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Composition, color and antioxidant properties of cocoa shell at different roasting temperatures

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Abstract

Cocoa shell is underexploited and is considered as waste. However, roasting cocoa is a fundamental step in producing cocoa products. Composition of the cocoa shell depends on the origin whereas cocoa processing involves fermentation, drying and roasting. However, scarce research has been conducted to analyze the composition of Malaysian cocoa shells at different roasting temperatures. Therefore, this research aims to determine the effect of different roasting temperatures (110, 120, 130, 140 and 150°C) on the proximate composition, colour and antioxidant activity of cocoa shell. Changes in antioxidant activity and polyphenol content were recorded. Fiber and moisture content were significantly (p<0.05) higher in unroasted cocoa shell. However, the protein was found to decrease significantly (p<0.05) after roasting at 150°C (10.93%). Fat content was found higher in roasted cocoa shell at 150°C (6.82%) compared to the unroasted cocoa shell (6.09%). The colour (L value) recorded significantly (p<0.05). the highest for unroasted cocoa shell (51.28) and the lowest at 150°C (47.93), DPPH radical scavenging activity of ethanolic extract of cocoa shell was significantly (p<0.05) higher in 0% ethanol concentration compared to other ethanol concentrations (20, 50, 80 and 100%). The roasted cocoa shell may be considered as a source of natural antioxidants for functional food development and intermediate food ingredient.

1. Introduction

Cocoa (*Theobroma cacao* L.) is an essential agricultural product in the economies of several countries such as Ghana, Ivory Coast, Nigeria, Indonesia and Malaysia. Malaysia is known as the fifth largest producer of cocoa beans in the world. Malaysia is also one of the leading producers of cocoa-based products in the world and is considered as the biggest in Asia (Othman *et al*., 2007). Cocoa beans contain biologically active compounds such as polyphenols and tocopherols that have a positive impact on human health.

The cocoa shell is obtained during the breaking and winnowing process of cocoa bean. Several tons of the cocoa bean shells are disposed of as waste every year. Cocoa shells are also utilized as animal feed, production of fuel briquettes, fertilization and improvement of soil, heavy metal sorption in wastewater, alcohol production, furfural, theobromine, thermoset resins, acoustic and thermal insulation panels, paper, pasta ingredients and to prevention of deposit formation in pipelines (Hamzat *et al*., 2011).

Cocoa bean roasting is another fundamental step in producing cocoa products, which causes several chemical changes, such as a change in water content, volatile acids, tannins, phenolic compounds, color, aroma and texture. The roasting process influences the ability of polyphenols to interact with protein thus leading to a decrease in astringency (Jati, 2009). Other than that, the extracting solvent has been reported to affect the total phenolic content and antioxidant capacity of cocoa beans significantly (Othman *et al*., 2007). It was reported that the water extract of *Terminalia chebuta* showed high antioxidant activity compared to methanolic extracts of *Lycopersicon esculentum* (Cai *et al*., 2004) Moreover, from a toxicological point of view, ethanol and water are safer for human consumption when compared to acetone, methanol and other organic solvents (Oktay *et al*., 2003). In addition, the extraction method is critical for the availability of phytochemicals in producing dietary supplements, nutraceutical products, food ingredients, pharmaceutical, and cosmetic products. Selecting the right solvent affects the amount and rate of polyphenols extracted (Dai and Mumper, 2010). In

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addition, various roasting conditions cause changes in flavanols, proanthocyanidin contents and antioxidant activity in the cocoa shell. However, a limited amount of research has been done on the effects of different roasting temperatures on Malaysian cocoa shells. Therefore, the objectives of this study were: (i) to determine the effects of different roasting temperatures on proximate composition and color; (ii) to evaluate the antioxidant activity at different concentrations of ethanolic extract for the roasted and unroasted cocoa shell.

2. Materials and methods

2.1 Cocoa bean samples

The preparation of roasted cocoa shells was carried out according to Harrington (2011). A batch of 400 g of unroasted cocoa beans was purchased from Jengka Pahang, Malaysia for each analysis and placed on a wired mesh tray. The wired mesh tray was then placed in the oven (Memmert, Germany) that had been preheated prior to the appropriate roasting temperatures (110˚C, 120˚C, 130˚C, 140˚C, 150˚C) for 45 mins. The selected roasting time and temperatures were selected based on previous studies done by Krysiak (2006). The mesh tray was used to promote even distribution of hot air around the cocoa beans during the roasting process. The roasted cocoa beans were then removed from the oven and left to cool at room temperature. The unroasted cocoa shell was used as a control. Shells of unroasted and roasted cocoa beans were removed manually before it was ground using a blender (Panasonic, MX-900M). The beans were tapped with the rolling pin to crack the shell, leaving only roasted cocoa nibs. The roasting process was conducted in triplicates for each roasting temperature with similar roasting time. The weight of cocoa bean during roasting was recorded as shown in Table 1.

Table 1. Weight of unroasted and roasted cocoa bean and shell

Type of cocoa	Weight (g)
Unroasted cocoa bean with shell	400.3±0.7
Roasted cocoa bean without shell	285.7±0.6
Roasted cocoa shell	58.9±0.4
Roasted cocoa shell powder	57.7±0.5

The roasted cocoa shells (58.9±0.4 g) were ground to a fine powder form (57.7±0.5 g) using a grinder (Model EM-11, SHARP, Johor, Malaysia). Approximately 5 g of cocoa shell powder was used in each analysis such as proximate analysis, colorimetry and radical scavenging activity (DPPH and TPC), packed and stored in the dark at room temperature before further analysis.

2.2 Proximate analysis

Protein, fiber, fat, moisture and ash content of cocoa shells were obtained by using AOAC methods (AOAC, 1997). Protein content determination was performed according to the Kjeldahl method. Fat content was calculated by weight loss after a 6-cycle extraction with petroleum ether using the soxhlet method. Moisture content was determined by drying a 3 g sample at 105°C to constant weight. Ash analysis was performed in a blast furnace at 550°C for 4 hrs. The crude fiber was determined according to AOAC methods (AOAC, 1997).

2.3 Color measurement

The color of the roasted cocoa shell at different roasting temperatures was measured using UltraScan PRO (HunterLab, USA) colorimeter after calibration against white and black glass standards. The colors were expressed in CIELAB color values (L, a, b) where the L-value represents the lightness to darkness gradation, a-value represents the greenness to redness spectrum and the b-value represents the blueness to yellowness spectrum. The color values (L, a, and b) are the three dimensions, which gives specific color values of the products.

2.4 Cocoa shell extraction for antioxidant analysis

In this study, different concentrations of ethanol (Merck, Darmstadt, Germany) (0, 20, 50, 80 and 100%) have been applied to the cocoa shell at different roasting temperatures (unroasted, 110, 120, 130, 140 and 150°C). The extraction of the cocoa shell was carried out using binary ethanol and water solvent extraction system according to Othman *et al*. (2007) with slight modification. Cocoa shell powder of 1 g was weighed accurately using analytical balance (Sartorius Goettingen, Germany) and transferred into a 100 mL conical flask and wrapped with aluminum foil to prevent light degradation during extraction. Extraction was carried out with 1:10 (w/v) sample-solvent ratio. The mixture was homogenized using a water bath shaker (SI500, Stuart, Staffordshire UK), rotating at 100 rpm for 1 hr at room temperature (21°C). The extract was then transferred to a 50 mL centrifuge tube and centrifuged at 2500 rpm for 10 mins. After that, the extracts were filtered using a Buchner funnel lined up with Whatman no. 1 filter paper. The filtrate was considered as cocoa shell extract and used for further analysis.

Figure 1 shows the preliminary study that has been done to obtain the optimum ethanolic extraction for the lowest roasting temperature at 110°C and the highest roasting temperature at 150°C for the unroasted and roasted cocoa shell. In this study, 0% ethanol concentration (only distilled water) showed significantly (p<0.05) the highest phenolic content for unroasted and roasted cocoa shells at 110°C and 150°C.

On the other hand, Figure 2 displays the preliminary study on antioxidant activity by using DPPH assay on different ethanol concentrations (0, 20, 50, 80 and 100%) on the unroasted and roasted cocoa shell at 110°C and 150°C. From Figure 2, antioxidant activity in 80% ethanol concentration displayed significantly (p<0.05) highest value. This result indicates that the use of appropriate solvent influence greatly the phenolic yield and antioxidant activity.

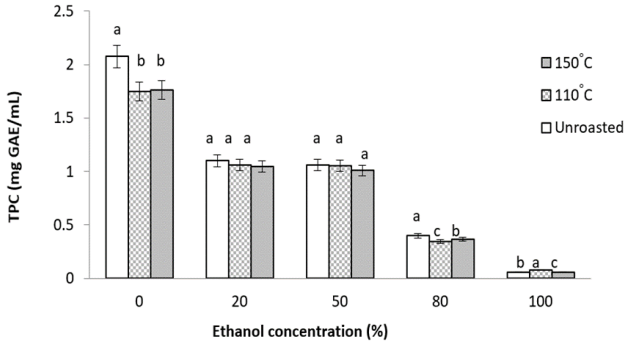

Figure 1. Total phenolic content of cocoa shells at different ethanol concentrations and roasting temperatures (unroasted, 110°C and 150°C). Values are presented as mean ± standard deviation of five measurements. Each column with different letters at different ethanol concentration (0 - 100%) indicates a significant different (p<0.05)

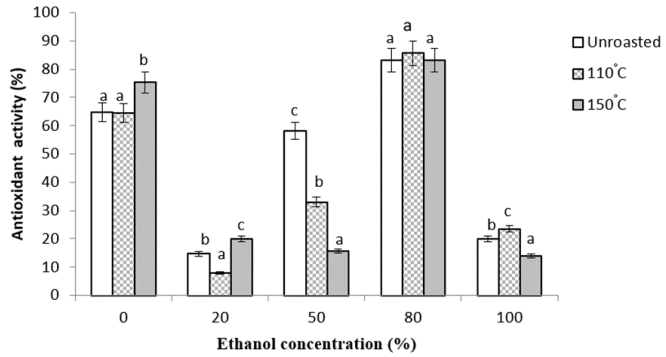

Figure 2. Antioxidant activities of cocoa shells at different ethanol concentrations and roasting temperatures (unroasted, 110°C and 150°C). Values are presented as mean ± standard deviation of five measurements. Each column with different letters at different ethanol concentrations (0 - 100%) indicates a significant different (p<0.05)

2.5 Total phenolic content analysis

In this study, total phenolic content was identified using Folin-Ciocalteu assay according to Martinez *et al*. (2012) with slight modification, in which the absorbance was measured at 725 nm (Harrington, 2011). Folin-Ciocalteu reagent was prepared by tenfold diluted with distilled water, where 10 mL of FCR was added into 100 mL of distilled water. On the other hand, a 7.5% sodium carbonate solution (Na_2CO_3) was prepared by dissolving 7.5 g anhydrous sodium carbonate completely in 100 ml distilled water. A volume of 200 µL of a sample (cocoa shell extract) was introduced into the test tubes, followed by adding 1.5 µL of Folin–Ciocalteau reagent. The mixture was allowed to settle for a better reaction for 5 mins. After 5 mins, 1.5 mL of a 7.5% sodium carbonate solution was added. The sample was incubated in a dark room at room temperature for 30 mins. The absorbance of the sample was measured at 725 nm using a spectrophotometer (Genesys 20 model 4001/4, Thermo Scientific, USA). TPC results were expressed in gallic acid equivalents (mg GAE/g) using a gallic acid standard curve.

2.6 DPPH radical scavenging capacity

The radical scavenging activity of the cocoa shell extracts was determined as described by Lai and Lim (2011) and Chan *et al*. (2012) with slight modification. In the preparation of the DPPH solution, 5.2 mg of DPPH powder was dissolved into 100 mL ethanol and sonicated for 30 mins for homogenization. Accurately 1 mL of DPPH was added to 1 mL extract in the aluminum foil coated test tubes and shaken vigorously. The tubes were kept in the dark at room temperature for 30 mins. The control was prepared as above without any extract. The changes in the absorbance of the samples were measured at 517 nm using a UV-160A spectrophotometer (Shimadzu Corp., Nagakyo-ku, Kyoto, Japan). Radical scavenging activity was expressed as the inhibition percentage. A graph of scavenging activity (%) versus concentration was plotted and the amount of free radical scavenger required for initial DPPH concentration by 50% (EC_{50} values) was obtained from the equation.

$$\% \text{ of inhibition} = (A_0 - A_1 / A_0) \times 100 \quad (1)$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

2.7 Statistical analysis

The experimental design was completely randomized. Each assay was performed in triplicate. Statistical analysis and comparisons among means were carried out using the statistical package Minitab 16 (Mtb EXE.Ink, USA, 2011). The collected data was examined by one-way analysis of variance (ANOVA, 2012). Tukey's post hoc test was used for comparisons of means, and differences are considered significant at the level of p<0.05.

3. Results and discussion

3.1 Proximate analysis

Table 2 shows the chemical compositions of the cocoa shell. The fiber in plants or food is an indication of various types of non-digestible carbohydrates and lignin

Table 2. Proximate composition of cocoa shell at different roasting temperatures

Roasting Temperature (°C)	Mean ± Std. Dev.				
	Fiber	Protein	Fat	Moisture	Ash
Unroasted	64.35±0.71^{a}	18.00±0.22^{a}	4.09±0.16^{d}	11.11±0.19^{a}	10.22±0.38^{a}
110	28.74±0.51^{e}	17.44±0.57^{a}	4.86±0.49^{cd}	7.33±0.57^{b}	10.11±0.19^{a}
120	28.81±0.63^{e}	17.17±0.25^{ab}	5.48±0.51^{bc}	6.33±0.57^{bc}	10.78±0.19^{a}
130	32.01±0.71^{d}	16.35±0.66^{b}	6.46±0.52^{ab}	5.89±0.19^{cd}	10.44±0.51^{a}
140	59.39±0.25^{c}	14.89±0.21^{c}	6.79±0.07^{a}	5.55±0.19^{cd}	9.99±0.57^{a}
150	61.21±0.81^{b}	10.93±0.01^{d}	6.82±0.26^{a}	4.89±0.19^{d}	10.89±0.38^{a}

Means followed by different letter within a same column indicates a significant different between samples (p<0.05).

(Akpabio and Ikpe, 2013). There was a significant difference (p<0.05) between fiber content of unroasted cocoa shell (64.35%) and roasted cocoa shell at roasting temperature 150°C (61.21%) followed by at 140°C (59.39%), 130°C (32.01%), 120°C (28.81%) and 110°C (28.74%), respectively. Current results show the fiber content in the cocoa shell (roasted and unroasted) higher than results from Agus *et al*. (2018), who stated that the fiber content 16.06% for roasted (140°C) and 13.86% for unroasted Malaysian cocoa shells. So, the fiber content can be affected by a different type of beans and quality of cocoa processing. Balentic *et al*. (2018) mentioned that the proportion of fiber in the cocoa shell is related to the roasting condition, whether there are roasted or not. However, one reason is that the total fiber included the "Klason lignin". Since we used roasted shells, the formation of Maillard products could augment this fraction. It may, therefore, is expected that the "Klason lignin" value in our study would be increased. This statement is in agreement with Redgwell *et al*. (2003) that shown the roasted cocoa shells contain approximately 40% of total dietary fiber, which is due to Klason lignin produced from Maillard reaction via roasting. According to Coimbra and Jorge (2011), the intake of food that is containing high dietary fiber related to less risk of several pathologies.

According to Table 2, the protein content (%) in Malaysian roasted and unroasted cocoa shell showed the significant decline from unroasted cocoa shell (18%) followed by 110°C (17.44%), 120°C (17.17%), 130°C (16.35%), 140°C (14.89%) and 150°C (10.93%), respectively. According to Serra Bonvehi (1998), Ecuador's unroasted cocoa shells contain 17.6% of protein, which is in agreement with our finding regarding 18% protein content for unroasted Malaysian cocoa shells. In contrast, Agus *et al*. (2018) concluded that the result of protein content in Malaysian cocoa shells is 25.07% for roasted (140°C) and 27.43% for unroasted, respectively. In addition, the protein content of Malaysian cocoa shells decreased from 18% to 10.93% after the roasting process at 150°C.

The fat content of cocoa shells at different roasting temperatures (110, 120, 130, 140, 150°C) is shown in Table 2. The significant (p<0.05) higher fat content (%) were found in the roasted cocoa shell at 150°C (6.82%) followed by 140°C (6.79%). On the other hand, the lowest fat content is related to the unroasted cocoa shell (4.09%), significantly (p<0.05). However, the migration of fat from the nib to the shell increased at a temperature of 150°C (6.82%), which is not a favorable condition, especially in cocoa butter production. The result was in agreement with Agus *et al*. (2018), who obtained 4.78% fat from unroasted Malaysian cocoa shells. Fat is essential macromolecules in cocoa production which contribute as a quality index for cocoa processor while purchasing of unroasted cocoa beans (Afoakwa *et al*., 2007).

Table 2 shows a significant (p<0.05) decreasing trend in moisture content of unroasted cocoa shell (11.11%) followed by 110°C (7.33%), 120°C (6.33%), 130°C (5.89%), 140°C (5.55%) and 150°C (4.89%). These results were in agreement with Joel *et al*. (2013), who reported that the average moisture contents in raw, dried and roasted beans were 28.80%, 18.14% and 7.11%, respectively. Moisture is required for the biochemical changes that affect the sensory and shelf quality of the cocoa products (Joel *et al*., 2013). According to Abiola and Tewe (1991), key factors for extending the shelf life of cocoa powder are the control of moisture content and the crystalline nature of the moisture in cocoa powder. However, no significant difference (p>0.05) was found between ash content and various roasting temperatures applied in this study.

3.2 Colorimetric

According to Table 3, the L-value was the highest at unroasted (51.28) followed by 110°C (50.23), 120°C (49.21), 130°C (48.85), 140°C (48.50) and finally 150°C (47.93), respectively. However, there was a significant (p<0.05) different between the L-value of unroasted (51.28) and roasted at 150°C (47.93) cocoa shell. Krysiak *et al*. (2013) mentioned that the cocoa bean browning rate is determined by the temperature and duration of temperature action (110-130°C). Zzaman and

Table 3. Effects of selected roasting temperatures on color parameters of cocoa shell

Color	Roasting temperatures (°C)					
	Unroasted cocoa shell	110	120	130	140	150
L	51.28±0.83^{a}	50.23±0.49^{ab}	49.21±0.53^{bc}	48.85±0.41^{c}	48.50±0.31^{c}	47.93±0.37^{c}
a*	7.54±0.09^{ab}	7.60±0.14^{ab}	7.31±0.19^{b}	7.48±0.20^{ab}	7.73±0.92^{a}	7.78±0.15^{a}
b*	8.78±0.16^{c}	9.43±0.11b^{c}	10.17±0.68^{ab}	10.5±0.44^{ab}	9.76±0.43^{abc}	10.95±0.73^{a}

Means followed by different letter within the same column indicates a significant difference between samples (p<0.05). L values measure the lightness of the cocoa shell. The a value refers to the redness of the cocoa shell. The b value measures the yellowness of the cocoa shell.

Yang (2014) observed a reduction in the L-value (darkening) of the cocoa beans at higher roasting temperatures, 200°C and 250°C.

In the current study, the reduction of L-value during the roasting process (110-150°C) could be due to the denaturation of protein, the amount of oil particles surrounded in protein matrix or low moisture content. All those parameters can be the cause of the dark color of cocoa shells during the roasting procedure. Zzaman and Yang (2014) stated that the increase in a-value was correlated with the decrease in the L value during the roasting process of cocoa beans. This statement supports our findings that roasting cocoa shell at 150°C was result in the lowest L-value (47.93) with the highest a-value (7.78) as a comparison to unroasted (7.54) and roasted cocoa shell at 110˚C (7.60), 120˚C (7.31), 130˚C (7.48) and 140˚C (7.73).

Table 3 also shows the variation in yellowness which is the b-value during cocoa shell roasting. The b-value was found to increase with the increase of roasting temperature. The highest b-value was found at 150˚C (10.95±0.73) followed by 130°C (10.59) and 120°C (10.17). A significant difference (p<0.05) was found at a roasting temperature of 150°C when compared between roasted (10.95) and unroasted cocoa shells (8.78). Zzaman and Yang (2014) indicated that time and temperature significantly (p<0.05) affect the color values (L, a, b) of cocoa beans during roasting. Moreover, De Muijnck (2005) stated that the color of cocoa powder is affected by the optical effect in which the fat on the solid particles influences the light absorption. The higher the fat content, the color of cocoa powder appear darker.

Therefore, changes in L, a and b values indicated a color change during the exposure of the cocoa shell powder to heat. These results are following De Muijnck (2005), stating that when cocoa powder is exposed to different roasting temperatures, discoloration occurs pertaining to the change in crystalline size or form of the cocoa butter. However, Krysiak (2006) mentioned that the roasting process is a crucial technological operation in the processing of cocoa beans since it influences the formation of characteristic brown color, mild aroma and texture of roasted cocoa beans. He also described that oxidation of polyphenol, polymerization, protein degradation, Maillard reaction and starch dextrinization contributes to the production of brown pigment which is an identifying mark of cocoa bean roasting. Davey *et al*. (2000) reported that the heating process which causes the phytochemicals (polyphenol) damage are a combination of components migration and contributes to loss or damage by variety chemical reactions involving enzymes, light and oxygen. According to Krysiak (2006), color alteration in roasted cocoa beans at 110°C, 135°C and 150°C is the effect of the brown pigment. Therefore, those causes could be due to color changes of roasted cocoa shells in our findings.

3.3 Evaluation of antioxidant activity of ethanolic cocoa shell extract

3.3.1 DPPH antioxidant activity and total phenolic content in cocoa shells at different ethanol concentrations and roasting temperatures

Different antioxidants testing methods resulted in different ranking orders of antioxidant capacity due to different experimental conditions. Therefore, two methods; DPPH and TPC were used in this experiment to compare the antioxidant activity of the unroasted and roasted cocoa shell at various temperatures such as 110° C, 120°C, 130°C, 140°C and 150°C. Ethanol and distilled water ranging from 0% to 100% (v/v) were used to extract the roasted and unroasted cocoa shell. DPPH radical scavenging activity and total phenolic content (TPC) were used as markers to determine the effect of the solvent at different concentrations and roasting temperatures.

Table 4 presents the effect of selected ethanol concentration (100%, 80%, 50%, 20% and 0%) on DPPH antioxidant scavenging of unroasted and roasted cocoa shell at different roasting temperatures (unroasted and roasted at 110°C, 120°C, 130°C, 140°C and 150°C) expressed as the mg of Gallic Acid Equivalent (GAE) per mL. In this study, lower EC_{50} value obtained from the DPPH assay indicates higher antioxidant activity. EC_{50} values of unroasted and roasted cocoa shell extract showed a decreasing trend when the ethanol concentrations were increased. The 0% ethanol concentration showed significantly (p<0.05) the highest

Table 4. Effects of different ethanol concentrations and roasting temperatures on DPPH scavenging activity (EC50) of cocoa shell

Roasting conditions (°C)	Ethanol concentrations (%)				
	0	20	50	80	100
Unroasted	83.26±0.00^{a}	64.77±0.00^{a}	58.22±0.02^{a}	20.08±0.01^{bc}	14.70±0.00^{bc}
110	85.74±0.00^{c}	64.48±0.00^{a}	33.04±0.01^{b}	23.58±0.01^{cd}	8.00±0.00^{a}
120	84.86±0.00^{b}	75.40±0.00^{b}	19.80±0.00^{c}	18.49±0.00^{ab}	12.52±0.00^{b}
130	85.74±0.00^{c}	79.77±0.00^{c}	35.52±0.01^{b}	17.18±0.00^{ab}	17.47±0.00^{cd}
140	84.72±0.00^{b}	74.96±0.00^{b}	40.76±0.00^{d}	25.91±0.00^{d}	16.45±0.00^{cd}
150	83.11±0.00^{a}	75.40±0.00^{b}	15.72±0.01^{c}	13.97±0.00^{a}	20.09±0.00^{d}

Results are expressed in Gallic Acid Equivalent (GAE). Values are expressed as mean ± standard deviation (n=3). Means followed by different letter within a same column are significantly different from one another at $p<0.05$.

EC_{50} (83.11 mg GAE/mL) at roasting temperature 150°C compared to 100% ethanol concentration, which showed the lowest EC_{50} (20.09 mg GAE/mL). Furthermore, the lowest EC_{50} in each ethanol concentration is related to the highest roasting temperature (150˚C). However, in 100% ethanol concentration, the lowest EC_{50} was at a roasting temperature of 110°C (8.00 mg GAE/mL) while the highest EC_{50} was found at a roasting temperature of 150°C (20.09 mg GAE/mL) with significant ($p<0.05$) different.

The antioxidant activity was determined by measuring the absorbance value of the DPPH solution after mixing with antioxidant compounds. A freshly prepared DPPH solution exhibits a deep blue color in the ethanol medium. Antioxidant molecules can quench DPPH free radicals (by providing hydrogen atoms or by electron donation, via a free radical attack on the DPPH molecule) and convert them to colorless (2, 2-di phenyl-1-hydrazine or substituted analogous hydrazine) which result in a decrease in absorbance at 517 nm.

Table 5 shows the effects of selected ethanol concentration (100%, 80%, 50%, 20% and 0%) on total phenolic content (TPC) of the unroasted and roasted cocoa shell at different temperatures expressed as the mg of Gallic Acid Equivalent (GAE) per ml. The total phenolic content in cocoa shell extracted from 0% ethanol concentration was significantly the highest ($p<0.05$) in unroasted (2.07 GAE/mL) and roasting temperatures compared to the cocoa shell extracted from other ethanol concentrations. These results proved that the most suitable extraction method to obtain a high total phenolic content of cocoa shells was without the presence of ethanol. There are numerous studies in which the antioxidant activity of the cocoa has been determined (Schinella *et al*., 2010). Though, several studies showed the antioxidant activity of the cocoa by-products rather than the cocoa shell itself.

Sun and Ho (2005) reported that antioxidant activity and yield of the phenolic content of buckwheat are affected by the different extracting solvent used. For example, the water extract of *Terminalia chebuta* showed high antioxidant activity, compared to methanolic extracts of *Lycopersicon esculentum* (Cai *et al*., 2004). Moreover, from a toxicological point of view, ethanol and water are safer than acetone, methanol and other organic solvents (Oktay *et al*., 2003).

Lee *et al*. (2003) compared the antioxidant effects of cocoa water extract against water extract obtained from red wine, green and black tea. They observed a high activity of the cocoa extract for both ABTS and DPPH tests, most likely due to high content in phenolics. In addition, Karim *et al*. (2014) stated that the antioxidant activity of cocoa shells could be reduced by various factors such as roasting and fermentation processes which might degrade the potentially active compound. As we can observe in current findings, total phenolic

Table 5. Effects of different ethanol concentration and roasting temperatures on total phenolic content (TPC) of cocoa shell

Roasting conditions (°C)	Ethanol concentrations (%)				
	0	20	50	80	100
Unroasted	83.26±0.00^{a}	64.77±0.00^{a}	58.22±0.02^{a}	20.08±0.01^{bc}	14.70±0.00^{bc}
110	85.74±0.00^{c}	64.48±0.00^{a}	33.04±0.01^{b}	23.58±0.01^{cd}	8.00±0.00^{a}
120	84.86±0.00^{b}	75.40±0.00^{b}	19.80±0.00^{c}	18.49±0.00^{ab}	12.52±0.00^{b}
130	85.74±0.00^{c}	79.77±0.00^{c}	35.52±0.01^{b}	17.18±0.00^{ab}	17.47±0.00^{cd}
140	84.72±0.00^{b}	74.96±0.00^{b}	40.76±0.00^{d}	25.91±0.00^{d}	16.45±0.00^{cd}
150	83.11±0.00^{a}	75.40±0.00^{b}	15.72±0.01^{c}	13.97±0.00^{a}	20.09±0.00^{d}

Results are expressed in Gallic Acid Equivalent (GAE). Values are expressed as mean ± standard deviation (n=3). Means followed by different letter within a same column are significantly different from one another at $p<0.05$.

content was reduced when the roasting temperatures were elevated.'

Awarikabey *et al*. (2014) stated that the antioxidant activities of Ghanaian unroasted cocoa nib (72.92±0.87 mg AAE/g) and the unroasted cocoa shell (55.26±0.72 mg AAE/g) were higher than the roasted cocoa shell (45.81±0.84 mg AAE/g) or cocoa shell that was subjected to infrared radiation (52.27±1.34 mg AAE/g) respectively. They suggested that as the beans undergo different temperature treatments, the phenolic content and antioxidant constituents are destroyed. In addition, high processing temperatures or longer processing time reduce the amount of polyphenols in cocoa. It is well recognized that the DPPH radical scavenging assay defined free antioxidants in products, while the Folin-Ciocalteu assay reagent determines both free and phenolic compounds (Singleton *et al*., 1999). The antioxidants in cocoa by-products may not contribute to radical scavenging activity in the DPPH assay.

According to Djikeng *et al*. (2018) who demonstrated that the decline in antioxidant activity of cocoa beans during roasting was associated with the destruction of polyphenols contained in them. On the other hand, Bauerin *et al*. (2016) confirmed that roasting is considered one of the stages in the processing of cocoa beans that leads to a high loss of phenolic compounds and a decrease in an antioxidant activity which is inconsistence with current results. Total phenolic content is significantly the lowest at 150°C compared to 140°C, 130°C, 120°C and 110°C, respectively. Similar conclusions were achieved by Arlorio *et al*. (2008) who was compared the antioxidant capacity of roasted and unroasted cocoa beans

In addition, Othman *et al*. (2007) reported that not only ethanolic extracts of cocoa bean showed higher scavenging activity than aqueous extracts, but also Malaysian beans (2.4±0.1 mg/mL) had a significantly (p<0.05) higher EC_{50} compared to Ghanaian beans (1.7±0.01 mg/mL).

In addition, different roasting conditions produced differences in flavanols, proanthocyanidin contents and antioxidant activity in the cocoa shell. In particular, Ioannone *et al*. (2015) stated that high temperature-short time (HTST) roasting at 145°C for 9 mins had better preserved the phenolic content of cocoa shells from Italy than low temperature-long time (LTLT) at 125˚C for 74 mins. Therefore, high temperature-short time processes (145°C for 9 mins) are recommended for preserving the functional properties of cocoa shell upon roasting in order of polyphenol bioavailability and function in human's metabolism. Thus, explaining the range of temperature chosen for this research. Ismail and Yee (2006) stated that the bioactive compounds found in the extract of cocoa shells could be flavonoids, which showed high antioxidant activity *in-vitro*.

4. Conclusion

The fiber content was found significantly (p<0.05) higher in the unroasted cocoa shell (64.35±0.71 g/100 g dry matter) than a roasted cocoa shell at 150°C (61.21±0.81 g/100 g dry matter). Moreover, significantly (p<0.05) higher protein, fat and moisture content were found in unroasted (18.00±0.22 g/100 g dry matter), 150˚C (6.82±0.26 g/100 g dry matter) and unroasted cocoa shell (11.11±0.19 g/100 g dry matter) respectively. Roasted cocoa shells had potential antioxidant activity due to the presence of the phenolic compound. The 0% ethanol concentration (only distilled water) showed significantly (p<0.05) the highest phenolic content compared to the other three selected roasting temperatures. Cocoa shells could be used as a natural antioxidant source in functional food development, such as nutraceuticals and preservatives in food formulations. Therefore, it is recommended to control all processes involved in cocoa processing such as roasting temperature so that the quality of cocoa beans and cocoa shells will not be reduced.

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