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Effects of Processing Time and Temperature on Flavanol and Procyanidin, Proanthocyanidin and Antioxidant Activity of Cocoa Bean in Taiwan

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Abstract Flavanol in cocoa is beneficial to cardiovascular health. This study investigated the effect of roasting conditions, temperature ranging from 110 to 150°C and time duration 15-35 min, on the level of flavanol and procyanidin (FP), total proanthocyanidin (PAC) and antioxidant activity of fermented cocoa beans in Southern Taiwan. The content of FP in unroasted and roasted cocoa bean was determined by the normal phase HPLC method and the antioxidant activity was assayed by DPPH and ABTS methods. High roasting temperature for long time decreased the content of FP and PAC in cocoa beans, where ranged from 55.30±1.95 mg (-)-epicatechin equivalents /g and 103.56±8.33mg procyanidin B1/g (at 110°C for 15 min) to 19.12±0.66 mg (-)-epicatechin equivalents /g and 51.58±0.86 mg procyanidin B1/g (at 150°C for 35 min), respectively. Meanwhile, as the roasting temperature rose, the DPPH and ABTS free radical scavenging ability of roasted cocoa bean extracts decreased gradually, varying from the maximum (IC_{50} = 104±2 µg/ml and 50.4±1.1mg TE/g) for unroasted bean to the minimum (IC_{50} = 144±4 µg/ml and 27.7±2.4 mg TE/g), and then slightly increased to IC_{50}=134±4 µg/ml and 31.5±0.7 mg TE/g, respectively at most severe roasting treatment, showing the formation of melanoidins and polyphenols caused the synergistic effect, leading to the rise of the antioxidant activity. The first order kinetic reaction and the Arrhenius equation were applied to forecast the change of FP concentration during roasting. The activation energy of FP degradation is 11.2 kcal/mol. The obtained kinetic equation could be useful to predict the remaining amount of FP in cocoa beans under the designed experiment. The cocoa products with higher flavanol content and antioxidant activity could be possible by setting appropriate roasting conditions.

Keywords: *Taiwan cocoa bean, flavanol, roasting, antioxidant activity, kinetics*

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1. Introduction

The history of the mankind enjoying the taste of chocolate has last for about 4000 years. Seeds of cocoa trees, or cocoa beans, originated from the Amazon Basin of South America, planted between latitude 23.5 °N and 23.5 °S, are the main ingredients of chocolate. Numerous studies have shown that polyphenol in the cocoa beans, especially the rich flavanols within, is beneficial to cardiovascular organisms. While Buijsse et al. [1] discovered that it held the potential to relieve high blood pressure; Heiss et al. [2] indicated its ability to improve the function of endothelial cells of blood vessels; Monagas et al. [3] substantiated its capability to prevent platelet agglutination and to reduce inflammation. The amount of flavanol is thus a health indicator of cocoa products and a focus on this study.

Producing chocolate products required a series of processes, successively including natural fermentation, drying, roasting, deshelling, grinding, refining, filling, and aging of chocolate. Cocoa processing played a decisive role in the remaining content of bioactive compounds in the cocoa bean, specifically the polyphenols, i.e., flavanols and proanthocyanidins. For fermentation, the polyphenols in cocoa beans are either polymerized or degraded by polyphenol oxidases in the catalytic browning reaction, further reacted with proteins and carbohydrates, and eventually led to the change of the color, flavor, and texture of cocoa beans. At the stage of drying, either sun-dried or (forced convection) oven-dried, whose operating temperature lies within 40-60°C, microorganisms continued to ferment, and the polyphenols as well kept gradually reacting with amino acids or carbohydrates. Roasting is another key step that is responsible for the change in the level of polyphenols, with its typical roasting temperature varying from 130 to 150°C, and the roasting time differing

among 10 to 40 min. The Maillard reaction associated with the roasting process darkened the color of cocoa beans, enriched the cocoa aroma and flavor, but severely lowered the level of polyphenols, with one claiming that the decrease was up to 50-80% [4,5]. Therefore, the low concentration of polyphenols hindered the pursuit of health care cocoa products.

The effect of fermentation, drying, and roasting on the level of polyphenols in cocoa under different operating conditions has intrigued numerous studies in recent years [4,6], with the most significant impact being under different roasting conditions. To discuss the relationship between roasting conditions and the level of proanthocyanidins, Ioannone et al. [7] conducted an experiment to decrease the water content of cocoa beans to the equivalent of 2% under different roasting temperatures, varying from 125 to 145°C. The result showed that under high temperature and short roasting time, the proanthocyanidin concentration remained more. Yet, the antioxidant activity was better at a low temperature and for long roasting time. Oracz et al. [8] discussed the change of flavanols and proanthocyanidins concentration from different origins of cocoa beans in a range of heating humidity varying from 0.3 to 5% and under various roasting temperatures (110-150°C). Under low-temperature and high-humidity conditions, more bioactive substances are remaining. Besides, different species of cocoa beans are found to contain a variety of polyphenol substances containing in nature. Quiroz-Ryes and Fogliano [9] indicated that both the concentration of total polyphenols and proanthocyanidins declined at high roasting temperature (150°C). The latter study led to an agreement that the roasting conditions play an important role to determine the quality and function of cocoa beans. Sacchetti et al. [10] proposed that a kinetic theory of the melanin formation during cocoa roasting, concluding that the activation energy of forming melanin (132 kJ/mol) was higher than that of oxidizing polyphenols (60-80 kJ/mol). Summa et al. [11] compared the free radical scavenging ability of extracts of different molecular weights between roasted and unroasted cocoa beans, showing that roasted cocoa bean extracts with molecular weights ranging from 5 to 10kDa possessed the highest antioxidant activity.

The cocoa industry of Taiwan has been uprising recently. Though the production scale is small and restricted, the interest in producing high quality and flavanol rich cocoa products enhanced the necessity of relevant studies. The functional properties and bioactive compounds of cocoa bean from Taiwan are however not constructed thoroughly yet. Moreover, there are so far no published studies that reported the profile of flavanol and procyanidin as well as their changes in Taiwan cocoa beans during commercialized roasting conditions. The outcomes of this study could be provided useful information for the cocoa industry in Taiwan to improve the roasting process to obtain flavanol rich cocoa bean and to enhance the antioxidant activity of cocoa bean as well as to create the competitive cocoa product with higher nutritional value.

The objective of this study was to study the changes in the flavanols and procyanidins, proanthocyanidins and free radical scavenging ability of Taiwan fermented cocoa beans under different commercialized roasting conditions, with the operating temperature set at 110, 130, 150°C, and the roasting time set for 15, 25, 35 min. Furthermore, to predict the concentration variation of these bioactive compounds, a reaction equation was developed through determining the value of kinetic coefficients, including the activation energy and the frequency factor.

2. Material and Methods

2.1. Chemicals

Procyanidin B1, (-)-epicatechin, methanol, butanol, hydrochloric acid, ammonium ferric sulfate, acetone, acetic acid, dichloromethane, 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All chemicals were of analytical grade.

2.2. Cacao Seeds Fermentation

Cacao pods were purchased from local farmers at Wanlaun and Neipu in Southern Taiwan. Cacao seeds were taken out from the ripe cacao pods and fermented spontaneously by naturally occurring bacteria in boxes containing three compartments vertically with each compartment the size of 50cm × 50cm × 50cm. Fresh cocoa seeds 80kg were covered with a sack in the top compartment initially for 48h and were moved to the middle compartment and blended for another 48h. Lastly, cocoa seeds were moved to the bottom compartment and stirred 2 times per day until the entire process had last for 168h. The temperature profile was recorded during fermentation and the highest temperature is around 48°C occurring at 120h. The fermented cocoa beans was oven-dried at 50°C until about 6% of moisture remaining. Dried cocoa beans were stored in refrigeration.

2.3. Experimental Design of Roasting

The experimental design is 3x3 completely factorial design. The 3 levels of the temperature was 110, 130 and 150°C and the 3 levels of the roasting time was 15, 25, and 35 min. The roasting process is conducted by using a conventional rotary dryer, which is set at 110, 130 and 150°C, respectively and held for 10min to reach equilibrium before the roasting process started. The fermented and dried cocoa beans in amount of 1000g were placed in perforated tray per each treatment for different time period. The roasted cocoa beans were cooled at ambient temperature for 2h and were kept in sealed plastic containers for each treatment in -25°C refrigerator until further analyses.

2.4. Defatting of Cocoa Beans

The fermented and roasted cocoa beans were randomly collected in amount of 50 grams from each roasting treatment and their shell were removed manually before grinding in the presence of liquid nitrogen to obtain the fine cocoa powder using blender machine. The ground cocoa powder was mixed with N-hexane with a ratio of

1 to 2 (w/v) with an ultrasonicator at 40°C for 30min. The mixture was shaken vigorously and then centrifuged for 10min at 10000rpm and the supernatant was removed. The defatting process was to be done in triplicate. The resulting precipitant was air-dried in a fume hood for 48h.

2.5. The Measurement of PAC

2.5.1. Preparing the Sample

0.2g of pre-processed cocoa powder with 20ml of 90% methanol were extracted in a 50ml centrifugal tube for 1h at room temperature with ultrasonic oscillation. The resulting supernatant was added to 25ml with demineralized water.

2.5.2. Producing the Standard Curve

Different concentrations of procyanidin B1 methanol solution were prepared: 0.01, 0.025, 0.05, 0.1, 0.15 and 0.20mg/ml. 1ml of the solution with different concentrations was added respectively to 6ml of butanol/hydrochloric acid (95/5, v/v) with the addition of 0.5ml of 2% ammonium ferric sulfate solution in each test tube. The tubes were heated in a hot water bath for 40min and then cooled in an ice water bath. The measurement of the absorbance of each resulting solution was performed in triplicate under 546nm wavelength in a 10mm cuvette with a spectrophotometer. With ddH_2O used for blank correction, the corresponding absorbance of different concentrations of procyanidin B1 solution was used to produce the standard curve.

2.5.3. Measuring the Samples

The measurement of PAC is according to the descripted method [12]. 1 ml of PAC extract was substituted for the procyanidin B1 solution. Absorbance were measured at 546nm.

2.6. The Measurement of FP

2.6.1. Preparing the Sample

The procedure of sample preparation was described by Counet and Collin [13] with several modifications. 0.2-0.5g fat-free cocoa powder was centrifuged with 50ml of a solvent of acetone/water/acetic acid (70:28:2, v/v) at room temperature for 1h for extraction. The supernatant was separated and kept, while the precipitant was extracted with repeated procedure. The resulting supernatant from the precipitant was mixed with the previous supernatant. The mixture was filtrated and was set to the volume of 100ml.

2.6.2. Normal-phase HPLC (NP-HPLC)

The crude extract was filtrated with a 0.45μm filter and the contents of flavanol and procyanidin are measured with NP-HPLC. Relevant information about normal-phase HPLC is descripted by Robbins et al. [14] as follows: Column: Develosil Diol 100Å 5μm, 250 × 4.6mm (Phenomenex, Torrance, CA; Cat. No. DI11546250W); Detection wavelength: UV 280nm; Mobile phase A: CH_2Cl_2/CH_3OH/H_2O/Acetic acid (82:14:2:2, v/v); Mobile phase B: CH_3OH/H_2O/Acetic acid (96:2:2, v/v); The flow rate of 1ml/min by using these two mobile phases (A and B) with a gradient elution. The gradient elution was as follows: from 100% to 82.4% A as time from 0 to 30 min, and then to 69.3% A as time from 30 to 45 min, and to 12.2% A as time from 45 to 50 min, and then to 50% A as time from 50 to 52 min. and to 100% A as time from 52 to 55 min, and maintained this gradient for another 5 min.

2.7. DPPH Radical Scavenging Activity

Defatted cocoa powder 2.00g were added to 20ml of acetone (50%) and the mixture was shaken at 50ºC for 30 min. With the solution extracted and centrifuged at 10000 rpm for 15 min, the resulting supernatant was collected and the concentration was adjusted to 100000ppm. DPPH· (α,α-diphenyl-β-picrylhydrazyl), a violet-colored stable free radical, was prepared as a 1mM solution. The solution was used to test the hydrogen donating ability of the samples. The maxima absorbance of the experiment was at 540nm with Trolox dilution used for the calibration curve as previously described by Chu and Lin [15].

2.8. ABTS Radical Cation Inhibition

This assay determined the capacity of sample to scavenge the ABTS radical cation followed the procedure described by Chu and Lin [15]. The ABTS radical cation was generated by reacting 1 mM ABTS with 0.5 mM hydrogen peroxide and 10 units/ml horseradish peroxidase in the dark at 30°C for 2 h. After 1 ml of ABTS radical cation was added to samples, the absorbance was recorded at 734 nm after 10 min.

2.9. The Kinetics of FP Changes in Cocoa Beans during Roasting

Reaction kinetics has applied to the biological materials for only several decades. Most of them occurred in foods are generally to be considered as either zero-order or first- order reaction [16]. The changes of FP concentration in cocoa beans during roasting conditions was assumed to be pseudo first-order reaction. The reason for adding a term pseudo to the order of the reaction is mainly due to the actual reaction mechanism in foods are more complex than that in chemicals. Thus, the kinetics of FP loss during roasting in cocoa beans is represented by:

$$-\frac{dC}{dt} = kC \quad (1)$$

Where C and t are the concentration of FP at time t during roasting conditions; k is the first-order rate constant. By integrating Equation (1), Equation (2) was obtained as follows:.

$$\ln\frac{C_0}{C} = kt \quad (2)$$

Where C_0 is the initial concentration of FP. The temperature dependence of rate constant (k) was evaluated by means of the Arrhenius equation:

$$k = k_0 e^{\frac{-E_a}{RT}} \quad (3)$$

Where Ea is the apparent activation energy; k_0 is the frequency factor; R is the universal gas constant; T is the roasting temperature in K.

2.10. Response Curve and Data Analysis

The polynomial curve with two independent variables: roasting temperature and time was used for modeling the experimental conditions. The dependent variables included the flavanol and procyanidin as well as proanthocyanidin in roasted cocoa bean. All the experimental data were used to fit the polynomial model as the following equation:

$$Z = a_0 + a_1X + a_2Y + a_3XY \quad (4)$$

Where Z is the predicted dependent variable; X is the roasting temperature; Y is the roasting time; a_0, a_1, a_2 and a_3 represented the regression coefficients for intercept, two first order terms and two way interaction effect respectively. Analysis of Variances (ANOVA) and Tukey's multiple range tests were applied to analyze the significant difference of the means among the treatments. The significant level (α) was set at 0.05. The polynomial regression model and Tukey's multiple range tests were performed by using the statistic computing software R. All the experimental tests were conducted at least in duplicate. The mean values with standard deviation were calculated for each treatment.

3. Results and Discussion

The quality of cocoa bean and the amount of polyphenols are not only affected by the origins of countries but also influenced by processing procedures. While the operating parameters of different drying methods and fermentation conditions are rather intractable, and hence increased the difficulties of maintaining expected outcome, roasting step turns out to be a relatively controllable process. Roasting, determining the final color, flavor, aroma, astringency, and sensory properties of cocoa products, triggered numerous reactions, such as the polyphenol oxidase catalyzing enzymatic and non-enzymatic browning reaction; the oxidation and polymerization reaction of cocoa polyphenols; the decomposition and Maillard reaction of proteins and carbohydrates. These reactions led to the decreasing level of bioactive substances along with the decrease of astringency of the cocoa beans. The emphasis of high polyphenols and flavanol concentration of cocoa products has grown to be an uprising trend and has won consumers' interest in recent years.

3.1. Effect of Roasting Conditions on FP

Temperature and time are two variables in the roasting experiment, with the operating temperature set at 110, 130 and 150ºC, and the roasting time was 15, 25 and 35 min, respectively. The level of FP shared a negative trend with roasting temperature and time. Figure 1 depicted the change of total flavanol and procyanidin concentration with respect to the roasting time and temperature. Experimental data indicated that the utmost level of total FP was 55.91±2.82 mg (-)-epicatechin equivalents /g through NP-HPLC measurement, which referred to the unroasted cocoa beans. For the low-temperature roasting process, at 110°C, the concentration didn't decrease significantly, while the level fell 65% to 19.12±0.66 mg (-)-epicatechin equivalents/g under high-temperature roasting conditions. The result is due to the enhancement of the redox activity of FP at high temperatures [17]. Besides, the present data, corresponding with other studies [7,8], showed that the rise of temperature was inversely proportional to the remaining FP level. For the roasting condition at 150°C, the FP concentration dropped steeply for the first 15 min, from 55.91±2.82 to 30.68±1.05mg (-)-epicatechin equivalents/g. Yet, the rate of the FP concentration declination had slowed down afterward, with the following 10 min decline to 23.51±0.81 mg (-)-epicatechin equivalents/g and the further 10 min lower to 19.12±0.66 mg (-)-epicatechin equivalents/g. At extreme heating temperature, the vigorous reaction with oxygen accelerated the polymerization and condensation among flavanol monomers, polyphenolic acid, and proanthocyanidins, resulting in the abrupt decrease of cocoa FP concentration at the beginning of the roasting process. The polynomial regression equation for total FP content (z) as a function of roasting temperature (x) and time (y) are expressed as the following equation: z = 137.71 - 0.63x - 0.83y - 0.00087xy (R^2 = 0.92). It could be used to predict total FP content under roasting conditions.

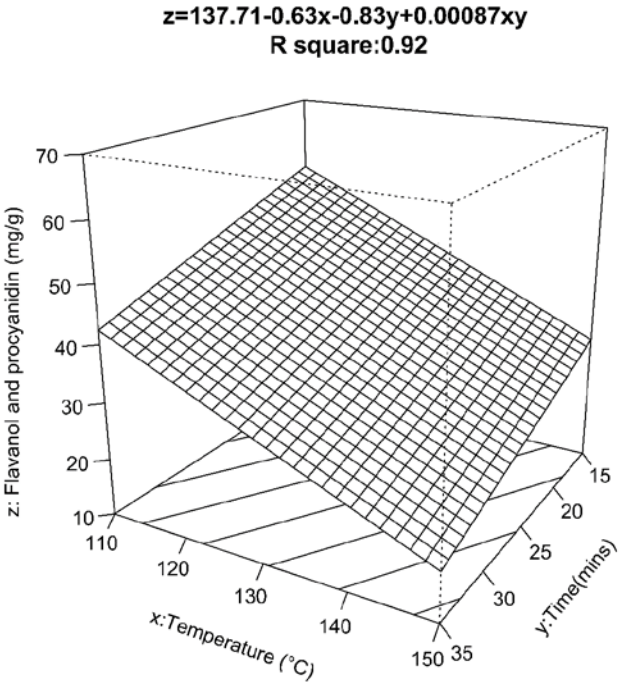

Figure 1. Polynomial regression curves display the effects of roasting temperature (X) and time(Y) on the flavanol and procyanidin content (Z) of cocoa bean

Table 1 presented the flavanol and procyanidin (DP1-DP10) concentration, in mg (-)-epicatechin equivalents/g, under different roasting conditions. The DP1 content in the cocoa beans was 43% of the total FP, while the concentration of FP with the degree of polymerization varying from DP6 to DP10 only took about 8%, showing that most FP existed in the form of monomers. This findings is similar to the previously reported results by Pedan et al. [6] who reported that the DP1 content in the

cocoa beans was 35% of the total FP (DP1-13). For cocoa beans roasted at 110°C and 15min, these monomers were possibly catechin or (-)-epicatechin, whose concentration lowered slightly from 24.09±1.31 to 20.53±0.73mg/g. However, for FP with a higher degree of polymerization, except for DP2 and DP4, the polymer concentration increased significantly ($p < 0.05$). One possible reason was that the heat accelerated the polymerization reaction. Under fixed temperature (110°C), the monomer concentration did not vary widely along with the increasing roasting time. On the other hand, for the roasting time set for 15 min, as the roasting temperature rose, the DP1 and DP2 levels gradually decreased from 20.53±0.73 to 17.80±0.63 mg/g and from 9.36±0.35 to 7.40±0.28 mg/g, respectively. Yet, for DP3 to DP10, their concentrations displayed a steep falling trend, indicating that the Maillard and polymerization reaction which occurred at higher temperature generated insoluble tannins. In summary, under severe heating conditions, the roasting temperature at 150°C and the roasting time 35 min, the DP1 level fell to 12.15±0.43 mg/g, which was half the amount of the unroasted cocoa beans, with the remaining concentration of DP2, DP3, DP4, and DP5 being 34, 32, 14 and 6%, respectively, of the unroasted cocoa beans. The result displayed the fact that the polymer concentration decreased seriously along with the degree of polymerization, which therefore explained the significant loss of total FP amount under intense roasting conditions.

3.2. Effect of Roasting Conditions on PAC

Total proanthocyanidin are containing in cocoa beans in nature. However, they may as well be produced by polymerization reactions of catechin and (-)-epicatechin during processing. These polymerized components are known as condensed tannins, which are generally soluble, except for those large-molecular weight tannins. As the source of the astringency taste of cocoa products, tannins also possess the ability to prevent cardiovascular disease and create health benefits. Figure 2 depicted the relationship between total proanthocyanidin and different roasting conditions, including two designed factors, the roasting temperature and the roasting time. Data showed that the highest concentration reached 123.12±9.29 mg procyanidin B1/g, referring to the unroasted cocoa beans. For high temperature and long roasting time, the level of total PAC decreased by 58%, remaining 51.58±0.86 mg/g. In general, intense heating conditions could be caused severe loss of the content of total PAC. Besides, the present results indicated that the PAC concentration dropped dramatically as the roasting time and temperature rose, falling from 103.56±8.33 to 51.58±0.86 mg/g. The declining trend of PAC concentration corresponded to that of the FP level. As for the roasting condition at 150℃, the content of total PAC dropped steeply for the first 15 min, from 123.12±9.29 to 77.41±3.13 mg/g. Yet, the falling rate of PAC content had slowed down afterward, with the following 10 min decline to 63.69±1.27 mg/g and the further 10 min lower to 51.58±0.86 mg/g. The obtained decreasing trend of PAC during roasting conditions of cocoa beans in this study is in line with the findings made by several studies who reported the higher the roasting temperature was applied to cocoa beans, the lower amount of phenolic compounds retained in cocoa bean [7,8,9]. The abrupt decrease of total PAC concentration at the beginning of the roasting process resulted from the vigorous reaction with oxygen and the polymerization and degradation reactions among flavanol monomers and polymers. The polynomial regression equation for proanthocyanidin content (z) as a function of roasting temperature (x) and time (y) are expressed as the following equation: $z = 163.41 - 0.45x - 0.87y + 0.013xy$ ($R^2 = 0.9$). The equation is useful to predict the PAC content under roasting conditions.

Table 1. Effects of Roasting Conditions on the Flavanol and Procyanidin Content (DP1-10) of Cocoa Beans

Roasting conditions		Flavanol and Procyanidin, mg (-)-epicatechin equivalents/g										
Temperature (°C)	Time (min)	DP1	DP2	DP3	DP4	DP5	DP6	DP7	DP8	DP9	DP10	Total
Unroasted cocoa bean		24.09 ±1.31^{a}	11.15 ±0.42^{a}	5.83 ±0.27^{b}	6.91 ±0.40^{a}	3.24 ±0.16^{b}	2.30 ±0.13^{b}	1.35 ±0.07^{b}	0.90 ±0.05^{b}	0.12 ±0.01^{e}	0.05 ±0.00^{cd}	55.91 ±2.82^{a}
110	15	20.53 ±0.73^{bc}	9.36 ±0.35^{b}	7.08 ±0.17^{a}	6.48 ±0.13^{ab}	5.13 ±0.24^{a}	2.94 ±0.12^{a}	1.64 ±0.08^{a}	1.07 ±0.05^{a}	0.64 ±0.04^{a}	0.45 ±0.02^{a}	55.30 ±1.95^{a}
	25	19.28 ±0.69^{bc}	9.30 ±0.35^{b}	6.15 ±0.15^{b}	6.08 ±0.12^{b}	2.53 ±0.12^{c}	1.91 ±0.08^{c}	1.33 ±0.07^{b}	0.89 ±0.04^{b}	0.52 ±0.04^{b}	0.13 ±0.01^{b}	48.09 ±1.65^{b}
	35	20.71 ±0.74^{b}	8.90 ±0.34^{b}	5.74 ±0.13^{b}	1.85 ±0.04^{d}	2.09 ±0.10^{cd}	1.50 ±0.06^{d}	1.04 ±0.06^{c}	0.72 ±0.03^{c}	0.38 ±0.03^{c}	0.14 ±0.01^{b}	43.05 ±1.53^{b}
130	15	20.93 ±0.74^{b}	10.12 ±0.38^{ab}	6.09 ±0.15^{b}	4.91 ±0.10^{c}	1.71 ±0.08^{d}	1.06 ±0.04^{e}	0.69 ±0.04^{d}	0.48 ±0.01^{d}	0.22 ±0.01^{d}	0.07 ±0.01^{c}	46.27 ±1.57^{b}
	25	18.77 ±0.66^{bc}	9.26 ±0.35^{b}	4.96 ±0.12^{c}	6.15 ±0.12^{b}	2.33 ±0.11^{c}	2.59 ±0.11^{b}	0.72 ±0.04^{d}	0.45 ±0.02^{d}	0.42 ±0.03^{c}	0.16 ±0.01^{b}	45.77 ±1.57^{b}
	35	14.52 ±0.52^{d}	6.26 ±0.24^{cd}	3.18 ±0.08^{d}	1.92 ±0.04^{d}	0.44 ±0.03^{e}	0.24 ±0.01^{f}	0.20 ±0.01^{e}	0.04 ±0.00^{e}	0.02 ±0.00^{f}	0.02 ±0.00^{de}	26.81 ±0.91^{cd}
150	15	17.80 ±0.63^{c}	7.40 ±0.28^{c}	2.96 ±0.08^{d}	1.76 ±0.04^{d}	0.39 ±0.01^{e}	0.17 ±0.00^{f}	0.18 ±0.01^{e}	0.03 ±0.00^{e}	0.01 ±0.00^{f}	0.00 ±0.00^{e}	30.68 ±1.05^{c}
	25	14.53 ±0.52^{d}	5.18 ±0.20^{d}	2.35 ±0.06^{e}	1.01 ±0.02^{e}	0.28 ±0.01^{e}	0.14 ±0.00^{f}	0.01 ±0.00^{e}	0.03 ±0.00^{e}	0.00 ±0.00^{f}	0.00 ±0.00^{e}	23.51 ±0.81^{de}
	35	12.15 ±0.43^{d}	3.76 ±0.14^{e}	1.88 ±0.04^{e}	0.99 ±0.02^{e}	0.21 ±0.01^{e}	0.09 ±0.01^{f}	0.02 ±0.00^{e}	0.03 ±0.00^{e}	0.00 ±0.00^{f}	0.00 ±0.00^{e}	19.12 ±0.66^{e}

Values are expressed as the means±SD. Different letters indicate significantly different ($p < 0.05$).

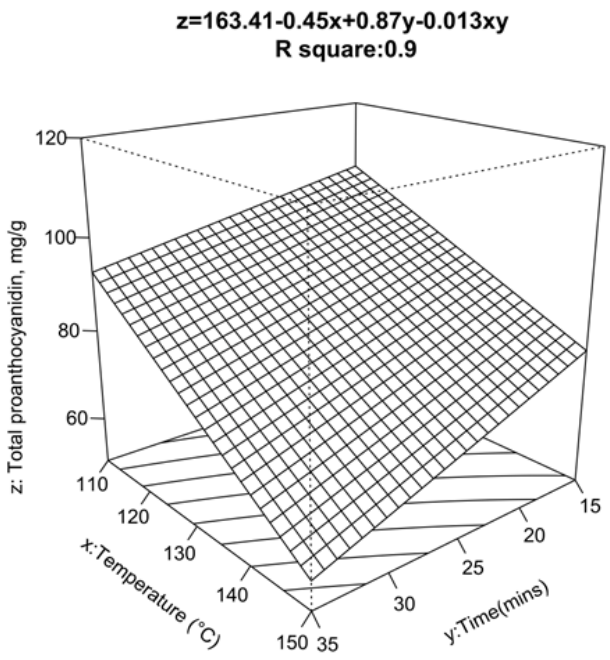

Figure 2. Polynomial regression curves display the effects of roasting temperature (X) and time(Y) on the total proanthocyanidin content (Z) of cocoa bean

3.3. Antioxidant Activity

The measurement result of the antioxidant activities in cocoa beans is affected by lots of factors, including the chosen solvents, the active components of the extracts, and the selected measurement method. As a result, it was necessary to measure the antioxidant activities through more than one approach. Figure 3 depicted the scavenging ability to DPPH free radicals of unroasted and roasted cocoa beans under nine different roasting conditions. The higher the value of IC_{50} was, the lower the ability of antioxidant activity. As the roasting temperature rose, the activity decreased gradually, varying from the maximum (IC_{50} = 104±2 μg/ml) of unroasted bean to the minimum (IC_{50} = 144±4 μg/ml), which corresponded to the roasting condition at 150°C and for 25 min. However, we could see that for the most intense roasting condition (at 150°C and for 35 min), the IC_{50} value decreased to 134±4 μg/ml, showing that the antioxidant activity was higher than the one at the same temperature but the roasting time for merely 25 min. It was found that under severe roasting conditions, the formation of melanoidins and phenolic compounds caused the synergist effect, leading to the rise of the antioxidant activity. This result is in accordance with the reported data by Mesias and Delgado-Andrade [18] who reported that the melanoidins have beneficial effects relating to health promoting properties, such as antioxidant activity. Besides, in general, the declination of the antioxidant activity was consistent with the decreasing of total FP and PAC concentration in cocoa beans.

Figure 4 displayed the result of the scavenging ability of ten kinds of cocoa bean extracts to the ABTS free radicals. As the roasting temperature increased, the antioxidant activity of the defatted cocoa beans started from the highest (50.4±1.1 mg TE/g), decreased to the lowest (27.7±2.4 mg TE/g) after 25 min of roasting at 150°C, and finally slightly increased to 31.5±0.7mg TE/g for 35 min roasting time. The scavenging ability between ABTS and DPPH free radicals was consistent with each other, and both were as well correlated to the content of total FP and PAC, except for one roasting condition at 150°C and 25min. These bioactive compounds, containing numerous tricyclic aromatic structure and hydroxide functional groups, formed a resonance structure to stabilize the free radicals and therefore possess the antioxidant activity [19,20].

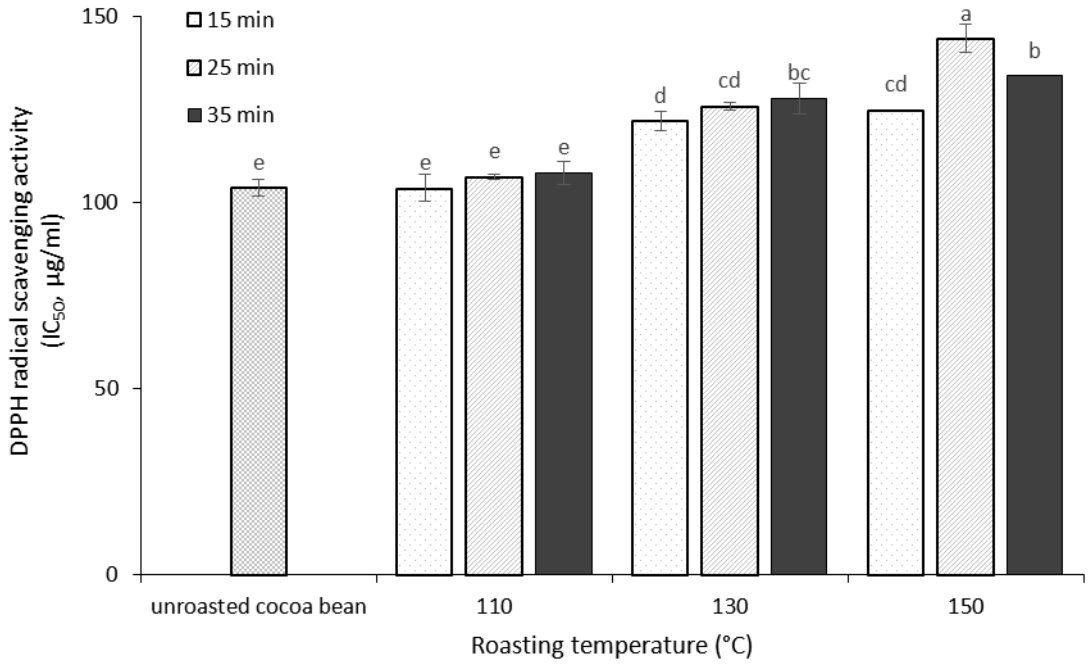

Figure 3. The effects of roasting temperature and time on DDPH radical scavenging activity of cocoa beans. Values are expressed as the means±SD and a common letter in the same graph are not significant different ($p < 0.05$)

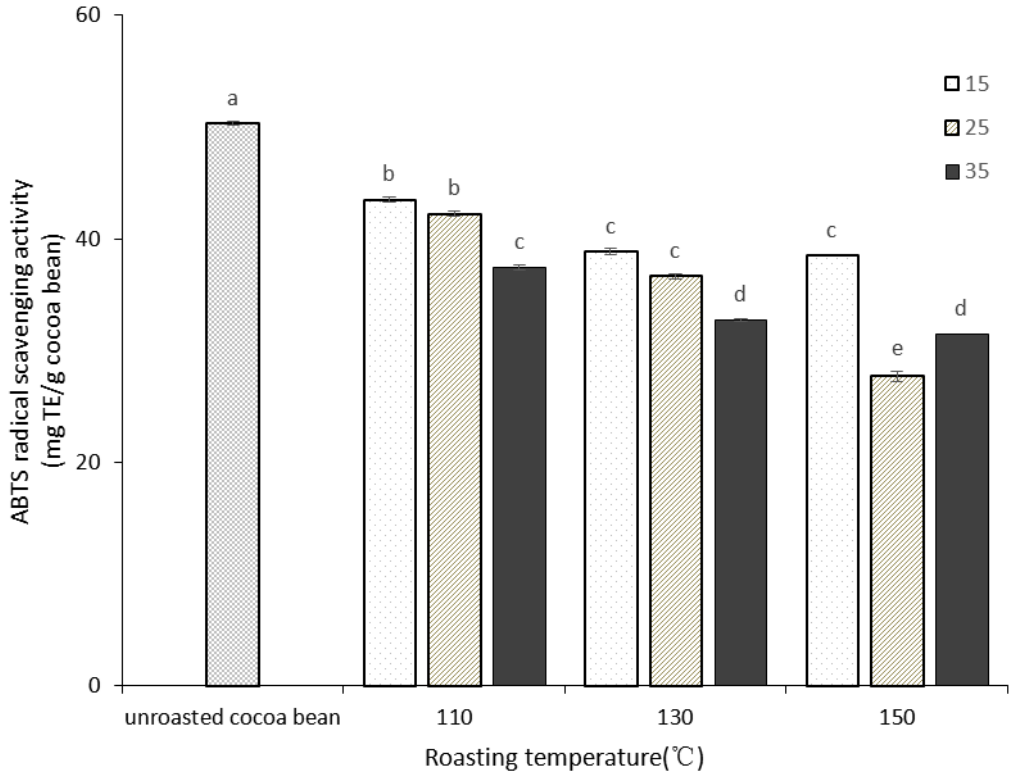

Figure 4. The effects of roasting temperature and time on ABTS radical scavenging activity of cocoa beans. Values are expressed as the means±SD and a common letter in the same graph are not significant different ($p < 0.05$)

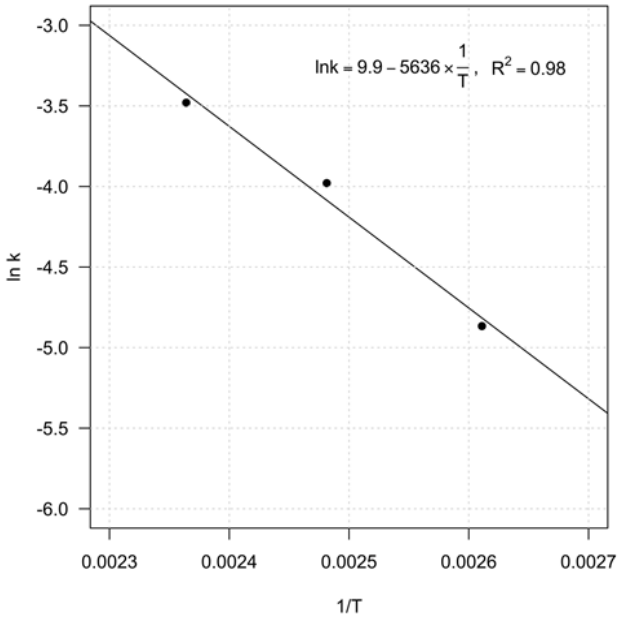

Figure 5. The relationship between the reaction rate constant (k) of FP in roasted cocoa bean and roasting temperature (T)

3.4. The Kinetic Equation for FP Change

Through transforming Equation (3) into log-form, Figure 5 presents the relationship between lnk and 1/T. The filled circle in the graph were the experimental data in this study. The obtained linear regression equation is

$$lnk = 9.9 - 5636\frac{1}{T} \quad (5)$$

where the two parameters are k_0 = 19930 min^{-1} and Ea = 11.2 kcal/mol with R^2 = 0.98. The activation energy of FP changes during roasting conditions was lower than that of polyphenol oxidation (14.34-19.12 kcal/mol) reported by Sacchetti et al. [10]. Combining Equation (3) with the results in Equation (5), the value of the rate constant under fixed roasting temperatures was determined. Then, the equation (2) could be used to estimate the amount of FP remaining in the cocoa bean during the roasting process.

4. Conclusions

The results showed that the level of FP and PAC in roasted cocoa bean both decrease with the increment of the roasting time and temperature. Meanwhile, as the roasting temperature rose, the DPPH and ABTS free radical scavenging ability of roasted cocoa bean extracts decreased gradually, varying from the maximum for unroasted bean to the minimum at relative lower thermal treatment, and then slightly increased at most severe roasting treatment, implying the formation of melanoidins and polyphenols caused the synergistic effect, leading to the rise of the antioxidant activity. The first order kinetic reaction and the Arrhenius equation were applied to forecast the change of total FP concentration during roasting with the determined parameters: k_0 and Ea. The obtained kinetic equation could be useful to predict the remaining amount of FP in cocoa beans under the designed roasting conditions. This will be an important information for producing cocoa bean with desired functional properties and chocolate products with positive benefits for human health.

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Conflicts of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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