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Polyphenol extracts from low quality cocoa beans: antioxidant, antibacterial and food colouring properties

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Abstract

Good quality cocoa beans are well studied and known as rich source of natural polyphenols. On the contrary, little research has been done regarding polyphenol extracts (PE) from low quality cocoa beans i.e. partially fermented dry cocoa beans (PFDCB) and unfermented dry cocoa beans (UFDCB). The following research was carried out to study the antioxidant, antibacterial, and food colouring properties of polyphenol extracts from PFDCB (PE-PFDCB) and UFDCB (PE-UFDCB). Antioxidant activity of PE-PFDCB and PE-UFDCB showed IC_{50} value of 1.1604 mg/ml and 0.2500 mg/ml respectively. Antibacterial activity of PE-PFDCB and PE-UFDCB was effective at inhibiting the growth of *Staphylococcus aureus* and *Salmonella typhimurium* at 25,000 ppm to 100,000 ppm respectively. As food colouring for yogurt, PE-PFDCB and PE-UFDCB showed the best color stability at 3% in refrigerated storage (2 - 5°C). As food colouring for syrup, 3% of PE-PFDCB and PE-UFDCB showed the best color stability at room temperature storage (24 - 25°C) and refrigerated storage (2 - 5°C) respectively. This study showed that polyphenol extracts from unfermented dry cocoa beans have higher polyphenol contents and stronger antioxidant activity than partially fermented cocoa bean, and can be used at 3% as natural functional food colors.

Introduction

World cocoa production averages 3.5 million tons per year from 2009 to 2012 and it was dominated by the Ivory Coast, Ghana, and Indonesia. These three countries were the first, the second, and the third largest cocoa producers, which account for 73% of overall the world's cocoa output (Manurung, 2010; Fairtrade Foundation, 2011; ICCO, 2012). World cocoa products such as cocoa powder, cocoa butter, cocoa liquor and cocoa beans are made of good quality well fermented cocoa beans (BKPM, 2010; Elshof, 2010; Keong, 2012). However, during cocoa beans processing a low-quality (partially fermented and unfermented) cocoa beans are also produced. For example 70% of cocoa beans production in Indonesia do not meet National Standard (SNI) and belongs to low quality products (Badan Kebijakan Fiskal, 2012).

The low-quality cocoa beans therefore pose not only a challenge but also an opportunity for investors to create a cocoa business with added-value. Some of the added-value include utilization of cocoa beans as source of organic acids, ethanol, polyphenols and food colours. Although good quality cocoa beans and their polyphenol content and functional activity have been well studied (Misnawi *et al.*, 2003; Othman *et al*., 2007), low quality cocoa beans have been paid less attention inspite of their abundance. Therefore the following research was carried out to compare the antioxidant and antibacterial activity of polyphenol extracts from low quality cocoa beans i.e. partially fermented and unfermented cocoa beans, and to study the properties of the extracts as natural food colors in food products with high acidity (yogurt and syrup).

Materials and Methods

Low quality cocoa beans

Partially fermented dry cocoa beans (PFDCB) and unfermented dry cocoa beans (UFDCB) were obtained from National Plantation PTPN - XII in Banjarsari and Blater, Jember - East Java, Indonesia respectively.

Chemicals

Ethanol (70%), petroleum benzene, BHT (Sigma), methanol, DPPH powder (Merck), potassium chloride buffer (pH 1.0), sodium acetate buffer (pH 4.5), sterile distilled water, sugar, commercial natural yogurt, distilled water, citric acid, citrate buffer solution (pH 3.0), antibiotics (chloramphenicol and ampicillin), Nutrient Agar (NA, Difco).

Source of bacteria

Salmonella typhimurium and *Staphylococcus*

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aureus (isolated from collection of Dr. Sarjito Hospital) were purchased from Microbiological Laboratory of Unimus, Semarang, Indonesia.

Defatted cocoa powder and polyphenol extracts (PE) preparation

Cocoa beans were sun dried to reduce water content and shelled to obtain the seed (nib). Nib was ground and defatted by soxhlet extraction with petroleum benzene (boiling point 40 - 60°C) for 4 - 6 h. The resulting defatted cocoa powder was dried by oven at 60°C for 16 h. Defatted cocoa powder was extracted by maceration in 70% ethanol (sample to solvent ratio of 1:3 (w/v)). The extraction process was carried out for 24 h at room temperature (25°C). Once the extraction was complete, it was filtered by vacuum (Misnawi *et al.*, 2003). The resulting polyphenol extracts was condensed by a rotary evaporator at 50°C and dried by vacuum oven at 60°C and designated as polyphenol extracts from low quality of cocoa beans (PE).

Determination of Total Polyphenol Content

Total polyphenol was determined by mixing 2.5 ml deionized water, 0.1 ml of PE, and 0.5 ml Folin-Ciocalteau reagent (Bintang, 2010). The mixture was then incubated in the dark for 8 minutes, after which it was mixed with 4.5 ml of 20% Na_2CO_3. Absorbance of the solution was measured at 765 nm. Tannic acid was used to generate a standard curve. Polyphenol contents were expressed as grams of Tannic Acid Equivalent (TAE) 100 g^{-1} DW.

Determination of total anthocyanin content in PE

Total anthocyanin content was determined by pH-differential method (Lee *et al*., 2005). Anthocyanin pigments undergo reversible structural transformations when the pH is changed from 1.0 to 4.5. At pH 1.0 it is in the form of colored oxonium which changes into colorless hemichetal at pH 4.5. Briefly, 1g of PE was diluted in 5 ml distilled water in 5 ml volumetric flask. A sample of 0.1 ml each was transferred into two glass tubes. One sample was adjusted with potassium chloride buffer pH 1.0, and the other sample with sodium acetate buffer pH 4.5 to make 5 ml total volume. After 15 minutes stand the absorbance of each sample was measured at 520 and 700 nm againts blank (distilled water). The absorbance of the diluted sample was calculated (A) by the following formula :

$$A: [(A_{520}\ nm - A_{700}\ nm)\ pH\ 1.0] - [(A_{520}\ nm - A_{700}\ nm)\ pH\ 4.5]$$

The monomeric anthocyanin pigment (MAP) concentration was expressed as mg cyanidin-3-glucoside g^{-1} in the original sample using the following formula:

$$MAP = (A \times MW \times DF \times 1000) / (\varepsilon \times L \times gram\ of\ sample)$$

Where MW is the molecular weight for cyanidin-3-glucoside (449,2 $g.mol^{-1}$), DF is dilution factor, and ε is the molar absorptivity (26,900 $L.mol^{-1}.cm^{-1}$), and L is pathlength in cm.

Antioxidant activity

The antioxidant activity was measured by DPPH assay (Bintang, 2010). PE were dissolved in methanol and made up into 5 ml volumes to give a concentration of 25, 50, 75 and 100 ppm phenolic equivalents. Each concentration of PE (0.1 ml) was added with 0.5 ml of 0.5 mM DPPH and 4 ml of methanol, mixed briefly, and incubated for 1 h in a dark. Absorbance was measured at a wavelength of 516 nm. BHT was used as reference antioxidant. Antioxidant activity was expressed in percentage and measured in triplicates and calculated by the following equation:

$$\text{Antioxidant activity (\%)} = \frac{\text{(absorbance of control – absorbance of sample)} \times 100}{\text{absorbance of control}}$$

IC_{50} value of antioxidant activity was determined from the regression equation between the concentration of PE and antioxidant activity (%).

Antibacterial activity

The antibacterial activity was measured by disk diffusion assay (Prihantini, 2000; Pratiwi, 2008). All works were carried out in the laminar air flow. One ml of bacterial suspension was seeded into petri dishes evenly and 15 ml of nutrient agar was subsequently added and allowed to harden. Once hardened, 7 wells of 5 mm diameter were created in each petri dish. Half ml of the following solution i.e. sterile distilled water, 25,000 ppm chloramphenicol, 25,000 ppm ampicillin, 25,000 ppm PE, 50,000 ppm PE, 75,000 ppm PE, and 100,000 ppm PE were added into the first to the seventh well respectively. One thousand ppm was equal to 1 mg/ml, so that the concentration of PE tested were equal to 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml respectively. All petri dishes were incubated at 37ºC for 24 h. Antibacterial activity was expressed as the inhibition zone in mm which was the average of triplicates measurement.

Determination of bacteriostatic and bactericidal activity

The bacteriostatic and bactericidal activity was determined by dillution method (Katsung, 1989;

Jawetz, 2005). Four test tubes were prepared and each tube contains 4.8 ml of sterile Nutrient Broth, 0.2 ml of PE (there are four concentration tested), and 0.2 ml bacteria. They were not incubated and served as control groups. Four other test tubes with the same contents were incubated at 37ºC for 24h as treatment groups. Optical Density (OD) of each tube was measured by spectrophotometer (λ = 480 nm). The difference (Δ) in OD between control and treatment groups was calculated. If $\Delta OD \leq 0$, it was further tested by measuring the OD after further incubation at 37ºC for 24 h. When the OD value ≤ 0, it is bactericidal and when the value ≥ 0, it is bacteriostatic.

Preparation and storage of PE added yogurt

PE of different concentration (1%, 2%, 3%) were added into commercial natural yogurt (no added color) in glass tubes. They were mixed well and stored in refrigerated temperature (2 - 5°C) for 4 weeks. Each week samples were taken for determination of color intensity.

Preparation and storage of PE added syrup

Three hundreds g of table sugar was added into 200 ml water, mixed by vortex, and heated to boiling. Three grams of citric acid was added during heating. The resulting syrup was allowed to cool before addition of PE. PE was added at different concentration (1%, 2%, 3%) into the syrup and mixed thoroughly. Syrup was then stored at room (25°C) or refrigerated temperature (2 - 5°C) for 6 weeks. Each week samples were taken for determination of color intensity.

Determination of color intensity of yogurt during storage

Color intensity of yogurt during storage was measured by Color Reader (Konika Minolta CR-100) method (Andarwulan *et al*., 2011), L^*, a^*, and b^* values were recorded. Chroma (C) and Hue (H) were calculated from the measured a^* and b^*, using the following equation: $C = (a^2 + b^2)^{1/2}$ and $H = \tan^{-1}[b/a]$.

Determination of color intensity of syrup during storage

Color intensity of syrup during storage was measured by spectrophotometer method (FAO, 1984). A total of 8.5 ml of citric acid solution pH 2 was put into a test tube and added with 1.5 ml of PE containing syrup. The mixture was mixed by vortex and the absorbance was measured in spectrophotometer at a wavelength of 520 nm.

Results and Discussion

Polyphenol and anthocyanin contents, antioxidant properties of PE

Polyphenol contents in PE-UFDCB showed a higher value (16,2 g TAE 100 g^{-1} DW) than PE-PFDCB (5,2 g TAE 100 g^{-1} DW). Further determination also showed that PE-UFDCB had a higher anthocyanin content (2.7 mg cyanidin-3-glucoside g^{-1}) than PE-PFDCB (0,8 mg cyanidin-3-glucoside g^{-1}). Polyphenols of cocoa beans consist of catechins (33 - 42%), leucocyanidins (23 - 25%) and anthocyanins (5%) (Misnawi *et al*., 2003). Anthocyanins gives PE-UFDCB a typical purplish colour. During cocoa bean fermentation, anthocyanins was hydrolized enzimatically into sugar and cyanidin which resulted in reduce anthocyanins content (Afoakwa *et al.*, 2012).

The antioxidant activity of PE-PFDCB showed IC_{50} value of 1160.7 ppm which was greater than IC_{50} value of PE-UFDCB (250 ppm) (Figure 1a, 1b). It indicated that higher polyphenol contents in PE-UFDCB also gave a stronger antioxidant activity. This is in line with the general knowledge that polyphenols are powerful antioxidant (Maleyki and Ismail, 2010). Furthermore, the antioxidant activity of BHT (Figure 1c) showed IC_{50} value of 540.9 ppm (0.5409 mg/ml) which was larger than IC_{50} value of PE-UFDCB (250 ppm or 0.2500 mg/ml), and smaller than IC_{50} value of PE-PFDCB (1160.7 ppm or 1,1607 mg/ml). It further confirmed that PE-UFDCB have a stronger antioxidant activity than BHT and PE-PFDB. Thus, PE-UFDCB is a powerful source of natural antioxidants which is potential to be used as food additives in beverages. Both PE-UFDCB and BHT are strong antioxidant due to their IC_{50} values <1 mg/ml (1 ppm).

Antibacterial properties

The inhibition areas of PE-PFDCB and PE-UFDCB showed a moderate and moderate to strong inhibitory activity respectively against *S. aureus* and *S. typhimurium* (Table 1). PE-UFDCB showed a stronger antibacterial activity than PE-PFDCB. Different antibacterial activity is most likely due to higher polyphenols and its anthocyanin content in each extract as well as different properties of cell wall structure of the two bacteria. The cell wall structure of gram positive *S. aureus* is more sensitive and simpler than gram negative *S. typhimurium*. A study of various pure phenolic compounds antibacterial activity by Puupponen-Pimiä *et al.* (2001) showed that in general *E. coli* is sensitive to phenolic compounds in contrast to *Salmonella* and lactic acid bacteria which are

Table 1. The test results of antibacterial activity of PE against gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*S. typhimurium*)

Test Bacteria	PE, standard, and control	Inhibition Zone (mm)			Average (mm)	Criteria
S. aureus	PE-PFDCB 25,000 ppm	8	7	8	7.7	moderate
	PE-PFDCB 50,000 ppm	8	8	10	8.7	moderate
	PE-PFDCB 75,000 ppm	11	12	15	12.7	strong
	PE-PFDCB 100,000 ppm	13	13	16	14.0	strong
	Chloramphenicol 25,000 ppm	20	19	22	20.3	very strong
	Ampicillin 25,000 ppm	24	32	31.2	29.1	very strong
	Sterile Aquadest 25,000 ppm	0	0	0	0.0	–
	PE-UFDCB 25,000 ppm	13.5	9.2	5.4	9.4	moderate
	PE-UFDCB 50,000 ppm	18	11	11.5	13.5	strong
	PE-UFDCB 75,000 ppm	15.5	15	16	15.5	strong
	PE-UFDCB 100,000 ppm	17	17.5	18.5	17.7	strong
	Chloramphenicol 25,000 ppm	24	21	27	24.0	very strong
	Ampicillin 25,000 ppm	30	29.2	32	30.4	very strong
	Sterile Aquadest 25,000 ppm	0	0	0	0.0	–
S. typhimurium	PE-PFDCB 25,000 ppm	6	6	6	6.0	moderate
	PE-PFDCB 50,000 ppm	7	7	8	7.3	moderate
	PE-PFDCB 75,000 ppm	8	7	9	8.0	moderate
	PE-PFDCB 100,000 ppm	11	13	13	12.3	strong
	Chloramphenicol 25,000 ppm	21	16	15	17.3	strong
	Ampicillin 25,000 ppm	18	15	14	15.7	strong
	Sterile Aquadest 25,000 ppm	0	0	0	0.0	–
	PE-UFDCB 25,000 ppm	14.4	13	11.2	12.9	strong
	PE-UFDCB 50,000 ppm	20	15	14	16.3	strong
	PE-UFDCB 75,000 ppm	21	17.5	18	18.8	strong
	PE-UFDCB 100,000 ppm	23	20.5	20	21.2	very strong
	Chloramphenicol 25,000 ppm	27	23	21	23.7	very strong
	Ampicillin 25,000 ppm	24.2	20	22	22.1	very strong
	Sterile Aquadest 25,000 ppm	0	0	0	0.0	–

Determination of the criteria: ≥ 20 mm: very strong, 10-20 mm: strong, 5-10 mm : moderate, ≤ 5 mm : weak.

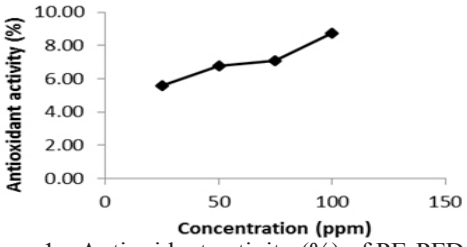

Figure 1a. Antioxidant activity (%) of PE-PFDCB, y = 0.0391 x + 4.6167

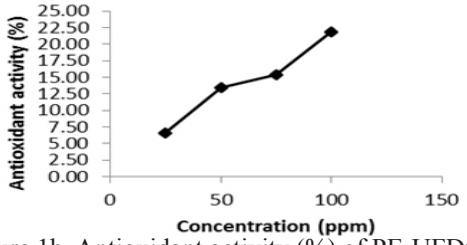

Figure 1b. Antioxidant activity (%) of PE-UFDCB, y = 0.1902 x + 2.4415

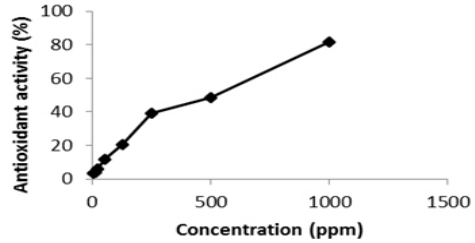

Figure 1c. Antioxidant activity of BHT as reference antioxidant, y = 0.0782x + 7.7052

resistant. In our study PE-PFDCB and PE-UFDCB at various concentrations showed bacteriostatic, but not bactericidal activity. The extracts were effective at inhibiting the growth of *S. aureus* and *S. typhimurium* in the range of 25,000 ppm to 100,000 ppm (Table 2).

Table 2. Minimal inhibitory and bactericidal levels

Bacteria	PE	Absorbance Value		ΔOD	Further Test (OD)	Antibacterial Activity
		Before Incubation	After Incubation			
S. aureus	PE-PFDCB 25,000 ppm	0,1549	0,1249	-0,0300	0,2557	Bacteriostat
	PE-PFDCB 50,000 ppm	0,2596	0,2218	-0,0378	0,2441	Bacteriostat
	PE-PFDCB 75,000 ppm	0,3372	0,3010	-0,0362	0,3188	Bacteriostat
	PE-PFDCB 100,000 ppm	0,3279	0,2798	-0,0481	0,3279	Bacteriostat
	PE-UFDCB 25,000 ppm	0,4260	0,0605	-0,3655	0,2840	Bacteriostat
	PE-UFDCB 50,000 ppm	0,4318	0,1427	-0,2891	0,3010	Bacteriostat
	PE-UFDCB 75,000 ppm	0,4437	0,2596	-0,1841	0,3372	Bacteriostat
	PE-UFDCB 100,000 ppm	0,4560	0,3565	-0,0995	0,3665	Bacteriostat
S. typhimurium	PE-PFDCB 25,000 ppm	0,1549	0,1249	-0,0300	0,2557	Bacteriostat
	PE-PFDCB 50,000 ppm	0,2596	0,2218	-0,0378	0,2441	Bacteriostat
	PE-PFDCB 75,000 ppm	0,3372	0,3010	-0,0362	0,3188	Bacteriostat
	PE-PFDCB 100,000 ppm	0,3279	0,2798	-0,0481	0,3279	Bacteriostat
	PE-UFDCB 25,000 ppm	0,4260	0,0605	-0,3655	0,2840	Bacteriostat
	PE-UFDCB 50,000 ppm	0,4318	0,1427	-0,2891	0,3010	Bacteriostat
	PE-UFDCB 75,000 ppm	0,4437	0,2596	-0,1841	0,3372	Bacteriostat
	PE-UFDCB 100,000 ppm	0,4560	0,3565	-0,0995	0,3665	Bacteriostat

When compared to antibacterial activity of the standard antibiotics (chloramphenicol and ampicillin) against *S. typhimurium*, 100,000 ppm of PE-PFDCB or 100,000 ppm of PE-UFDCB had the same criteria as the standard antibiotics (Table 2). PE-PFDCB and PE-UFDCB at 100,000 ppm had a strong and very strong antibacterial activity respectively and similar to both Chloramphenicol and Ampicillin at 25,000 ppm. On the other hand, the antibacterial activity of PE-PFDCB and PE-UFDCB against *S. aureus* at different concentrations tested were found to be less active than standard antibiotics. At the same low concentration as the standard (25,000 ppm), PE-PFDCB or PE-UFDCB showed moderate to strong antibacterial activity which is less active than the antibiotics. Such results are in line with many existing studies regarding lower antibacterial activity of natural extracts than pure antibiotic (at the same concentration as pure antibiotics).

When PE-PFDCB was compared to PE-UFDCB at each different concentration tested against both *S. typhimurium* and *S. aureus*, in general PE-UFDCB showed a stronger antibacterial acitivity than PE-PFDB. This again was likely due to the polyphenolic content of PE-UFDCB which was higher than PE-PFDCB. As anthocyanins content in PE-UFDCB was higher than PE-PFDCB it was further indicated that at least anthocyanin contributed in the difference in antibacterial strength. A study by Puupponen-Pimiä *et al.* (2001) showed that anthocyanin has a stronger inhibiton zone against *E. coli* than other pure poliphenolic class such as certain flavones and flavonols. Another study on the antibacterial activities between acetone extract and ethanol extract of cocoa pod husk against *E. coli*, *S. typhimurium*, *S. aureus*, dan *S. mutans* showed no inhibition and inhibition at 50,000 to 200,000 ppm respectively (Sartini *et al.*, 2007) indicating that different class of poliphenols in the extracts exhibited different antibacterial activities.

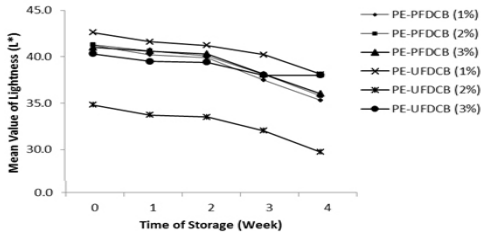

Figure 2a. Mean value of lightness (L*) of PE-PFDCB and PE-UFDCB at various concentrations during storage

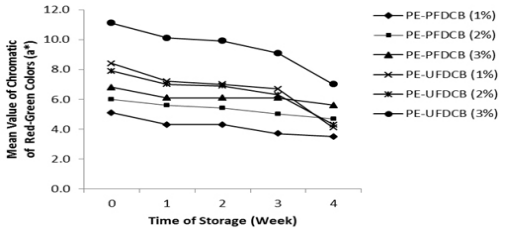

Figure 2b. Mean value of chromatic of red-green colors (a*) of PE-PFDCB and PE-UFDCB at various concentrations during storage

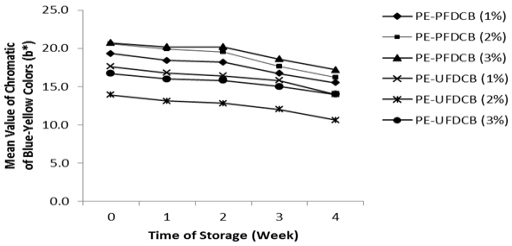

Figure 2c. Mean value of chromatic of blue-yellow colors (b*) of PE-PFDCB and PE-UFDCB at various concentrations during storage

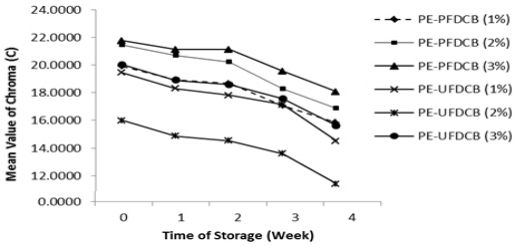

Figure 2d. Mean value of chroma (C) of PE-PFDCB and PE-UFDCB at various concentrations during storage

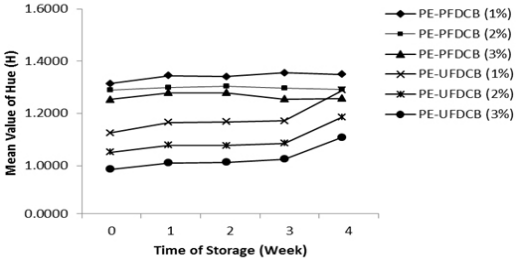

Figure 2e. Mean value of hue (H) of PE-PFDCB and PE-UFDCB at various concentrations during storage

Apart from the polyphenolic classes, the concentration of PE tested was also important. For example a study by Bubonja-Sonje *et al.* (2011) observed no inhibition zone of cocoa powder phenolic extract. However the cocoa bean phenolic extracts showed antibacterial activity with the same method. The difference in activity was partly due to the difference in concentration tested. The the cocoa powder phenolic extracts were tested at very low concentration (0.2 to 6.4 mg/ml) compared to the high concentration (100 mg/ml) of cocoa bean polyphenolic extracts. Another study applying cocoa powder (2.5% w/v) which contains 12% polyphenol combined with pulse electric field treatment in liquid whole egg/skim milk mixed beverages have been shown to increase log cycle reduction of *B. cereus* than pulse electric field treatment alone indicating polyphenol cocoa powder contribution (Pina-Pérez *et al*., 2009).

Overall our study here showed that low quality cocoa bean polyphenolic extracts at 100,000 ppm had a higher antibacterial activity which are similar to 25,000 ppm Chloramphenicol and Ampicillin. Our results also suggested that PE-UFDCB and PE-PFDCB are potential natural food preservatives.

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Color intensity of PE-PFDB and PE-UFDCB in yogurt during storage

Color intensity of PE-PFDCB and PE-UFDCB in yogurt during storage consisted of lightness (L^*), red-green colors (a^*), blue-yellow colors (b^*), chroma (C) and hue (H). The L^* values showed the brightness, a^* and b^* values showed the chromatic colors, while chroma and hue showed the chromatic colors intensity.

The mean value of L^* of PE-PFDCB and PE-UFDCB at various concentrations decreased during storage (Figure 2a). This decrease could be due to the destruction of pigments in PE-PFDB and PE-UFDCB. The brightness of PE-PFDCB and PE-UFDCB also diminished and made yogurt color faded and paled. The high fat content in milk and other dairy products such as yogurt can mask pigments in PE-PFDCB and PE-UFDCB. The presence of protein and available reducing sugars in the yogurt also could trigger the Maillard reaction which reduced brightness.

The mean value of chromatic colors (a^* and b^* value) of PE-PFDCB and PE-UFDCB at various concentrations was in the range of positive values. This means that the color of both extracts contain redness and yellowness. There was a gradual continuous decline of chromatic colors during storage (Figure 2c, 2b) which indicated that pigments in PE-PFDCB and PE-UFDCB lost the red and yellow colors. Visually, yogurt color faded in the fourth week. The mean value of chroma of PE-PFDCB and PE-UFDCB at various concentrations also decreased during storage (Figure 2d). This might be affected by the decrease in chromatic colors of PE-PFDCB and PE-UFDCB. The decrease occurred continuously during storage and indicated that pigments in PE-PFDCB and PE-

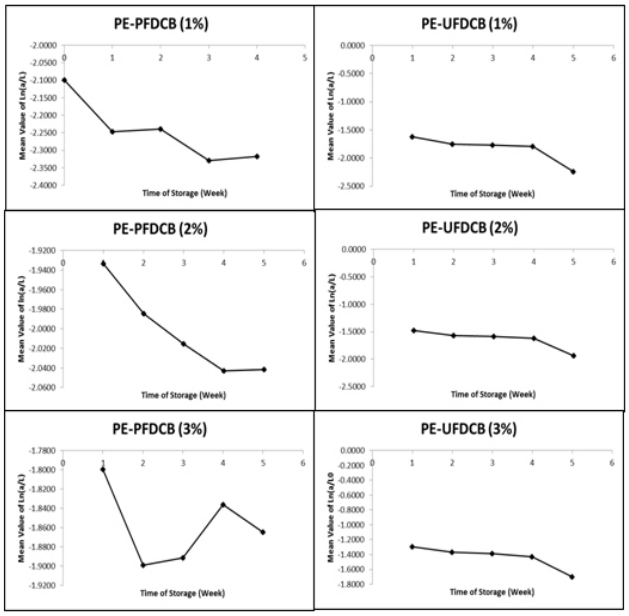

Figure 3. The relationship between decline of Ln (a/L) value and storage time

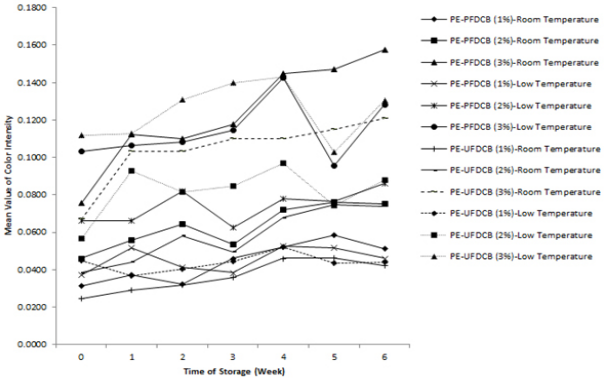

Figure 4. Color intensity of PE at various concentrations and temperatures during storage

UFDCB lost the chromatic colors intensity.

The mean value of Hue of PE-PFDCB and PE-UFDCB at various concentrations represented the color of anthocyanin (pelargonidin, cyanidin, peonidin), red and red-yellow (according to plot colors on the Hue, Saturation and Value color system). The average value of Hue of PE-PFDCB and PE-UFDCB at different concentrations increased during storage (Figure 2e). The increase indicated that pigments in PE-PFDCB and PE-UFDCB turned to yellow due to a shift in equilibrium to pseudobasa and khalkone. Overall, 3% of PE-PFDCB and PE-UFDCB had high L^*, a^*, b^* values and low Hue. It showed both of them had high lightness, redness, yellowness, strong chromatic color intensity, and chromatic color. Thus, 3% of PE-PFDCB and PE-UFDCB during storage showed the best color stability. Our results were similar to that by Hassani and Sharifi (2012) who found that 3% anthocyanin extract of barberry fruit in jelly dessert, milk dessert, soft ice cream, hard ice cream, and yogurt showed the best color stability

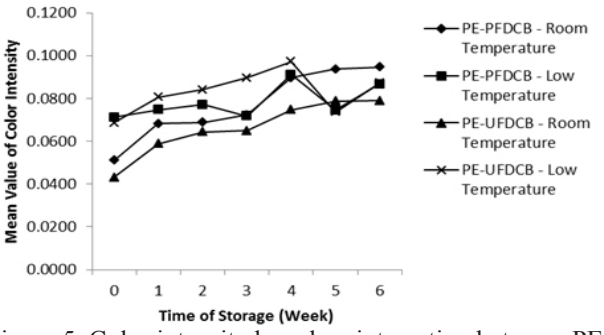

Figure 5. Color intensity based on interaction between PE and temperature

during storage (brightness, the degree of red, yellow degree, chromatic colors and intensity). The extracts were stable at storage temperature of 0 - 4°C for jelly dessert, milk desserts, and yogurt and -21°C for soft ice cream and hard ice cream.

The relationship between the decline in the value of a/L and storage time was a negative linear relationship which showed the color stability of PE and pigment half-life in PE during storage (Figure 3). The relationship between Ln (a/L) with storage time could represent the kinetics of degradation of pigments in PE-PFDCB and PE-UFDCB. This relationship was relatively more gentle and stable for PE-UFDCB than PE-PFDCB.

Color intensity of polyphenol extracts from cocoa beans in syrup

Three percent of PE-PFDCB at room temperature and PE-UFDCB at refrigerated storage showed the most stable color intensity among the twelve combined treatment (Figure 4). Color intensity of PE-PFDCB at various concentrations was more stable at room temperature storage, while the color intensity of PE-UFDCB was more stable at refrigerated storage. However, there was a decrease in the color intensity of either PE-PFDCB or PE-UFDCB at low temperature in the fifth week (Figure 5).

Temperature has an influence on anthocyanins in food. The exact mechanism of anthocyanin degradation by temperature is not fully understood, but color degradation is probably due to changes in anthocyanin pigments in red flavium cations into khalkone, carbinol and eventually becomes colorless (Puspita *et al*., 2004). As explained in the previous discussion that the change in color intensity (especially anthocyanin) is not only affected by temperature but also by other factors such as pH, oxidizing agents, and light. In this case, light is likely to be the factor that may affect the change.

Conclusions

Polyphenol extracts from unfermented dry cocoa

beans have higher polyphenol contents and stronger antioxidant and antibacterial activity than from partially fermented cocoa beans and can be used as natural functional food colors.

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