

Antioxidant, Antityrosinase Activity and Physicochemical Properties of Manufactured Chocolates in Taiwan Affected by Roasting Treatments

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Abstract Roasting is an important step in cocoa processing that affects the functional and organoleptic properties of chocolate products. The effect of roasting treatment on antioxidation, antityrosinase activity, and physicochemical properties of 12 manufactured chocolates from Southern Taiwan fermented cocoa beans was investigated. The cocoa beans were roasted at different fixed temperatures ranging from 90 to 150°C for different time duration 15-35 min. The results showed that more severe thermal treatment decreased the contents of total phenolic compounds and proanthocyanidins, from the highest 20.57± 0.86mg GAE /g defatted chocolate and 9.20±0.49 mg CyE /g defatted chocolate to the lowest 11.48±0.23 mg GAE /g defatted chocolate and 4.14±0.41 mg CyE /g defatted chocolate, respectively. Meanwhile, roasting treatment also reduced DPPH and ABTS radical scavenging activities in temperature and time-dependent manners. In addition, the low-roasting chocolate revealed the potent inhibitory action on mushroom tyrosinase activity at 2.5 mg/g defatted chocolate. However, chocolate produced from beans roasted at 130°C for 25 min was ranked the highest consumer acceptability in the sensory evaluation. Taken together, the optimization of cocoa bean roasting conditions can improve the health benefits of chocolate, while the low-roasting chocolate acts as a potent candidate for tyrosinase inhibitors.

Keywords: Taiwan chocolate, antioxidation, antityrosinase, sensory evaluation, roasting treatment

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1. Introduction

In recent years, the impression of cocoa products has gradually transferred from flavor-oriented leisure snacks into health-oriented bioactive substances. While polyphenols in the cocoa beans are highly beneficial to human health, low-processed cocoa beans are considered the best effort to preserve the optimal amount of bioactive compounds [1]. Besides the polyphenols being responsible for the astringency and bitter taste of cocoa products, further health benefits of polyphenols are as follows: 1. Cardioprotective effect: promote the NO generation and therefore relax smooth muscles; reduce the blood pressure. 2. Prevention of Parkinson's disease and Alzheimer's disease: improve the cognitive function of elders. 3. Protective effect on intestinal probiotics and emotional regulation. 4. Obesity prevention: increase adiponectin and glucose transporters and reduce lipid deposition and insulin resistance [2]. Due to the multiple benefits of polyphenols mentioned above, polyphenol-rich chocolate has received increasing attention as a functional food among chocolate consumers nowadays.

Studies have shown that the content of polyphenols in cocoa beans varies dramatically within different species. While some researchers estimated its concentration 6-8% dry weight, the experimental result of others was sometimes 2-3 times higher [3]. The amount of epicatechin, a type of polyphenols, in the Criollo strain is only two-thirds of that in the Forastero strain, for instance [4]. Notwithstanding different strains affect the total polyphenols concentration, the processing, involving the fermentation, drying, roasting, oil extracting, and alkali treatment of cocoa powder, is the main cause of the decreasing polyphenols level. Among the processing procedures, roasting is especially decisive for the level change of polyphenols. The typical roasting temperature varies from 130°C to 150°C, with the roasting time differing among 10 to 40 min. The Maillard reaction associated with the roasting process darkens the color of cocoa beans, enriches the cocoa flavor, but severely lowers the level of polyphenols 50-80% [3,5]. Ioannone et al. [6] roasted cocoa beans at different fixed temperatures (range from 125°C to 145°C) until the moisture content was down to about 2%, finding that cocoa beans with higher roasting temperature and shorter roasting time had higher levels of proanthocyanidins but lower antioxidant activities. Oracz et al. [7] discussed

the influence of different roasting temperatures (110-150°C) and humidity (0.3-5%) on the properties of cocoa beans. Results showed that cocoa beans with lower roasting temperature and higher humidity retained more bioactive compounds. Meanwhile, different origins of cocoa beans affected the polyphenols concentration as well [3].

As a new place for cocoa plantation, the cocoa industry of Taiwan has been uprising recently. Though the manufacturing scale small and restricted, the deep interest in producing high-quality and polyphenol-rich cocoa products accentuates the necessity of relevant researches. Research regarding the physicochemical and functional properties as well as bioactive compounds of Taiwanese chocolate are however not constructed thoroughly yet. Moreover, few reviews have studied the influence of roasting conditions on bioactive compounds and the tyrosinase inhibition of chocolate products.

Our objective is to compare the antioxidation activity, color change, sensory property, and tyrosinase inhibition among 12 manufactured chocolates. Each, obtained from Southern Taiwan fermented cocoa beans, is manufactured under different roasting treatments, with the operating temperature set at 90, 110, 130, 150°C and the roasting time set for 15, 25, 35 min. In addition, we predict the concentration variation of these bioactive compounds polyphenol and proanthocyanidins by polynomial regression equations fit by the experimental data.

2. Material and Methods

2.1. Chemicals

Folin-Ciocalteu reagent, 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), cyanidine chloride, kojic acid, mushroom tyrosinase, were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). L-3,4-dihydroxyphenylalanine (L-DOPA) was obtained from Acros Organics (Geel, Belgium). All chemicals were of analytical reagent grade.

2.2. Cacao Seeds Fermentation

Cacao pods were harvested from Wanlaun and Neipu in Southern Taiwan and transported to Chia Nan University of Pharmacy and Science. Cacao seeds were taken out from the ripe cocoa pods and fermented spontaneously by naturally occurring bacteria in wooden boxes. The entire fermentation process had last for 7 days. The fermented cocoa beans were sun-dried until about 6% of moisture remaining. Dried cocoa beans were stored in refrigeration until further used.

2.3. Cocoa Bean Roasting

The roasting process was conducted by using a conventional rotary dryer, which was set at 90, 110, 130 and 150°C respectively and hold for 10 min to reach equilibrium before the roasting process started. The fermented and dried cocoa beans in amount of 1 kg were placed in

perforated tray per each treatment for different designed roasting time period. The roasted cocoa beans were cooled at ambient temperature for 2 h and were kept in sealed plastic containers for each treatment in refrigeration.

2.4. Chocolate Samples Preparation

The roasted cocoa beans were initially cracked and winnowed, being ground into paste-like cocoa, and then refined for 24 h. After the addition of sugar, 30% of the total amount of cocoa, it takes another 24 h for further refine grinding. Then the tempering process was conducted. The cocoa liquor was heated to 48°C, then chilled to 27°C, and then raised to 32°C eventually. The tempered cocoa liquor was immediately filled into a plastic mold (40 mm * 40 mm * 5 mm). The finished chocolates were kept in sealed glass container and stored in a cabinet at 18°C with the humidity of 55% for 7 days. The aged chocolates are then delivered to 44 untrained panels to conduct the sensory assessment or further analysis.

2.5. Defatting Samples

The 12 manufactured chocolates were put and melted at 50°C in an oven, and then extracted 2 times with n-hexane at a ratio of 1:10 (w/v) for 30min using ultrasonicator, and then were centrifuged at 10000 rpm for 15min. The precipitate (defatted chocolate powder) was collected and dried in hood for 48h. 1.00 g of defatted chocolate powder were extracted using 50% acetone aqueous at a ratio 1:10 for 30 min at 50°C in a ultrasonicator, and then were centrifuged at 10000 rpm for 15min. The supernatant was further analyzed.

2.6. Total Polyphenolics Assay

Total polyphenol content (TPC) were determined as gallic acid equivalents (GAE). The different concentrations of sample were added to a 10 mL volumetric flask, to which 2 mL sodium carbonate (20% (w/v)) was added. After 5 min, 0.1 mL Folin-Ciocalteu reagent (50% (v/v)) was added and the volume was made up to 10 mL with H₂O. After 1 h incubation at 30 °C, the absorbance was measured at 750nm and compared to a gallic acid calibration curve [8].

2.7. Proanthocyanidins Assay

Proanthocyanidins were determined by the method described by Belščak et al. [9]. The reaction mixture consisting of 0.25 mL of defatted chocolate extract, 1.5 mL of n-BuOH/HCL (95:5, v/v) and 50 µL of a 2% solution of NH₄Fe(SO₄)₂ · 12H₂O in 2M HCl. The solution was capped, shaken and heated for 1h at 95°C in a water bath. The mixture was cooled at room temperature and the absorbance was measured at 550nm using ELISA reader (Molecular Devices VMax, MA, USA). The quantity of proanthocyanidin was determined from a standard curve of cyanidin chloride following the same procedure, and indicated as mg cyanidine chloride equivalents CyE/g defatted chocolate.

2.8. Determination of DPPH Radical Inhibition

The effect of samples on the DPPH radical was estimated as described previously [8]. The samples were added to a methanolic solution (1 mL) of DPPH radical (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min; the absorbance of the resulting solution was then measured at 517 nm.

2.9. Determination of ABTS Radical Cation Inhibition

As previously described [10], this assay determined the capacity of sample to scavenge the ABTS radical cation. 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.

2.10. Determination of Mushroom Tyrosinase Activity

The tyrosinase inhibitory activity was determined with the degree of inhibition on tyrosinase-catalyzed oxidation of L-DOPA as previously described [11]. The reaction mixture consisting of 0.1 mL of samples, 0.1 mL of mushroom tyrosinase (1000 Unit/mL) and L-DOPA (3.8 mM) was added in this order to read the absorbance at 475 nm for 5 min. The value in the absence of samples was represented as the control. The inhibition of tyrosinase activity was calculated with the following formula: Inhibition (%) = $(1 - (\text{OD}_{475} \text{ in sample} / \text{OD}_{475} \text{ in control})) \times 100\%$.

2.11. Color Determination

The color of chocolate samples was measured using a colorimeter (ColorLite GmbH sph870-6-uk 400-700nm, Germany). The results were displayed in accordance with the parameters L^* , a^* , b^* , C^* and h^* , where L^* indicates lightness, range from 0 (black) to 100 (white); a^* indicates redness, range from green (-50) to red (+50), b^* indicates yellowness, range from blue (-50) to yellow (+50), C^* represents chroma, the intensity of color was calculated by the following formula, $C^* = (\text{a}^{*2} + \text{b}^{*2})^{1/2}$, and h^* represents hue angle was calculated by the following formula, $h^* = \tan^{-1}(b^*/a^*)$. The numerical value of hue angle is a useful indicator of browning. High values of h^* imply more browning. The chocolate sample were placed in a cylindrical and optical glass cuvette and the color of each sample was measured in 5 replicates. The instrument was calibrated with a standard white tile before the measurement [12].

2.12. Sensory Evaluation of Chocolates

Folin-Ciocalteu Sensory evaluation of chocolate was performed by 44 untrained panelists using the point score method in our Lab. The attributes of chocolate include

appearance, aroma, flavor, sweetness, the intensity of acidity, bitterness, astringency, and overall acceptability. The organoleptic properties were presented on a nine-point hedonic scale, according to which like extremely or extremely strong = 9, dislike extremely or extremely weak = 1, and recorded their scores [1].

2.13. Statistical Analysis

Each experiment was performed at least triplicate and averaged. All data were presented as mean \pm SD. Analysis of Variances (ANOVA) and Tukey's Test were applied to analyze the significant difference of the means among the treatments. The significant level (α) was set at 0.05. All statistical analysis were performed by using the statistical computing software R.

3. Results and Discussion

Roasting plays a crucial role in the chocolate manufacturing process. Precursors of the Maillard reaction such as reducing sugar, amino acid, and peptide, react with each other during roasting, contributing to the generation of flavor and brown pigment [3,13]. However, the roasting process is also known as a key step to harmful the phenolic compounds. Recently, due to healthcare perspective, the processing of foods has a trend to be low-processed or unprocessed. Our research followed the trend of low-roasting process, the roasting temperature lowering to 90°C, and the roasting duration shortening to 15 min, which is far lower than the commercialized roasting temperature 130-150°C, and the roasting time 30-40 min. We, therefore, explored the functional and organoleptic properties of 12 chocolates manufactured from Southern Taiwan cocoa beans that were roasted under designed conditions.

3.1. Total Polyphenol and Proanthocyanidins Contents

Many reports have noted that naturally occurring bioactive compounds, such as total polyphenol and proanthocyanidins, are thought to be responsible for protective health benefit against human diseases. Figure 1 shows the effect of roasting treatments on the total polyphenol contents of 12 manufactured chocolates. The results indicate that more severe heat treatment decreased TPC from the highest 20.57 \pm 0.86mg GAE /g defatted chocolate to 11.48 \pm 0.23 mg GAE / g defatted chocolate. The polynomial regression equation for total polyphenol content (z) as a function of roasting temperature (x) and time (y) are expressed as the following equation: $z = 32.75 - 0.12x - 0.036y - 0.00052xy$ ($R^2 = 0.98$). It could be used to predict TPC under roasting treatments.

The obtained data showed that chocolates produced from beans roasted at relatively higher temperatures and longer times may result in a lower concentration of polyphenols (Figure 1). These results are consistent with previously reported trends for the roasting effect on TPC decrease in a temperature and time- dependent manner. Significantly decrease was observed at over temperature

of 130°C and higher. The presented data and Oliviero et al. [5] observed that cocoa beans were roasted at 150 °C for 30 min a losing 44% and 57% in TPC, respectively. This indicates that Southern Taiwan chocolate may retain more polyphenol after a series of cocoa processing steps.

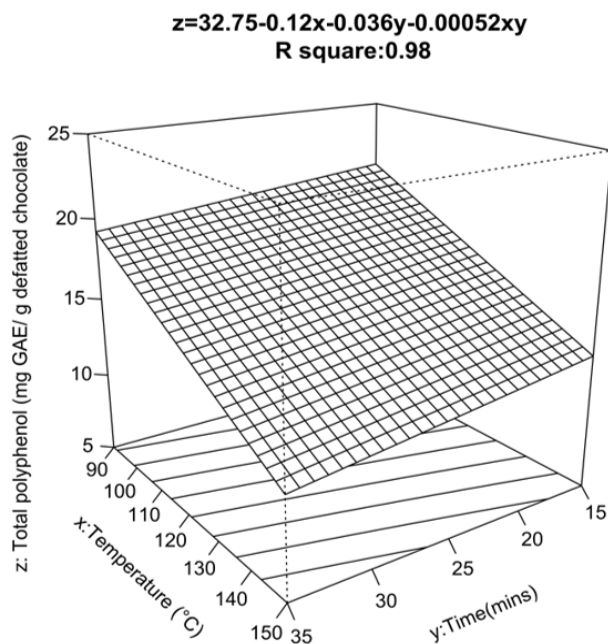


Figure 1. Polynomial regression curve showing the effects of roasting temperature (X) and time(Y) on the total polyphenol content (Z) of 12 manufactured chocolates.

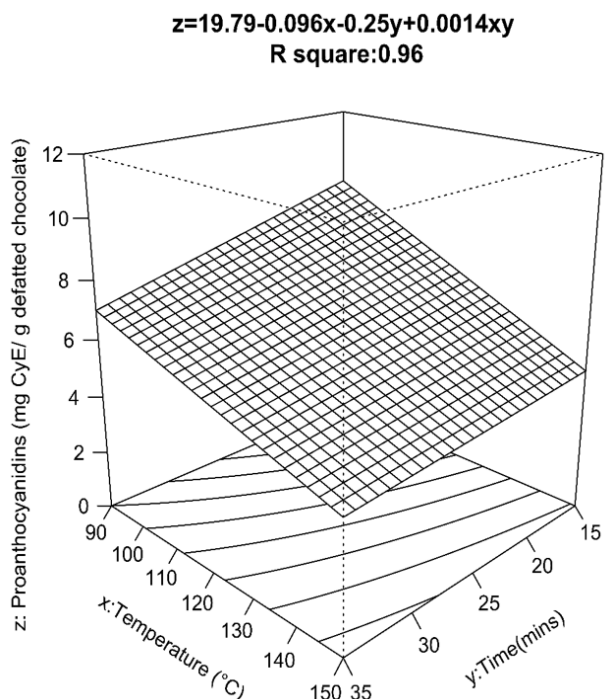


Figure 2. Polynomial regression curve showing the effects of roasting temperature (X) and time(Y) on the proanthocyanidins content (Z) of 12 manufactured chocolates

Proanthocyanidins has not only beneficial effects on human health, but is also responsible for the pleasant sensory profile of chocolate [14]. Figure 2 shows the effect of roasting conditions on the Proanthocyanidins contents. The results showed that roasting conditions

significantly decreased the proanthocyanidins contents in a temperature and time-dependent manner, from the highest 9.20 ± 0.49 mg CyE /g defatted chocolate decreased to 4.14 ± 0.41 mg CyE /g defatted chocolate. These data from Figure 1 and Figure 2 confirm the observation of more severe thermal treatment decreased the contents of total polyphenol and proanthocyanidins. The polynomial regression equation for proanthocyanidins content (z) as a function of roasting temperature (x) and time (y) are expressed as the following equation: $z = 19.79-0.096x-0.25y+0.0014xy$ ($R^2 = 0.96$). The equation is useful to predict the proanthocyanidins content under roasting treatments. Proanthocyanidins has a beneficial effect on vasculoprotection and include the monomeric forms, (-)-epicatechin and (+) catechin, and the oligomeric form of the monomeric units, the basic structure (C6-C3-C6) two aromatic rings and oxygenated heterocycle in the middle [15]. Severe roasting treatment caused a significant ($p < 0.05$) decrease in the level of TPC and proanthocyanidins which exhibited a rapid degradation rate (Figure 1 and 2). The losses of free phenolics could be due to the fact that these compounds are susceptible to oxidative degradation at high roasting temperature [12]. While the content of proanthocyanidins is likely the result of the balance of degradation reactions and formation polymerization reaction [16].

3.2. Antioxidant Capacity

Two different assays of ABTS and DPPH are used to determine the antioxidant activity, which are associated with free radical scavenging. Figure 3(A) shows ABTS radical scavenging activities of 12 manufactured chocolates. The roasting treatment significantly decreased the ABTS radical inhibition in a temperature and time-dependent manner, from the highest 5.06 ± 0.26 mg TE /g defatted chocolate decreased to 2.27 ± 0.39 mg TE /g defatted chocolate.

In addition, DPPH radical scavenging activities of 12 manufactured chocolates are measured. As seen in the Figure 3(B), also exhibited a temperature and time-dependent manner decrease on DPPH radical scavenging activity. A decline of DPPH radical scavenging activity, from the lowest IC₅₀ 128 μ g /g defatted chocolate to the highest value 245 μ g/g defatted chocolate was displayed. As shown from Figure 1, Figure 2, and Figure 3(B) the results show that the lowest roasting process retain the highest TPC, proanthocyanidins, ABTS, and DPPH radical scavenging activity. Our data implies that the antioxidant activity is directly related to TPC and proanthocyanidins, which are affected by the roasting treatment. The main polyphenolic compounds present in cocoa bean are epicatechin, catechin, and proanthocyanidins, which consist of aromatic compounds characterized by OH group having antioxidant activity [17]. These observations demonstrate that the antioxidant activities are significantly affected with the levels of total polyphenols in chocolate. Free radicals play an important role to induce pathological event such as inflammation, aging and carcinogenesis. In present study, the low-roasting chocolates rich in phenolic compounds could be a potential functional food with strong free radical scavenging activity.

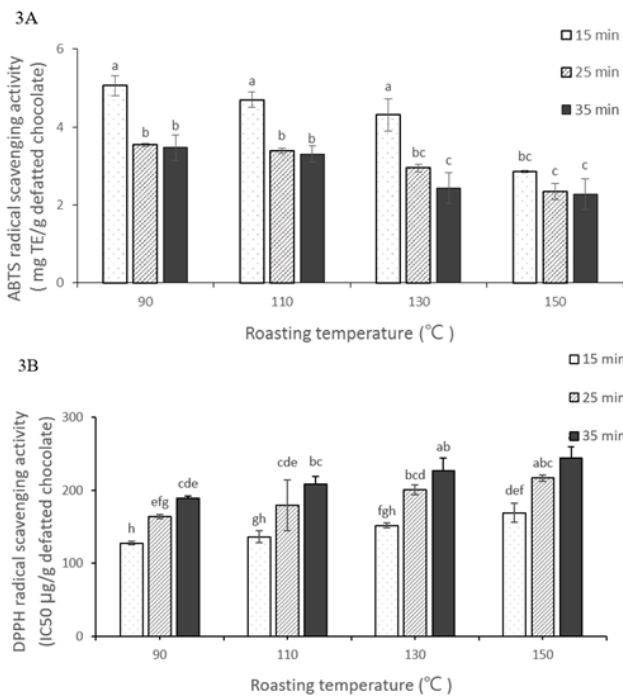


Figure 3(A). The effects of roasting temperature and time on ABTS radical scavenging activity of 12 manufactured chocolates. **(B).** The effects of roasting temperature and time on DPPH radical scavenging activity of 12 manufactured chocolates. Values are expressed as the means±SD and a common letter in the same graph are not significant different ($p < 0.05$).

3.3. Mushroom Tyrosinase Inhibitory Activity

Tyrosinase, a copper-containing enzyme, is a key enzyme for melanin formation, which being the most important factor of mammalian skin color. The melanin formation owing to tyrosinase activity after sunlight exposure causes dermatological disorders associate with age spots, melasma, and freckles [11]. In addition, unfavorable enzymatic browning of food by tyrosinase leads to a decrease in nutritional quality and economic loss of food products [18]. The effect of 12 manufactured

chocolates on mushroom tyrosinase activity is displayed in Figure 4. The concentration at 2.5 mg/g defatted chocolate, more severe thermal treatment lowers the inhibitory effect, from $37.14 \pm 2.13\%$ to $3.89 \pm 1.86\%$. The inhibitory effect of chocolate roasted at 90 °C for 15 min on mushroom tyrosinase activity is the highest inhibition than the others. Obviously, low-roasting chocolate exhibited significant inhibitory effect on tyrosinase activity. (-)Epicatechin, (+) catechin, kaempferol, quercetin, and ellagic acid present in cocoa bean that show the competitive inhibitory activity of tyrosinase [14,19]. In addition, the action of inhibitory activity is suggested that the copper ion of tyrosinase is chelated by hydroxyl group of these bioactive compound and through OH groups bind to the active site of tyrosinase, lead to steric hindrance or change conformation [18,20]. In other words, the potent inhibitory action on mushroom tyrosinase of low-roasting chocolate could be contribute to these bioactive compounds such as epicatechin, catechin, ellagic acid, quercetin, kaempferol, and other unknown compounds. Severe thermal treatment decreasing the inhibitory effect is suggested that these bioactive compounds were lost by degradation or polymerization reactions. Thus, low-roasting chocolate especially, could be a potent whitening reagent with antityrosinase activity.

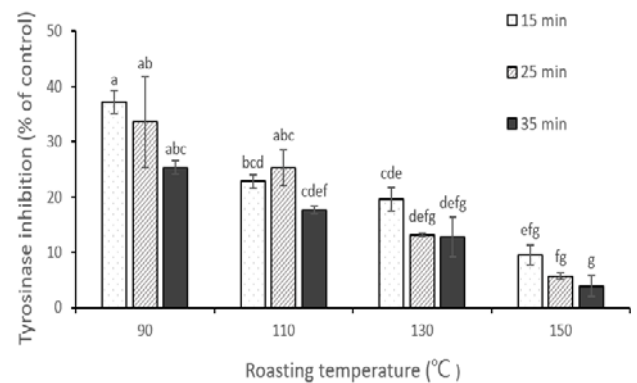


Figure 4. The effects of roasting temperature and time on the inhibition of mushroom tyrosinase activity of 12 manufactured chocolates. Values are expressed as the means±SD and a common letter in the same graph are not significant different ($p < 0.05$).

Table 1. L^* , a^* , b^* , C^* , h^* values for 12 manufactured chocolates under roasting treatments

Roasting treatments		Color parameters				
Time (min)	Temperature (°C)	L^*	a^*	b^*	C^*	h^*
15	90	32.36 ± 0.07^a	7.36 ± 0.09^e	2.61 ± 0.12^i	7.80 ± 0.09^e	19.51 ± 0.87^j
	110	31.21 ± 0.12^f	7.32 ± 0.05^e	2.86 ± 0.14^h	7.85 ± 0.02^e	21.36 ± 1.09^b
	130	31.94 ± 0.09^c	8.01 ± 0.05^c	5.97 ± 0.09^e	9.99 ± 0.02^d	36.71 ± 0.59^e
	150	30.89 ± 0.40^e	7.98 ± 0.20^c	6.67 ± 0.31^c	10.40 ± 0.34^c	39.85 ± 0.75^c
25	90	32.18 ± 0.03^{ab}	7.38 ± 0.04^e	2.48 ± 0.11^i	7.78 ± 0.04^e	18.55 ± 0.79^j
	110	32.14 ± 0.10^b	7.73 ± 0.05^d	3.82 ± 0.07^e	8.63 ± 0.07^f	26.28 ± 0.37^e
	130	30.97 ± 0.17^e	8.08 ± 0.06^{bc}	6.43 ± 0.13^d	10.32 ± 0.09^c	38.51 ± 0.58^d
	150	31.74 ± 0.08^{cd}	8.17 ± 0.13^{ab}	7.73 ± 0.19^a	11.25 ± 0.21^a	43.40 ± 0.56^a
35	90	31.89 ± 0.03^c	7.44 ± 0.04^e	2.53 ± 0.10^i	7.86 ± 0.02^e	18.79 ± 0.80^{ij}
	110	31.48 ± 0.04^e	7.80 ± 0.07^d	5.21 ± 0.08^f	9.38 ± 0.06^e	33.74 ± 0.53^f
	130	31.64 ± 0.34^{de}	8.27 ± 0.26^a	7.22 ± 0.17^b	10.98 ± 0.30^b	41.11 ± 0.58^b
	150	30.55 ± 0.10^h	8.11 ± 0.08^{bc}	7.24 ± 0.12^b	10.87 ± 0.13^b	41.72 ± 0.40^b

Values are expressed as the means±SD (n = 5)
Different letters indicate significantly different ($p < 0.05$).

Table 2. The organoleptic properties of 12 manufactured chocolates under roasting treatments

Roasting treatments		Organoleptic properties							
Time (min)	temperature (°C)	Appearance	Aroma	Flavor	Sweetness	Acidity	bitterness	Astringency	Acceptability
15	90	5.44 ^a	4.21 ^c	3.91 ^{bc}	2.60 ^c	4.95 ^a	6.23 ^a	6.07 ^a	2.91 ^e
	110	5.81 ^a	4.63 ^{abc}	4.74 ^{abc}	3.98 ^{ab}	4.53 ^a	5.12 ^{abc}	5.26 ^{abc}	4.02 ^{bcd}
	130	6.07 ^a	5.35 ^{abc}	5.14 ^{ab}	4.40 ^a	3.88 ^a	4.56 ^{bc}	4.74 ^{bc}	4.74 ^{abc}
	150	5.65 ^a	5.67 ^a	4.95 ^{abc}	4.44 ^a	5.02 ^a	4.67 ^{bc}	4.67 ^{bc}	4.26 ^{bcd}
25	90	5.77 ^a	4.44 ^{abc}	3.95 ^{bc}	3.16 ^{bc}	4.42 ^a	5.72 ^{ab}	6.51 ^a	3.23 ^{de}
	110	6.14 ^a	4.84 ^{abc}	4.67 ^{abc}	3.86 ^{ab}	4.42 ^a	5.26 ^{abc}	5.30 ^{abc}	3.86 ^{cde}
	130	6.05 ^a	5.51 ^{ab}	5.60 ^a	4.72 ^a	4.37 ^a	4.07 ^c	4.26 ^c	5.67 ^a
	150	6.26 ^a	5.07 ^{abc}	4.35 ^{abc}	3.60 ^{abc}	4.47 ^a	4.79 ^{abc}	4.98 ^{bc}	3.72 ^{cde}
35	90	5.26 ^a	4.23 ^{bc}	3.70 ^c	3.05 ^{bc}	5.02 ^a	5.77 ^{ab}	6.14 ^{ab}	3.14 ^{de}
	110	5.63 ^a	4.88 ^{abc}	4.91 ^{abc}	4.09 ^{ab}	4.84 ^a	5.00 ^{abc}	5.26 ^{abc}	4.37 ^{bcd}
	130	6.14 ^a	5.23 ^{abc}	5.49 ^a	4.58 ^a	4.88 ^a	4.02 ^c	4.86 ^{bc}	5.16 ^{ab}
	150	5.85 ^a	4.40 ^{abc}	3.91 ^{bc}	3.58 ^{abc}	4.19 ^a	4.86 ^{abc}	4.19 ^c	3.67 ^{cde}

Values are expressed as the means (n = 44)

Different letters indicate significantly different ($p < 0.05$).

3.4. Color Parameters

Color of chocolate is a typical sensory perception to consumer and affects its acceptability. The color of the chocolate is represented by the L^* , a^* , b^* , C^* and h^* value. As given in Table 1, the results showed that the L^* value decreased gradually as the roasting temperature and time increased from 32.36 to 30.55 and the value of a^* , b^* , C^* and h^* generally increased with higher roasting temperatures and longer times. The changes in color parameters are highly related to the Maillard reactions during roasting that lead to synthesis of brown pigments. The parameters L^* and h^* were chosen as the strength of browning. The findings of the L^* value are consistent with the reported trends by Żyzelewicz et al. [1]. They studied the color changes of chocolate made from cocoa liquor containing roasted and unroasted beans and revealed that a decrease in L^* value (from 31.59 to 30.04) with the increased amount in cocoa liquor from roasted beans. The h^* value peaked at roasting temperature 150 °C and time for 35 min and exhibited a significant difference ($p < 0.05$) comparing to the others. The highest h^* value of the chocolate could be attributed to the oxidation and polymerization of polyphenols, degradation of proteins and dextrinization of starch yielding more brown pigments as reported by Krysiak [21].

3.5. Sensory Evaluation

The results of the sensory evaluation for 12 manufactured chocolates, including appearance, aroma, flavor, sweetness, acidity, bitterness, astringency, and acceptability, are shown in Table 2. Except for appearance and acidity, there exists significant differences among the organoleptic properties of 12 manufactured chocolates. The overall preference analysis has the worst and best rating of chocolate made by roasting at 90°C for 15min (2.91 points) and 130 °C for 25 min (5.67 points), respectively. Low-roasting chocolates result in the high intensity of bitterness and astringency rating, while an increase in temperature and duration of roasting improve their sensory attributes as given in Table 2. These results confirm the observation of odor threshold values by Frauendorfer and Schieberle [22], the changes induced by roasting at temperature above 130°C should be

noticeable to consumers. Stanley et al. [16] indicated that cocoa aroma related compounds increased with roasting temperature above 100°C, whereas deleterious sensory-related compounds formed at 150°C for 30min or higher roasting conditions. This may be also the reason for chocolate from beans roasted at temperature 150°C for 35 min with the rating only 3.67 points of consumer acceptability. In addition, according to the data in Table 2, Figure 1 and Figure 2, thermal processing of chocolate at relatively high temperature may contribute to favorable physicochemical, and organoleptic changes result in a decrease of polyphenols concentration [3]. Therefore, a proper design of roasting conditions could be optimized the chocolate both of health promoting compounds and acceptable sensory quality.

4. Conclusions

Cocoa bean roasting is an important step in chocolate processing and results in the generation of desirable flavor and aroma compounds, as well as color changes contributing to high consumer acceptability. However, the low-roasting chocolate could be retained more content of the polyphenol and proanthocyanidins, and significant antioxidative effect. In addition, our findings provide a new insight that the low-roasting chocolate seems to be effective in the inhibition of mushroom tyrosinase. The inhibitory action on tyrosinase may be attributed to polyphenolic components presenting in the chocolate. In summary, the low-roasting chocolate exhibited a potency in radical scavenging and tyrosinase inhibition, could be as the potent candidate of health functional food. Further investigation of low-roasting chocolate on the inhibition of melanin formation and related to unknown bioactive compounds will be identified in our further.

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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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