between 5 to 12 g kg⁻¹ and conching with temperatures up to 68°C.

Keywords: Cocoa bean, chocolate, conching, alkalization, antioxidant, polyphenol

INTRODUCTION

Recently, cocoa bean polyphenols have attained much more attention, owing to their antioxidant activity and their possible beneficial implications for human health (Wollgast and Anklam, 2000a) such as the treatment and prevention of cancer (Wollgast and Anklam, 2000b), cardio vascular disease (Wollgast and Anklam, 2000b; Kattenberg, 2000) and other pathologies. In vitro experiments using the TROLOX assay and animal models suggest that most of cocoa bean's polyphenol fractions have antioxidant activity and are beneficial to human health (Osakabe *et al.*, 1998a, 1998b, 2000; Lee *et al.*, 2003). The effects include inhibition of hydrogen peroxide and superoxide anion formation and thus protection against lipid peroxidation and deterioration (Kattenberg, 2000), inhibition of oxidative stress and reduction of low-density lipoprotein (LDL) oxidation associated with cardio vascular disease (Lee *et al.*, 2003), protection against gastric lesion (Osakabe *et al.*, 1998a, 1998b), antiulceric and antimutagenic effects (Osakabe *et al.*, 2000), inhibition of tumour promotion and carcinogenesis (Sanbongi *et al.*, 1997) and antimicrobial activity (Sanbongi *et al.*, 1998; Kattenberg, 2000). However, the amounts and

types of the polyphenols, namely flavan-3-ols and procyanidins, in the gastro intestinal tract following cocoa consumption are influenced by the stability of these compounds in both acidic and alkaline environments (Zhu *et al.*, 1998).

Cocoa beans are rich in polyphenols, it has been found that unfermented cocoa beans contain ca. 135 g kg⁻¹ of polyphenolic compounds (Misnawi *et al.*, 2002); in which three groups of the polyphenols can be distinguished: namely catechins or flavan-3-ols constituting 37%, anthocyanins 4% and proanthocyanidins 58% (Wollgast and Anklam, 2000b). Polyphenols in cocoa products are mostly responsible for the astringent sensation and they also contribute to the bitter taste along with alkaloids, some amino acids, peptides and pyrazines (Bonvehi and Coll, 2000, 1997; Luna *et al.*, 2002; Misnawi *et al.*, 2005) present in cocoa. On the other hand, their quantity must be sufficient to have beneficial preservative effects (Misnawi *et al.*, 2003). Cocoa polyphenols can act as an antioxidant in cocoa and chocolate products.

Alteration of cocoa polyphenols during the process of chocolate manufacture should be expected, particularly during alkalization, roasting, grinding, refining and conching. However, knowledge of these changes through out the

*Corresponding author

Email: iccri@iccri.net, misnawi@yahoo.com

processes is limited (Wollgast and Anklam, 2000a). Alkalization, also known as 'Dutching', is a treatment that is sometimes used before or after grinding to modify the colour and flavour of cocoa products. The process involves soaking the nib or the cocoa mass in potassium or sodium bicarbonate and raising the pH level from 5.5 to 6.0 and up to 8.2. By varying the ratio of alkali to nib or cocoa mass, a wide range of colours, from red-brown to dark mahogany-red to extremely dark can be produced. Concentration of alkali, reaction time, temperature, moisture content, intermixture of alkali, along with possible sequenced treatment by various alkali concentrations, influence the colour and flavour properties of the resultant cocoa. This research aimed to evaluate the effect of alkalization on antioxidant activity of the polyphenol content and physical properties of chocolate produced.

MATERIALS AND METHODS

Chocolate preparation

Chocolate was prepared according to a standard recipe of the Indonesian Coffee and Cocoa Research Institute (ICCRI) as shown in Table 1. Fermented Fine flavour Cocoa bean was used for cocoa liquor and butter preparation. Formulated chocolate was refined to about 20 micron using a five rolls refiner for three circulations. Chocolate was then conched for 22 hours at temperatures of 40 – 80°C, depending on the experimental treatment. An amount of sodium bicarbonate was applied at the beginning of conching. Chocolate tempering was settled up according to Mulato *et al.* (2004) before moulding and cooling at temperature of 32 – 34°C and 10 – 12°C, respectively.

Design of the study and statistical analysis

This study was carried out using Response Surface Methodology (RSM) design with two variables in terms of alkali concentration and conching

temperature. The former and the latter were arranged in the range of 1 – 15 g kg⁻¹ and 40 – 80°C, respectively. Combination of the two variables is presented in a matrix treatment as shown in Table 2. Data obtained were analysed for variance and then followed by contours plotting.

Total polyphenol

Total polyphenol was determined spectrophotometrically using the modification method of Singleton and Rossi (1965) not in the list and Misnawi *et al.* (2003) in terms of sample preparation. Two hundred fifty milligrams of defatted cocoa powder from the chocolate formulation after conching treatment was added with 40 ml of 80% aqueous acetone, placed in a 125 ml conical flask and the mixture was sonicated in a Sonicor C-125 (Sonicor Inst., New York, USA) for 30 min. During sonication, the extraction mixture was kept cold by filling the sonicator vessel with ice water. Sonication was preferred over shearing as an aid in solubilizing polyphenol, since shearing promotes browning of the polyphenol extract by oxidation (Shamsuddin and Dimmick, 1986).

A clear extract was obtained by vacuum filtration of the mixture through Whatman no. 1 filter paper. The residue and all glassware were washed with 80% aqueous acetone and total volume of the extract was made up to 100 ml in a volumetric flask. One milliliter of the extract was pipetted into a 100 ml volumetric flask and diluted with 70 ml of distilled water. The extracted polyphenol was then reacted with 5 ml of 2 N Folin-Ciocalteu's reagent for 2 min. Then 15 ml of saturated Na₂CO₃ solution was added to stabilize the color formed. The blue color was allowed to develop for at least 2 hrs and its absorbance was measured at 765 nm. (-)-Epicatechin standard of nine known concentrations of 1 to 9 mg l⁻¹ was used for reference calculation.

Antioxidant activity

Antioxidant activity was analysed by using DPPH (1,1-diphenyl 2-picrylhydrazyl) method according to Molyneux (2004). Defatted cocoa powder of 0.2 g was added with 20 ml of absolute ethanol, stirred for 10 min and centrifuged for 3 min at 5,000 rpm. One millilitre of supernatant was added with 0.5 ml of DPPH reagent and allowed to stand for 20 min. Absolute ethanol was then added to the mixture to obtain a volume of 5 ml. After vortex, its absorbance was measured at a wavelength of 517 nm. The same procedure was applied to the reagent without cocoa powder as reference. Antioxidant activity was calculated as:

Table 1: Chocolate recipe

Ingredient	Proportion (%)
Cocoa liquor	23.5
Cocoa butter	23.5
Milk powder	17.6
Refined sugar	35.0
Salt	0.05
Vanillin	0.1
Lecithin	0.3

Table 2: Matrix of the treatment of alkali concentration and conching temperature combination

Treatment sequence	Treatment code	Alkali concentration (g kg ⁻¹) ¹⁾	Conching temperature (°C) ²⁾
1	2	8.0	40.0
2	6	1.0	40.0
3	3	15.0	60.0
4	3	15.0	60.0
5	4	15.0	80.0
6	8	1.0	53.3
7	1	15.0	40.0
8	11	10.3	53.3
9	5	1.0	80.0
10	1	15.0	40.0
11	4	15.0	80.0
12	10	10.3	80.0
13	5	1.0	80.0
14	7	1.0	66.7
15	2	8.0	40.0
16	9	5.7	80.0
-	Control	0.0	50.0

¹⁾ range of 1 – 15 g kg⁻¹dough

²⁾ range of 40 – 80°C.

Chocolate hardness

Hardness was measured by determining the maximum force on the centre of the chocolate. The chocolate was prepared by moulding to a size of 15 mm x 30 mm of long and wide, and 15 ml high. A digital texture analyser RHEO – TEX SD-700 (Ogawa Seiki, Tokyo, Japan) was used to measure the force for 7 mm penetration at 20°C, with a single probe needle of 10 mm. Measurement was obtained from five replications.

Fat melting point

Fat melting point was measured by using Melting Point Aparatus Electrothermal Type 9400 (United Kingdom). Fat obtained from the respective chocolate treatments was extracted through soxhlet extraction (AOAC, 1998). Amount of fat (200 mg) was setted in a glass compartment and cooled at 18-20°C for 24 hours. The compartment was then loaded to the apparatus. Final temperature was settled at 40°C with increments of 2°C min⁻¹. The melting point was recorded at a temperature when cocoa butter starts to melt. All measurements were carried out on five replications.

RESULTS AND DISCUSSION

Total polyphenol

Analysis of variance on total polyphenol showed that alkali concentration and temperature of conching significantly influenced ($p < 0.01$) total polyphenol in the resultant chocolate. Improvement of alkali concentration and conching temperature gradually reduced polyphenol concentration (Figure 1). There was no significant interaction effect between alkali concentration and conching temperature on the polyphenol concentrations. However, the decrease in polyphenol concentration due to conching temperature at alkali concentration lower than 5 g kg⁻¹ was slower, indicating that the reduction of polyphenol was suggested mainly to be triggered by the presence of alkali.

Unfermented Forastero cocoa beans contain 120 – 180 g kg⁻¹ of polyphenolic compounds (Kim and Keeney, 1984; Wollgast and Anklam, 2000). During cocoa fermentation, polyphenols are subjected to biochemical modification through polymerization and complexation with protein, hence decreasing solubility and astringency

(Bonvehi and Coll, 1997). At the same time, anthocyanins are hydrolyzed to anthocyanidins. The latter compounds polymerize along with simple catechins to form complex tannins. Polyphenol content in dried fermented cocoa bean is 63 g kg⁻¹ (Misnawi *et al.*, 2004). Polyphenol concentration in chocolate of this experiment was found at 26.7 g kg⁻¹ which is due to dilution with other ingredients in terms of sugar and milk in the formulation.

Improvement of alkali concentration during alkalization is suggested to increase the intensity of polyphenol oxidation. In the same way, heat applied during conching also increases the oxidation intensity. These two conditions resulted in significant reduction of polyphenol concentration after conching at 80°C with alkali concentration of 15 g kg⁻¹ to be at 22.5 g kg⁻¹. However, the reduction is suggested to be caused more by oxidation rather than by hydrolyzation since most of the cocoa bean's polyphenol are condensed tannin which is resistant to hydrolysis.

Condensed tannins are proanthocyanidins and are high molecular weight polymers. Their monomeric unit is a flavon-3-ol (e.g. catechin and epicatechin), with a flavon-3, 4-diol or leucoanthocyanidin molecule as its precursor. Oxidative condensation occurs between carbon C-4 of the heterocycle and carbon C-6 or C-8 of adjacent units. Most of the literature on condensed tannin refers only to oligomeric proanthocyanidins (dimeric, trimeric, and tetrameric), because of difficulty in analyzing highly polymerized molecules (Bravo, 1998). Cocoa bean polyphenol is composed of ca. 37% catechins, ca. 4% anthocyanins and ca. 58% proanthocyanidins (Wollgast and Anklam, 2000b).

Antioxidant activity

Antioxidant activity in chocolate significantly decreased as conching temperature increased from 40°C to 80°C. The activity also decreased due to the increase in alkali concentration, although the decrease was not as intense as that due to conching temperature (Figure 2).

Most of the antioxidant activity in the chocolate comes from polyphenol content. All fractions of cocoa bean polyphenols have been identified to have antioxidant property (Steinberg *et al.*, 2002). However, Jinap and Misnawi (2002) found that roasting of cocoa liquor up to 120°C for 45 min did not significantly reduce its polyphenol antioxidant activity.

Results of this study proved that the reduction of cocoa polyphenol antioxidant activity is higher with application of heat for longer times, which in this experiment was 24 hours, and in the complex system where sugar, milk, salt, vanillin and alkali

are present. Nevertheless, in chocolate applied with high concentration of alkali, the reduction was more intense. Figure 2 shows that an increase in temperature from 40°C to 80°C in conching with 1 g of sodium bicarbonate reduced antioxidant activity from 84% to 67% (17% reduction), meanwhile for that with 15 g of sodium bicarbonate the activity was reduced from 78% to 55% (23% reduction). Reduction of the antioxidant activity due to alkali improvement from 1 g to 15 g at conching temperature of 40°C and 80°C was calculated to be 7% and 18%, respectively. Reduction of the polyphenol antioxidant activity was clearly triggered by heat and alkali synergistically.

Chocolate hardness

Conching as well as alkalization is suggested to influence chocolate hardness through its effects on fat covering, hydrolysis and saponification. Results of this study showed that chocolate hardness was influenced significantly ($p < 0.01$) by alkali concentration, but not by conching temperature ($p > 0.05$). An increase in alkali concentration was followed by the increase in chocolate hardness (Figure 3). Chocolate hardness is important for quality product especially for those distributed in tropical areas. This study showed that chocolate hardness produced from conching with alkali concentration 15 g kg⁻¹ was 1,846 g, about 3.7 times harder than that of the control. Hardness of the control treatment was 498 g.

Improvement of chocolate hardness by increase in alkali concentration was suggested to correlate with the increase in cocoa butter melting point. Alkali was suggested to neutralize free fatty acids presents in the mixture through saponification mechanism. Splitting of triglycerides during processing was suggested to alter fat crystallization and compounds produced in terms of glycerol and fatty acids would have a lower melting point than their glycerides.

Fat melting point

Results of this study showed that alkali concentration and conching temperature influenced fat melting point ($p < 0.01$); there was significant interaction ($p < 0.05$) between alkali concentration and conching temperature. An increase in alkali concentration of up to 12 g kg⁻¹ at low conching temperature i.e. at 40°C was significant following increase in fat melting point. Low alkali concentration produced lower melting point at all conching temperature; however, higher alkali concentrations produced higher melting points at low conching temperatures, but lower melting points at high conching temperatures.

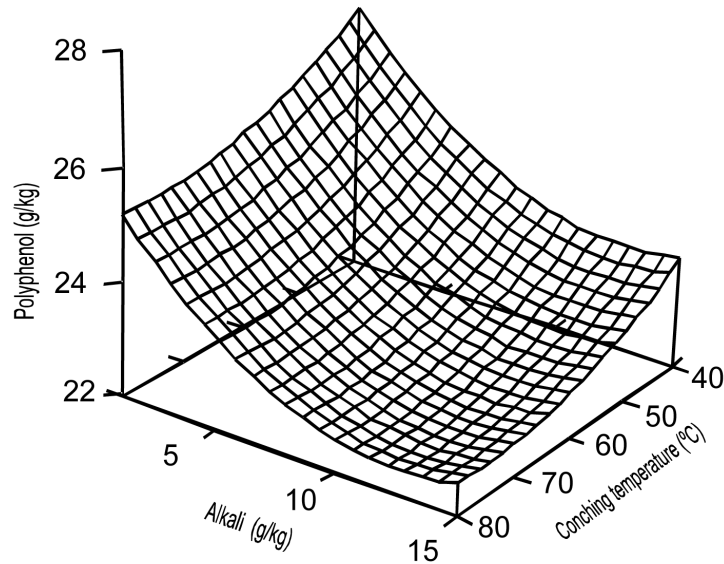


Figure 1: Profile of the effect of alkali concentration and conching temperature on polyphenol concentration in chocolate

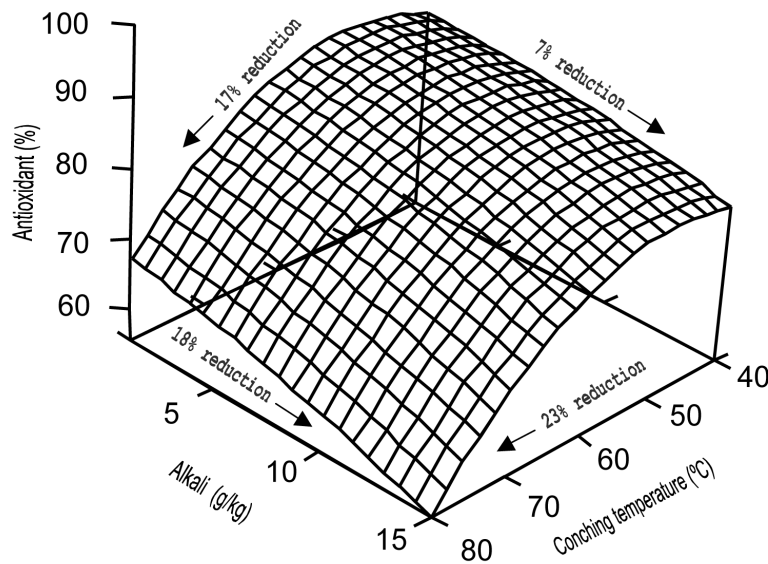


Figure 2: Profile of the effect of alkali concentration and conching temperature on antioxidant activity of cocoa powder

Fat fraction in the chocolate is a mixture of cocoa butter and milk fat. Chocolate quality with melting point $>35^{\circ}\text{C}$ could be found through alkalization at alkali concentration of $>5\text{ g kg}^{-1}$ at conching temperature of 40°C , however, in higher conching temperatures up to $\pm 68^{\circ}\text{C}$, higher alkali concentrations are needed. Alkali presence in the mixture is suggested to neutralize free fatty acids causing low melting point. In addition, the high

conching temperature was suggested to promote fat hydrolyses producing more fatty acids.

Cocoa butter constitutes $\pm 35\%$ of unsaturated fatty acids in terms of oleic acid (Beckett, 2000), and that of milk fat contains $\pm 25\%$ (Haylock and Dodds, 1999). These unsaturated fatty acids are less stable and tend to hydrolyze faster at a higher temperature producing more glycerol and free fatty acids.

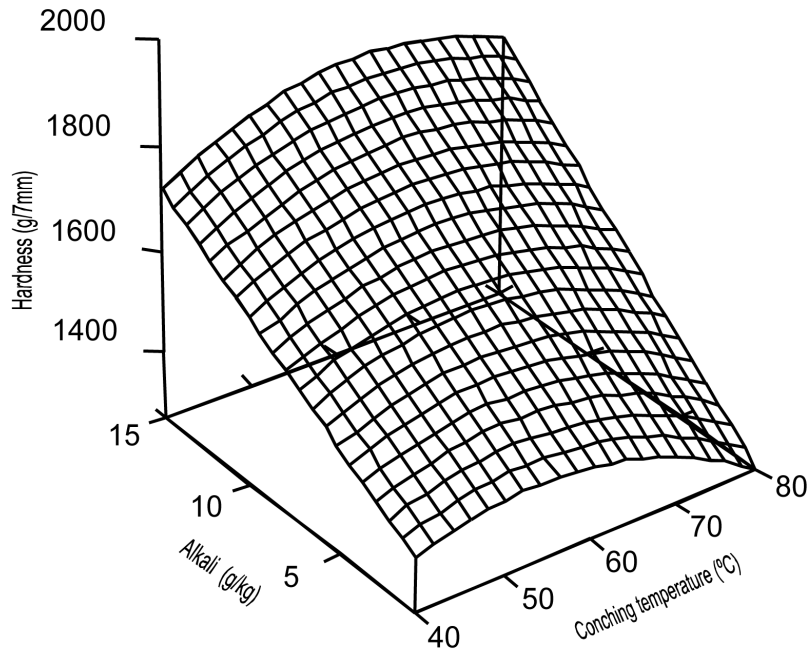


Figure 3: Profile of the effect of alkali concentration and conching temperature on chocolate hardness

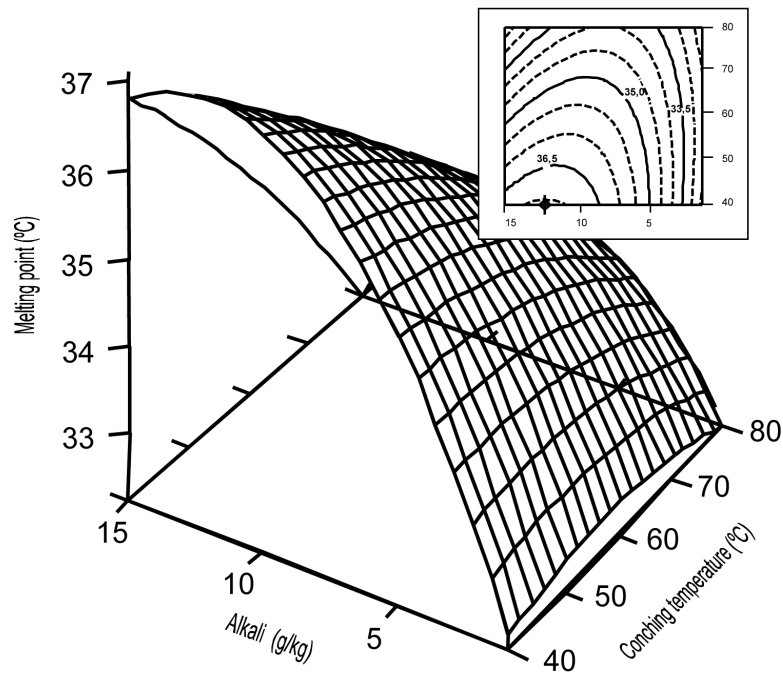


Figure 4: Profile of the effect of alkali concentration and conching temperature on melting point of extracted fat from chocolate (inset: two dimension contour)

CONCLUSION

Antioxidant activity and polyphenol concentration in chocolate were reduced by alkalization and application of heat during conching. Improvement of conching temperature from 40°C to 80°C with 1 g of sodium bicarbonate reduced 17% antioxidant activity, whereas that with 15 g of sodium bicarbonate resulted in a reduction of 23%. Reduction of the antioxidant activity due to alkali concentration improvement from 1 g to 15 g at conching temperature of 40°C and 80°C was 7% and 18%, respectively. However, a better quality chocolate with better hardness was obtained at alkali concentrations of 5 – 12 g kg⁻¹ with conching temperatures not more than 68°C.

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