Journal of Food and Nutrition Research, 2021, Vol. 9, No. 9, 457-468
Available online at http://pubs.sciepub.com/jfnr/9/9/2
Published by Science and Education Publishing
DOI:10.12691/jfnr-9-9-2

Flavan-3-ols Monomer and Oligomer, and Flavor Profiles of Chocolates Produced from Fermented Cocoa Beans under Different Roasting Degree in Taiwan

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Received July 23, 2021; Revised August 29, 2021; Accepted September 08, 2021

Abstract Taiwan cocoa beans were fermented in a wooden box by natural occurring microorganisms for seven days. Six types of chocolate were manufactured from the fermented cocoa beans under different roasting degree. The fermentation parameters including the count of microorganisms, fermenting mass temperature, pH value of the pulp and cotyledons of cocoa bean along with the fermentation time were determined. Furthermore, the flavan-3-ols monomer and oligomer (FMO) of chocolates were determined by normal phase HPLC and the volatile aroma compounds of the six types of chocolate were analyzed by electronic nose to identify the important flavors and odors. Primary component analysis (PCA) was performed to distinguish the flavor profiles among the chocolates. The sensory assessment was conducted to evaluate the organoleptic properties of the six types of chocolate and their overall acceptability. The results showed that the total amount of FMO and proanthocyanidins in the chocolate both decrease with the intensity of the roasting degree of cocoa beans. Twenty-three important volatile aroma compounds were identified by electronic nose analysis. Differences in the amount of each compound were determined in the investigated chocolate and these are expected to cause sensory differences among the six types of chocolate. As a result, chocolates produced from beans under medium roasting condition (130°C and 25min) rated the highest score of overall acceptability among the chocolates. It was significantly higher than those of chocolate produced from light roasting beans. On the other hand, chocolate produced from cocoa bean under light roasting degree was retained more content of the bioactive compounds and could be a potent candidate of health functional food. PCA was successful to discriminate the flavor profiles of the six types of chocolate produced from beans under different roasting degree and could be used as a tool to recognize the origin of chocolate product. These results of this study could provide an important approach for producing chocolate products with desired flavor properties and bioactive compounds that have positive benefits for human health.

Keywords: *Taiwan chocolate, FMO, roasting degree, flavor profiles, electronic nose, sensory assessment, PCA*

Cite This Article: Ying Chun Lin, and Youk Meng Choong, "Flavan-3-ols Monomer and Oligomer, and Flavor Profiles of Chocolates Produced from Fermented Cocoa Beans under Different Roasting Degree in Taiwan." *Journal of Food and Nutrition Research*, vol. 9, no. 9 (2021): 457-468. doi: 10.12691/jfnr-9-9-2.

1. Introduction

Chocolate products require a series of processing, including fermentation, drying, roasting, winnowing, grinding, refining, molding, and aging. During fermentation, the amounts of bioactive substances in the cocoa beans decrease, especially for polyphenols, which are flavan-3-ols monomer and oligomer (FMO) as well as proanthocyanidins (PAC). Numerous researches have pointed out the health benefits of polyphenols, such as the prevention of Parkinson's and Alzheimer's disease, the protection of cardiovascular and intestinal probiotics, emotion regulation, and obesity prevention [1]. Due to the multiple benefits of polyphenols, chocolate products with rich polyphenols are gaining increasing attention among chocolate consumers [2].

Fermentation affects the chocolate flavor. Polyphenol oxidases polymerize or degrade polyphenols, making these resultants react with proteins and carbohydrates, and eventually change the color, flavor, and texture of cocoa beans. Meanwhile, flavor precursors such as free amino acids, short-chain peptides, and reducing sugar are generated during fermentation, which are important substances that affect the chocolate flavor while roasting [3]. Roasting is another essential procedure that affects the flavor and the amount of polyphenols. Typical roasting temperature is between 130°C and 150°C, and the roasting time ranges from 10 to 40 min. Maillard reaction, which occurs during roasting, darkens the color of the cocoa beans, enriches the aroma and flavor, yet seriously reduces

the level of flavan-3-ols monomer and oligomer as well as proanthocyanidins. Some researches even indicated that the level of these bioactive compounds drops up to 50-80% [4,5]. Ioannone et al. [6] roasted the cocoa beans in different temperatures (125-145°C) and roasted them until the level of moisture declined to 2%. They discovered that cocoa beans with higher temperature and shorter roasting time remain at a higher level of proanthocyanidins yet have a poorer antioxidant capacity. Oracz et al. [7] discussed the influence of roasting temperature (110-150°C) and humidity condition (0.3-5%) on cocoa beans, showing that cocoa beans with lower roasting temperature and higher humidity retain more bioactive substances. Intense roasting not only decreases the level of polyphenols, which is an obstacle to the pursuit of chocolate healthcare products but also harms the chocolate flavor. Meanwhile, the genotype and the growth environment of cocoa trees affect the level of polyphenols in cocoa beans as well [3].

The cocoa plantation is uprising agriculture in Taiwan. As the plantation gradually spreads from the south to the middle parts of Taiwan, the increasing planting area as well represents the growing production of cocoa beans. This attracts the government and private academic institutions to devote themselves successively to cocoa relevant research, e.g., the choice of fermentation strains and drying methods, the regulation of roasting temperature and time, the change of the bioactive substances, and the health benefits of the chocolate products, all aiming to strengthen the competitive advantage of Taiwan cocoa beans. However, few have discussed the aroma compounds and the flavor characteristic profiles of chocolate products made from Taiwan cocoa beans. In particular, the change of the level of bioactive compounds and the aroma compounds as well as the flavor characteristic profiles under low and high roasting temperature conditions are not completed yet, which highlights the necessity of our research.

The present study was designed to prepare six types of chocolate from the fermented cocoa beans in Taiwan under different roasting degree. The fermentation parameters including the count of microorganisms, fermenting mass temperature, pH value of the pulp and cotyledons of cocoa bean during the fermentation duration were determined. Furthermore, the flavan-3-ols monomer and oligomer (FMO), the volatile aroma compounds, and the sensorial assessment of the six types of chocolate were analyzed. In addition, PCA was performed to distinguish the flavor profiles among the six types of chocolate. These results could provide an important approach for producing chocolate products with desired flavor properties and bioactive compounds that have positive benefits for human health.

2. Material and Methods

2.1. Chemicals

Cycloheximide, chloramphenicol and penicillin G potassium salt were purchased from Cyrusbioscience, Inc. Taiwan. Glucose (Panreac Química S.L.U.), Peptone (BD Bacto™), Yeast Extract (MdBio, Inc.), Agar, Bacteriological (Acumedia), MRS BROTH (CONDA), Calcium carbonate (EMSURE). Chlortetracycline hydrochloride, procyanidin B1, (-)-epicatechin, dichloromethane, methanol, acetone, acetic acid, butanol, hydrochloric acid, and ammonium ferric sulfate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All chemicals were of analytical grade.

2.2. Cocoa Bean Fermentation

The cocoa pods were harvested in the morning and transported to the laboratory immediately by local farmer in Pingtung County of the South Taiwan. The seeds with mucilaginous pulp were taken out from the cocoa pods on the condition of not harming the cocoa beans. 75 kg cocoa beans were immediately transferred to a wooden box and the spontaneous fermentation process was initiated by natural microorganisms. For every 24 h, 25 g of cocoa beans were sampled to measure the microbial population and 25 g of cocoa beans were also sampled for every 12 h to determine the pH value of pulp and cotyledon. The fermentation was lasted for 7 days and the fermenting mass temperature profiles were recorded. When the fermentation process was terminated, drying was conducted at 50°C in a dryer until about 6% of moisture remaining in the cocoa beans. Dried cocoa beans were stored in refrigeration until further used.

2.3. Microbial Population Determination

The yeast medium was YEPA (2% glucose, 2% peptone, 1% yeast extract, 1.5% agar, pH = 5.6, containing 100 mg/L chloramphenicol and 50 mg/L chlortetracycline to inhibit bacterial growth). The lactic acid bacteria (LAB) medium was MRSA (55 g/L MRS Broth, 1.5% agar, 0.5%$CaCO_3$, containing 100 mg/L cycloheximide to suppress yeast growth and 100 mg/L cysteine-HCl to produce anaerobic conditions during incubation). The acetic acid bacteria (AAB) medium was GYCA (5% glucose, 1% yeast extract, 5% $CaCO_3$, 1.5% agar, pH = 5.6, containing 100mg/L cycloheximide and 50 mg/L penicillin to inhibit the growth of yeast and bacteria, respectively). The cocoa beans were taken out 25g every 24h from fermentation box and mixed with 225 ml of distilled water and homogenized in a stomacher, followed by the series of decimal dilutions. The three microorganisms, yeast, LAB and AAB, were enumerated by surface spread plate inoculation on YEPA, MRSA and GYCA, respectively. The growth of yeast incubated at 30°C for 3 days. LAB were grown at 37°C for 3 days, AAB were incubated at 30°C for 5 days. At the end of incubation, the number of colony forming units (CFU) was recorded for each type of microorganism [8].

2.4. pH Determination

The pH measurements of pulp and cotyledon of cocoa beans were according to the methods of Lin and Choong [9].

2.5. Cocoa Bean Roasting

The roasting conditions were set at 90°C for 15min and 90°C for 25min, which is referred to as light roasting; 110°C for 25min and 130°C for 25min, which is referred to as medium roasting; 150°C for 25min and 150°C for 35min, which is referred to as heavy roasting. The roasting process was based on the methods described by Chu and Lin [10].

2.6. Chocolate Preparation

The roasted cocoa bean samples (1.2kg each) with different roasting degree were initially cracked and winnowed by a machine to separate the cotyledons and the cocoa bean shells. The cotyledons were used to produce paste-like cocoa liquors by the cocoa grinding machine and the six types of chocolate were prepared by the methods of Chu and Lin [10]. After demolding from the chocolate mold, the individual chocolate was wrapped by aluminum foil and then stored at 18°C in a cabinet for 7 days. The finished chocolates are delivered to 38 untrained panels to conduct the sensorial evaluation or further analysis.

2.7. Chocolate Defatting

The six types of chocolate (10g each) were ground in the presence of liquid nitrogen using blender machine (Pulverisette 11, Fritsch GmbH, Germany) to obtain the fine powder. The ground fine powder was mixed with n-hexane at a ratio of 1 to 2 (w/v) for 30min with a ultrasonicator at 40°C. The mixture was then centrifuged at 10000rpm for 10min and the supernatant was removed. The defatting process was repeated in triplicate. The collected precipitate was air-dried in a fume hood for 48h.

2.8. Flavan-3-ols Monomer and Oligomer Determination

0.2g fat-free powder each from different roasting degree chocolates was centrifuged with 50ml of a solvent of acetone/water/acetic acid (70:28:2, v/v) at 25°C for 1h for extraction. The supernatant was kept and the precipitant was extracted with repeated procedures. The collected supernatant was mixed with the previous one. The resulting mixture was filtrated and adjusted the volume to 100ml. This extract was filtrated with a 0.45μm filter and the FMO content including different degree of polymerization (DP) from 1 to 10 were measured by NP-HPLC. Relevant information of NP-HPLC is descripted by Lin et al. [11] 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 was 1ml/min with a gradient elution by the combination of mobile phase A and B. The gradient elution was as follows: from 100% to 82.4% of A as time from 0 to 30 min, and then to 69.3% of A as time from 30 to 45 min, and to 12.2% of A as time from 45 to 50 min, and then to 50% of A as time from 50 to 52 min. and to 100% of A as time from 52 to 55 min, and maintained this gradient for an additional 5 min.

2.9. PAC Determination

0.2g fat-free powder each from different roasting degree chocolates with 20ml of 90% methanol were extracted in a 50ml centrifugal tube for 1h at room temperature with ultrasonic oscillation. The collected supernatant was adjusted to 25ml with demineralized water. The procyanidin B1/methanol solution were prepared at different concentrations: 0.01, 0.025, 0.05, 0.1, 0.15 and 0.20mg/ml. 1ml of the solution each 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 was conducted in triplicate under 546nm 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 establish the standard curve for PAC determination. The measurement of PAC was based on the methods described by Lin et al. [11]. 1 ml of extracted solution each from the six types of chocolate was substituted for the procyanidin B1 solution.

2.10. Flavor Profiles Identified by Electronic Nose Analysis

The flavor profiles of the six types of chocolate produced from cocoa bean with different roasting degree were measured using an Electronic Nose Heracles II (Alpha M.O.S. Inc., Toulouse, France). The Heracles II was equipped with an auto-sampling system, an integrated cooled trap with thermo-desorption system, a dual column configuration system: a non-polar column (MXT5: 5% diphenyl, 95% methylpolysiloxane, 10 m length and 180 μm diameter) and a slightly polar column (MXT1701: 14% cyanopropylphenyl, 86% methylpolysiloxane, 10 m length and 180 μm diameter) coupled to 2 flame ionization detectors for data acquisition [9,12,13]. An aliquot of each different roasting degree chocolate sample (5g) was placed in a 20 mL vial and sealed with a magnetic plug. The vial was placed in a shaker oven where it remained at 60°C for 10 min. Next, a syringe pierced the silicone septum of the magnetic plug and sampled 5 ml of the head space. The 5ml headspace aliquot was trapped at 20°C for 50sec, and subsequently, the temperature of the trap was increased up to 240°C in 30sec heating duration and then the sample was injected at 125μL/sec. The temperature program started at 50°C (held for 2 sec) and increased up to 80°C at 1°C/sec, and then increased up to 250°C at 2°C/sec, the final temperature was held for 21 sec. The total acquisition time was 138sec. For calibration, an alkane solution (from n-hexane to n-hexadecane) was used to convert retention time in Kovats indices and identify the aroma compounds and their sensory attributes using AroChemBase library software. Meanwhile, Alpha Soft (V14.2, Alpha M.O.S, Toulouse, France) software was used for instrument control and raw

data processing. The six types of chocolate were analyzed in quadruplicate

2.11. Sensory Evaluation

Sensory evaluation of each chocolate manufactured from cocoa bean under different roasting degree was conducted by 38 untrained panelists using the point score method in our lab. The attributes of chocolate include odor, flavor, acidity, astringency, and overall acceptability. Each panelist was requested to refresh their palate by using polenta congee between the test samples to remove the remaining odor and flavor in their mouths. 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 results [2,10].

2.12. Statistical Analysis

Each measurement was conducted at least triplicate and averaged. All data were presented as means ±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. PCA was used to discriminate the flavor profiles of different roasting degree chocolates. The statistical computing software R 4.0 was used to perform statistical and principal component analysis.

3. Results and Discussion

The amount of bioactive compounds and the flavor profiles of chocolate are not only affected by the origins of countries but also influenced by processing steps. While the fermentation parameters and drying methods are rather intractable and multifarious, and hence increased the difficulties of maintaining expected quality of chocolate products, roasting step turns out to be a relatively controllable process. Roasting degree of cocoa beans determined the final color, flavor, odor, astringency, the amount of bioactive compounds and consumer acceptability of chocolate products. Roasting 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 on the high levels of flavan-3-ols concentration and specific flavor profiles of chocolate products has grown to be an uprising trend and has caught consumers' eyes in recent years. In this study, we explored the changes of the flavan-3-ols monomer and oligomer (DP1-10), proanthocyanidins and the flavor profiles of chocolates produced from Taiwan fermented cocoa beans under different roasting degree.

3.1. Temperature and pH Change during Fermentation

The temperature change of cocoa mass during natural spontaneous fermentation process was shown in Table 1. The center temperature of fermenting cocoa mass started at 25.4°C, gradually increased and reached the peak temperature 48.1°C at 156h, and then decreased to 44.1°C at the end of fermentation process. The change of cocoa mass center temperature was mainly due to biochemical reactions of metabolites of natural occurring microorganisms during cocoa bean fermentation. The yeast transformed the substances in mucilaginous pulps into ethanol and carbon dioxide. Afterward, the AAB oxidized ethanol into acetic acid. This reaction is an exothermal process, liberating heat and leading to a fast temperature increase. The temperature rose to even 50°C of the fermenting cocoa mass and eventually caused the death of the seed embryo [14,15,16]. The observations in this study were not in accordance with the study made by Brito et al. [17], who indicated that the peak temperature appeared at around the fifth day of natural fermentation. In addition, Schwan and Wheals [18] reported that the maximum temperature happened at the fourth day during cocoa fermentation. As seen in Figure 1, the population of AAB at 120h was largely increased up to 6.9 log CFU/g of cocoa bean and maintained at this level until the termination of fermentation. This may be the reason for the peak temperature of fermenting cocoa mass occurred at 156h.

The pH in pulp of cocoa bean remained relatively constant from 3.92 at the beginning of fermentation and slightly increase in pH up to 4.16 at the end of fermentation as shown in Table 1. However, the initial value of pH in cotyledon of cocoa bean was around 6.42. Later, it remained relatively constant (6.24-6.56) until 96 h, and then drastically decreased to a value of 4.52 at the end of the fermentation process. The change in the pH value is associated with the biochemical reaction, where LAB converts citric acid and carbohydrates into lactic acid, resulting in a slight increase in pH during fermentation. However, AAB oxidize ethanol and lactic acid into acetic acid, which results in a small decrease in pH value of the pulp [19]. At the end of fermentation, a slightly increased pH in pulp was possibly due to the evaporation of volatile acids, such as acetic acid; or that these acids were penetrating into the cotyledons, and then gradually absorbed by the cotyledons. As a result, the pH in cotyledons drastically decreased. These findings concurred with the previous studies [8,20].

Table 1. Cacao bean fermenting mass center temperature, pH of pulp and cotyledon change during natural spontaneous fermentation

	Fermentation time (h)														
	0	12	24	36	48	60	72	84	96	108	120	132	144	156	168
Temperature, °C	25.4	25.3	26.3	27.8	27.9	28.9	30.4	34.1	35.1	35.6	37.9	44.2	45.1	48.1	44.1
pH of pulp	3.92	3.94	3.92	3.96	3.94	3.92	3.82	3.82	3.83	3.85	3.97	4.22	4.13	4.17	4.16
pH of cotyledon	6.42	6.52	6.45	6.56	6.42	6.51	6.49	6.39	6.24	6.10	6.02	5.84	5.18	4.66	4.52

3.2. Microbial Population Change during Fermentation

The microbial succession in the cocoa bean fermentation process has been established [21,22,23]. At the first beginning of fermentation, many species of yeasts proliferate, leading to production of ethanol and secretion of pectinolytic enzymes. Meanwhile, LAB and AAB appear in the fermenting cocoa mass. The microbial population of the three microorganisms during fermentation are shown in Figure 1. The initial pH in pulp about 3.92, mainly due to citric acid and other organic acids, favor yeasts utilize pulp substances under low oxygen level conditions. The yeast population remained at 7.0-7.6 log CFU/g of bean during the first 48 h, then gradually decreased to 4.3 log CFU/g of bean for the next 96 h after that there is a dramatic decline to 2.0 log CFU/g of bean at the end of fermentation. The LAB with the amended conditions exhibit the fastest growth rate during the 48-72h period of fermentation and reach a peak population 8 log CFU/g of bean and remain at high level of 6.7-7.1 log CFU/g of bean throughout the fermentation process. When increase the aeration frequency of the fermenting cocoa mass and the temperature rises above 37°C, AAB became the dominant microorganisms, and the population reached a peak at 120h with 6.9 log CFU/g of bean. This stage in the microbial succession is reflected in an increase in acetic acid. The exothermic reactions of AAB raise the temperature of the fermenting cocoa mass up to 48.1°C at 156h. The AAB population remains relatively constant until the end of fermentation. The observations in AAB population during cocoa bean fermentation were not very consistent with several studies [18,22]. They reported that AAB population reached a peak at 88h with 1.2×10^7 CFU/g of pulp and followed a decrease trend in the number of AAB to zero at the end of the fermentation. The reason for the difference in AAB population may be due to the local climatic and fermentation operating conditions that influence the species and the sequence of microorganisms involved in cocoa bean fermentation.

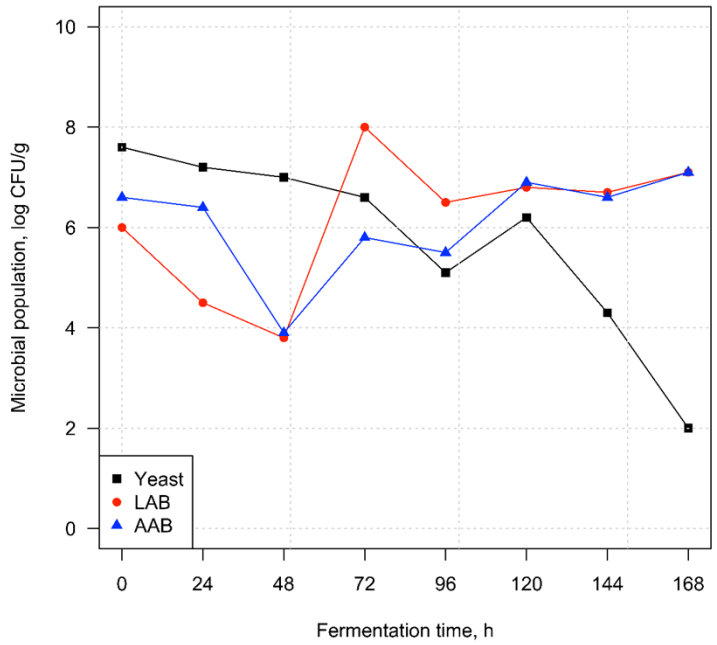

Figure 1. Microbial population of the yeast, LAB and AAB varied with the fermentation time.

Table 2. Flavan-3-ols monomer and oligomer (DP1-10) and proanthocyanidins of the six types of chocolate produced from fermented cocoa beans under different roasting conditions

Flavan-3-ols monomer and oligomer, mg (-)-epicatechin equivalents/g	Roasting conditions (temperature-time)					
	90°C 15min	90°C 25min	110°C 25min	130°C 25min	150°C 25min	150°C 35min
DP1	22.83±1.24^{a}	21.03±1.14^{ab}	19.64±1.07^{b}	16.31±0.88^{c}	15.44±0.83^{cd}	12.71±0.69^{d}
DP2	9.78±0.37^{a}	9.41±0.36^{a}	7.11±0.27^{b}	5.19±0.20^{bc}	4.07±0.16^{c}	3.58±0.13^{c}
DP3	6.67±0.30^{a}	6.66±0.31^{a}	5.26±0.25^{a}	2.80±0.13^{b}	2.29±0.11^{b}	1.25±0.06^{b}
DP4	6.45±0.37^{a}	6.73±0.40^{a}	6.04±0.35^{a}	4.28±0.25^{ab}	2.44±0.14^{bc}	0.75±0.04^{c}
DP5	2.78±0.13^{a}	2.86±0.14^{a}	2.32±0.11^{a}	1.20±0.06^{a}	3.22±0.15^{a}	0.17±0.01^{a}
DP6	1.87±0.11^{a}	1.88±0.11^{a}	1.66±0.10^{a}	0.53±0.03^{b}	0.32±0.02^{b}	0.05±0.00^{b}
DP7	1.27±0.07^{a}	1.34±0.07^{a}	0.98±0.06^{ab}	0.51±0.02^{bc}	0.02±0.00^{c}	0.02±0.00^{c}
DP8	0.92±0.05^{a}	0.96±0.06^{a}	0.74±0.04^{ab}	0.31±0.02^{bc}	0.00±0.00^{c}	0.00±0.00^{c}
DP9	0.14±0.01^{a}	0.14±0.01^{a}	0.11±0.01^{a}	0.03±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
DP10	0.07±0.01^{a}	0.06±0.01^{a}	0.04±0.00^{a}	0.01±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Total (DP1-10)	52.76±2.67^{a}	51.05±2.58^{a}	43.89±2.24^{a}	31.16±1.59^{b}	27.80±1.41^{bc}	18.51±0.93^{c}
PAC, mg procyanidin B1/g	109.85±2.09^{a}	105.19±3.12^{a}	101.16±3.41^{a}	82.93±3.87^{b}	69.00±1.54^{bc}	59.47±4.25^{c}

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

3.3. Effect of Roasting Degree on FMO and PAC

Temperature and time are two variables in the roasting design, with the operating temperature set at 90-150°C, and the roasting time was 15-35min. Table 2 presented the flavan-3-ols monomer and oligomer (DP1-10) and proanthocyanidins concentration of chocolate produced from fermented cocoa beans under different roasting degree. For cocoa beans roasted at 90°C and 15min, the total FMO concentration was 52.76±2.67mg (-)-epicatechin equivalents/g defatted chocolate, while increase the roasting time to 25min at 90°C the total FMO concentration was slightly decreased to 51.05±2.58 mg (-)-epicatechin equivalents/g defatted chocolate. However, there was no significantly ($p<0.05$) difference in FMO concentration between the two light roasting conditions. For cocoa beans roasted at 110°C and 25min, the total FMO concentration was 43.89±2.24mg (-)-epicatechin equivalents/g defatted chocolate, while rise the roasting temperature to 130°C the total FMO concentration was obviously decreased to 31.16±1.59 mg (-)-epicatechin equivalents/g defatted chocolate. There exists a significantly ($p<0.05$) difference in total FMO concentration between the two medium roasting conditions due to the different roasting temperature. For cocoa beans roasted at 150°C and 25min, the total FMO concentration was 27.80±1.41mg (-)-epicatechin equivalents/g defatted chocolate, while increase the roasting time to 35min at 150°C the total FMO concentration was decreased to 18.51±0.93 mg (-)-epicatechin equivalents/g defatted chocolate. However, there was no significantly ($p<0.05$) difference in FMO concentration between the two heavy roasting conditions. This observation indicated that more severe heat treatment decreased FMO from the highest 52.76±2.67mg (-)-epicatechin equivalents/g defatted chocolate for light roasting to the lowest 18.51±0.93 mg (-)-epicatechin equivalents/g defatted chocolate for heavy roasting. The results implied that chocolates produced from beans roasted at relatively higher temperatures and longer times resulted in a lower concentration of total FMO. The findings are consistent with previously reported trends for the roasting effect on total polyphenol decrease in a temperature and time-dependent manner [10]. Significantly decrease in total FMO concentration of chocolate was observed at over temperature of 130°C ($p<0.05$). In addition, the DP1 content was about 41-43% of the total FMO for light roasting, 45-52% of the total FMO for medium roasting, 56-69% of the total FMO for heavy roasting, while the concentrations of total FMO (DP6-10) were less than 8% for all roasting conditions, showing that most FMO existed in the form of monomers. The results also displayed the fact that the polymer concentration decreased seriously along with the degree of polymerization, which therefore explained the significant loss of total FMO amount under heavy roasting conditions. This observation is similar to the previously study by Pedan et al. [24] who indicated that the DP1 content in the cocoa beans was 35% of the total FMO (DP1-13).

Proanthocyanidins are existing in cocoa beans in nature and they are responsible for the unpleasant taste of astringency in the chocolate products. On the other hand, they also possess the ability to prevent cardiovascular disease and create health benefits. For cocoa beans roasted at 90°C and 15min, the PAC concentration was 109.85±2.09 mg procyanidin B1/g defatted chocolate, while increase the roasting time to 25min at 90°C the PAC concentration was slightly decreased to 105.19±3.12 mg procyanidin B1/g defatted chocolate. However, there is no significantly ($p < 0.05$) difference in PAC concentration between the two light roasting conditions. For cocoa beans roasted at 110°C and 25min, the PAC concentration was 101.16±3.41 mg procyanidin B1/g defatted chocolate, while rise the roasting temperature to 130°C the PAC concentration was decreased to 82.93±3.87 mg procyanidin B1/g defatted chocolate. There exists a significantly ($p < 0.05$) difference in PAC concentration between the two medium roasting conditions due to the different roasting temperature. For cocoa beans roasted at 150°C and 25min, the PAC concentration was 69.00±1.54 mg procyanidin B1/g defatted chocolate, while increase the roasting time to 35min at 150°C the PAC concentration was decreased to 59.47±4.25 mg (-)-epicatechin equivalents/g defatted chocolate. However, there is no significantly ($p < 0.05$) difference in PAC concentration between the two heavy roasting conditions. In general, intense heating conditions could be caused severe loss of the content of total PAC. The present results indicated that the PAC concentration dropped dramatically as the roasting time and temperature rose, falling from 109.85±2.09 mg procyanidin B1/g defatted chocolate to 59.47±4.25 mg (-)-epicatechin equivalents/g defatted chocolate. The declining trend of PAC concentration corresponded to that of the FMO level. 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 [6,7,25].

3.4. Effect of Roasting Conditions on Aroma Compounds

Flavor and odor are of great importance when considering the consumer acceptability of chocolate products. The characteristic flavors and odors of chocolates are due to a mixture of hundreds of aroma compounds [26]. These compounds comprise of acids, alcohols, aldehydes, furans, esters, hydrocarbons, pyrazines, ketones, ethers, and others. Each of them possesses particular flavor attributes. For instance, most esters provide a fruity/flowery odor and pyrazines usually generate roasted odor. Therefore, the amount and type of aroma compounds are the key of chocolate quality, and they depend not only on the genotype and the origins of the cocoa tree, but also on how the beans have been processed. Consequently, the fermentation and roasting process both play a decisive role for determining the flavor of the chocolates; the former generated flavor precursors that develops the aroma attributes, and the latter produced aroma compounds in the roasted cocoa beans [3]. Twenty-three important aroma compounds and their odor description were detected by electronic nose analysis. Aroma compounds such as acetic acid, 2,3-butanediol, acetaldehyde, ethanol, propanal, cis-3-hexenol,

2-methylfuran, 2-methylbutanal, propyl acetate, 1-butene, ethyl 2-methylbutyrate, trimethylpyrazine, homofuraneol, ethyl isobutyrate, 2-methyl-2-cyclopenten-1-one, pentane, tert-butylmethylether, 2-methylbutane, ethenyl-dimethylpyrazine, methyl 2-methylbutanoate, ethylbenzene, nonan-2-one, 1s-(-)-alpha-pinene were identified and their peak area of each aroma compound were calculated as shown in Table 3. The total peak area of aroma compounds of chocolate produced from cocoa beans with heavy roasting degree were significantly greater than those of chocolates produced from cocoa beans with light and medium roasting degree ($p<0.05$). The results showed that the higher the roasting temperature was applied to cocoa beans, the higher amount of aroma compounds generated in cocoa bean and the resulted chocolates.

Table 3. The peak area of each compound of the six types of chocolate produced from cocoa beans under different roasting conditions identified by electronic nose

Compound	Description of odor	Roasting conditions (temperature-time)					
		90°C-15min	90°C-25min	110°C-25min	130°C-25min	150°C-25min	150°C-35min
Acetic acid	Pungent, Vinegar	38976 ±5393^{c}	51870 ±3533^{bc}	74250 ±11177^{bc}	65477 ±1880^{bc}	142877 ±6602^{a}	98325 ±5984^{ab}
2,3-Butanediol	Fruity, Onion	29799 ±7826^{b}	18652 ±2855^{b}	30988 ±5753^{ab}	22443 ±3151^{b}	62411 ±4726^{a}	48472 ±2437^{ab}
Acetaldehyde	Fresh, Fruity	23910 ±2239^{b}	26494 ±2754^{b}	121584 ±15984^{b}	109968 ±1145^{b}	245732 ±51359^{a}	321989 ±12954^{a}
Ethanol	Alcoholic, Pungent	13125 ±1290^{b}	14011 ±1468^{b}	65008 ±8931^{b}	59185 ±620^{b}	141054 ±32392^{a}	190489 ±9013^{a}
Propanal	Pungent, Solvent	11712 ±1557^{b}	11311 ±88^{b}	14440 ±1138^{ab}	11937 ±378^{b}	23125 ±2225^{a}	14940 ±774^{ab}
cis-3-Hexenol	Fresh, Grassy	11629 ±2496^{b}	6346 ±670^{b}	12516 ±2731^{ab}	8198 ±877^{b}	25495 ±1022^{a}	19437 ±802^{ab}
2-Methylfuran	Burnt, Chocolate	7934 ±1127^{ab}	7345 ±113^{ab}	8688 ±825^{ab}	7217 ±569^{b}	14049 ±1876^{a}	7549 ±777^{ab}
2-Methylbutanal	Cocoa, Almond	4736 ±967^{b}	1823 ±103^{b}	2601 ±973^{b}	2718 ±814^{b}	10834 ±2931^{a}	9351 ±1230^{ab}
Propyl acetate	Caramel, Fermented	4014 ±821^{ab}	3128 ±249^{ab}	6216 ±1162^{a}	3000 ±196^{ab}	2646 ±119^{b}	1552 ±341^{b}
1-Butene	-	3128 ±291^{b}	2708 ±204^{b}	4015 ±507^{b}	3769 ±75^{b}	8107 ±1213^{a}	9704 ±360^{a}
Ethyl 2-methylbutyrate	Apple, Fruity	2571 ±518^{c}	3422 ±303^{abc}	3223 ±181^{bc}	4034 ±340^{ab}	3491 ±341^{abc}	4491 ±150^{a}
Homofuraneol	Caramelized	1837 ±512^{a}	1608 ±54^{a}	1410 ±238^{a}	1532 ±132^{a}	1247 ±561^{a}	1802 ±236^{a}
Trimethylpyrazine	Cocoa, Earthy	1417 ±186^{bc}	895 ±81^{bc}	756 ±61^{c}	1343 ±101^{bc}	2654 ±118^{a}	2464 ±126^{ab}
Ethyl isobutyrate	Fruity, Strawberry	1198 ±644^{a}	232 ±2^{a}	337 ±127^{a}	270 ±144^{a}	717 ±806^{a}	1917 ±804^{a}
2-Methyl-2-cyclopenten-1-one	-	1139 ±148^{bc}	640 ±178^{c}	1052 ±170^{bc}	1142 ±318^{bc}	1962 ±599^{ab}	2380 ±70^{a}
Pentane	Alkane	836 ±74^{b}	683 ±58^{b}	832 ±111^{b}	787 ±30^{b}	1787 ±445^{ab}	3067 ±56^{a}
tert-Butylmethylether	-	793 ±61^{c}	816 ±24^{c}	1577 ±318^{c}	1591 ±213^{c}	3310 ±575^{b}	5038 ±327^{a}
2-Methylbutane	Almond, Herbaceous	720 ±69^{c}	689 ±43^{c}	1228 ±207^{bc}	1041 ±15^{bc}	2160 ±133^{a}	1694 ±118^{ab}
Ethenyl-dimethylpyrazine	Floral, Sweet	714 ±565^{a}	1460 ±255^{a}	841 ±298^{a}	424 ±26^{a}	1510 ±639^{a}	1842 ±78^{a}
Methyl 2-methylbutanoate	Apple, Fruity	654 ±400^{a}	718 ±19^{a}	648 ±12^{a}	791 ±38^{a}	1080 ±568^{a}	1599 ±794^{a}
Ethylbenzene	Floral, Sweet	423 ±106^{b}	561 ±25^{b}	513 ±38^{b}	536 ±48^{b}	582 ±54^{b}	838 ±87^{a}
Nonan-2-one	Baked, Earthy	40 ±81^{c}	29 ±40^{c}	23 ±40^{c}	249 ±8^{bc}	506 ±31^{a}	484 ±27^{ab}
1S-(-)-alpha-pinene	Herbaceous	35 ±40^{a}	100 ±0^{a}	68 ±11^{a}	79 ±11^{a}	65 ±44^{a}	84 ±11^{a}
Total		161347 ±21915^{b}	155800 ±7492^{b}	352235 ±39652^{b}	315421 ±16775^{b}	697412 ±82914^{a}	776699 ±61590^{a}

- not determined
Values are expressed as the means ± SD.
Different letters in the same row indicate significantly different ($p < 0.05$).

The concentrations (%) of each aroma compound of chocolates produced from cocoa beans under different roasting conditions were calculated by the peak area of individual aroma compound divided by the total peak area. For cocoa beans roasted at 90°C and 15-25min, the dominant aroma compound was acetic acid present in concentrations from 24.16 to 33.35% of total volatile substances. As the roasting temperature and roasting time increased, the acetic acid concentration in the resulted chocolate gradually decreased to 13.12% of total aroma substances for most intensive roasting condition (150°C and 35min). The reason for this may be due to the acetic acid volatilized more at higher temperature. The outcome was reflected in the sensorial evaluation results as displayed in Figure 4. The chocolate produced by cocoa bean under light roasting degree (90°C and 15-25min) was rated the lowest point of overall acceptability by the panel. This is probably due to high level of acetic acid that will interfere with chocolate acceptability [22,27]. The odor description of each aroma compound were also shown in Table 3. The chocolate produced by cocoa bean under heavy roasting degree (150°C and 25min) was characterized by the highest peak area of 2-methylbutanal among the tested samples. As a result, its consumer acceptability was rated with higher point (Figure 3). This is confirmed by Owusu et al. [28], who reported that the most important odor in chocolate was 2-methylbutanal and 3-methylbutanal, with a cocoa, chocolate attribute.

The high peak area of pyrazines, such as trimethylpyrazine and ethenyl-dimethylpyrazine, occurred in the chocolate produced from heavy roasting conditions. These compounds were an indicator of Maillard reaction during high temperature roasting. This observation was agreed with the statements by Żyżelewicza, et al. [2].

3.5. Flavor Profiles Analysis by PCA

PCA was applied to distinguish the flavor profiles of the six types of chocolate under different roasting conditions. The PCA can sort out the data based on their similarities and differences, by reducing the number of dimensions without losing much of information, and can define the number of “principal components.” In general, principal component 1 (PC1) and principal component 2 (PC2) are generated and are sufficient to explain the maximum variance in all original information [29,30]. PCA classifies the data to distinct groups that best describe the relationships among the tested samples. PC1 describes the statistical relationship that accounts for the greatest amount of variation, followed by PC2, PC3, etc.; each corresponds to a decreasing variation in the sample set [31]. The information of components in PCA including the eigen value, the percentage of variance for each principal components and the cumulative percentage was shown in Table 4. PC1 explained 61.87% of the total variance in the sample, whereas PC2 explained an additional 13.44% and PC3 further explained another 8.76%. In summary, PC1, PC2 and PC3 are adequate to explain 84.08% of the total variance. The factor loadings for aroma compounds of chocolate are shown in Table 5. Higher loading scores indicate a tighter association with the corresponding principal component. Loadings with an absolute value higher than 0.24 in PC1 represent a strong impact on the flavor of chocolate. 1-Butene, ethanol, acetaldehyde, tert-butylmethylether, trimethylpyrazine, 2-methylbutane, pentane and 2-methylbutanal which are among volatile compounds had the greater influence on the total explained variance. On the other hand, PC2 are mainly influenced by the following volatile compounds, such as 2-methylfuran, 1s-(-)-alpha-pinene, ethylbenzene, ethyl 2-methylbutyrate and propanal. In addition, PC3 are mainly influenced by the following aroma compounds, such as homofuraneol, 1s-(-)-alpha-pinene and ethyl isobutyrate. PCA has a great advantage to provide a better visualization of the variation in the tested samples and to find correlations between the different variables [32]. The PCA score and loading plot are shown in Figure 2 and Figure 3. The score plot showing the six types of chocolate produced from different roasting conditions and displaying the layout of the original data for PC1 and PC2, depicts 75.31% of the total variance. Chocolate samples were independently separated in a different quadrant with the exception of an overlapping occurred in the second and the third quadrant for chocolate produced from beans under the light roasting (90°C-15min; symbol ○). Chocolates produced from beans under heavy roasting (150°C- 35min; symbol ■) and (150°C - 25min; in symbol □) differ in PC2 but have similarities in PC1. On the other hand, chocolate samples produced from beans under medium roasting (130°C- 25min; symbol ▲) and light roasting (90°C - 25min; symbol ●) have similarities in PC2 but differ in PC1. The flavor profiles of chocolates produced from beans under medium roasting (110-130°C and 25min) and light roasting (90°C and 15-25min) were more tightly aggregation than that of chocolates produced from beans under heavy roasting (150°C and 25-35min). This finding is confirmed by the results of the total peak area of the volatile aroma compounds in the chocolate produced from beans under heavy roasting were significantly higher than those of chocolates produced from beans under light and medium roasting ($p < 0.05$). In addition, the chocolate samples from beans under heavy roasting condition (150°C - 25min) differs from all the others, having positive values in PC1 and PC2, indicating a high content of 2-methylbutanal and trimethylpyrazine, which are important odorants for chocolate [33]. The PCA loading plot of aroma compounds in the six types of chocolate from different roasting degree was shown in Figure 3. It gives a better visualization of the distribution of these aroma compounds. 1-Butene, ethanol, acetaldehyde, tert-butylmethylether, trimethylpyrazine, 2-methylbutane, pentane and 2-methylbutanal are positioned at the right side with similar weighting scores on PC1, and 1s-(-)-alpha-pinene, ethylbenzene, and ethyl 2-methylbutyrate are positioned at the bottom with higher weighting scores on PC2.

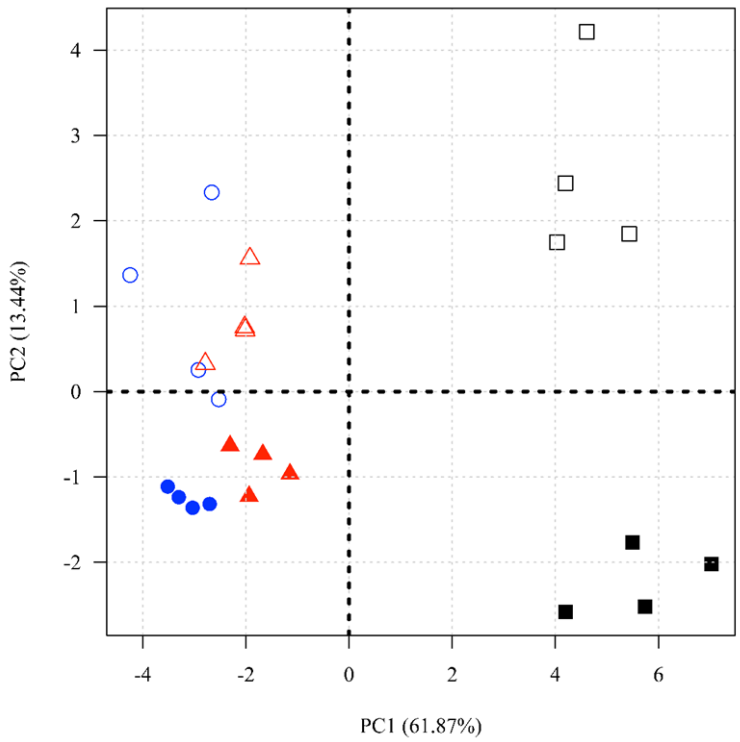

Figure 2. PCA score plot of PC1 and PC2 showing the distribution of the six types of chocolate produced from different roasting degree. Symbols: □ and ■ for heavy roasting; △ and ▲ for medium roasting; ○ and ● for light roasting

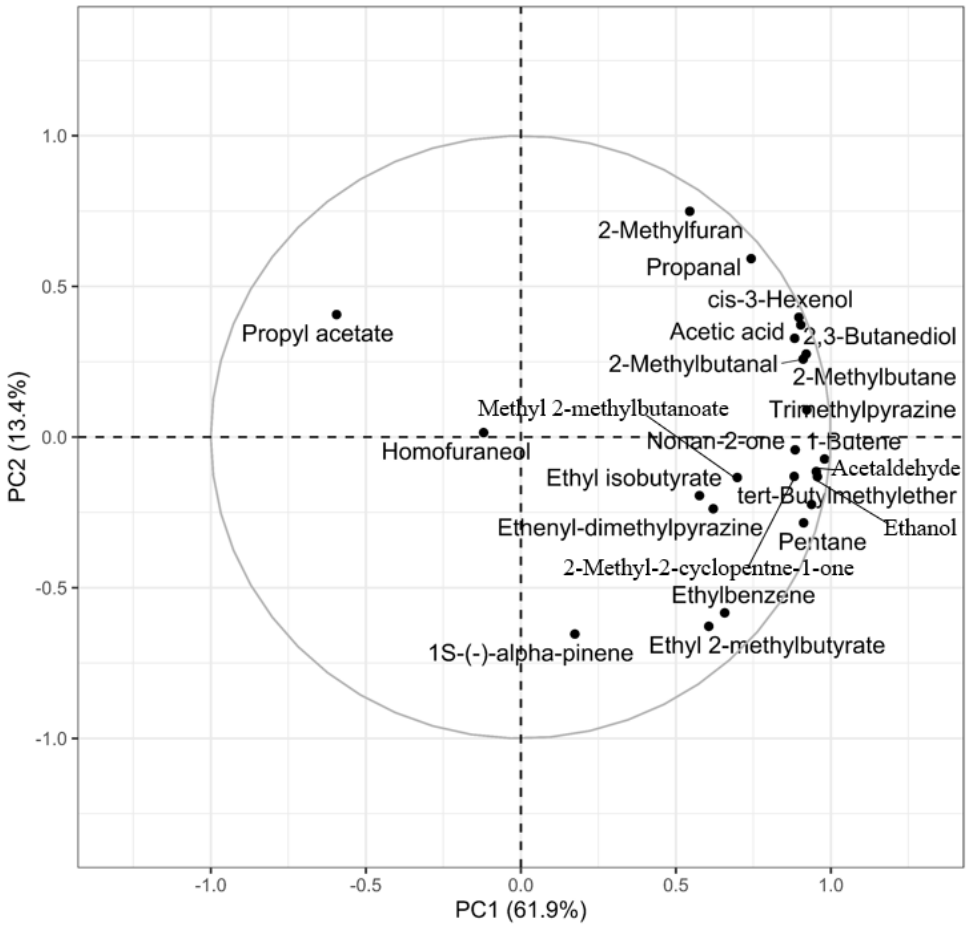

Figure 3. Loading plot of the first two principal components for aroma compounds of the six types of chocolate from different roasting degree

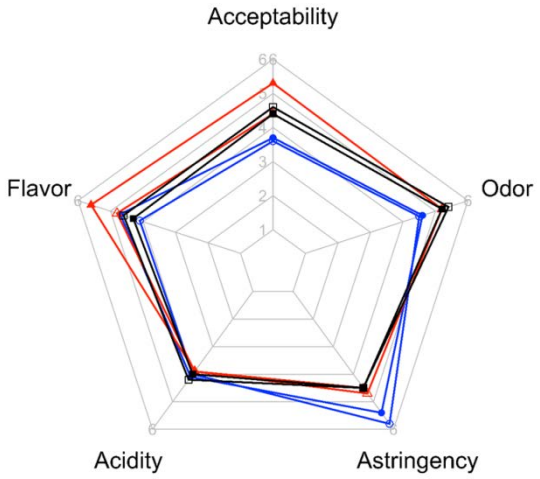

Figure 4. Sensorial assessment of the six types of chocolate from different roasting degree. Symbols: □ and ■ for heavy roasting; △ and ▲ for medium roasting; ○ and ● for light roasting. Assessed in a nine-point hedonic scale, where 9 = like extremely or extremely strong; 1 = dislike extremely or extremely weak

Table 4. Information of components in principal component analysis

	Eigen value	% of Variance	Cumulative,%
PC1	14.2307	61.87%	61.87%
PC2	3.0924	13.44%	75.32%
PC3	2.0156	8.76%	84.08%
PC4	0.9771	4.25%	88.33%
PC5	0.7461	3.24%	91.57%
PC6	0.6212	2.70%	94.27%
PC7	0.4611	2.00%	96.28%
PC8	0.2509	1.09%	97.37%
PC9	0.2046	0.89%	98.26%
PC10	0.1437	0.63%	98.88%
PC11	0.0929	0.40%	99.29%
PC12	0.0635	0.28%	99.56%
PC13	0.0474	0.21%	99.77%
PC14	0.0249	0.11%	99.88%
PC15	0.0168	0.07%	99.95%
PC16	0.0062	0.03%	99.98%
PC17	0.0032	0.01%	99.99%
PC18	0.0012	0.00%	100.00%
PC19	0.0000	0.00%	100.00%
PC20	0.0000	0.00%	100.00%
PC21	0.0000	0.00%	100.00%
PC22	0.0000	0.00%	100.00%
PC23	0.0000	0.00%	100.00%

3.6. Sensorial Assessment

The results of the organoleptic attributes for the six types of chocolate produced from beans roasted at different roasting degree, including odor, flavor, acidity, astringency, and acceptability, are shown in Figure 4. The odor and acidity attribute for chocolate produced from beans roasted at 150°C and 25min had the highest score of 5.39 points and 4.21 points respectively, but there were no significantly difference with that of the others ($p<0.05$). The flavor attribute of chocolate produced from beans roasted at 130°C and 25min had the highest score of 5.63 points and was significantly ($p<0.05$) higher than that of chocolates produced under the most light roasting degree (4.13 points) and the heavy roasting condition (4.32 points). The astringency attribute of chocolate produced from beans roasted at 130°C and 25min had the lowest score of 4.47 points and was significantly ($p<0.05$) lower than that of chocolate produced under the most light roasting condition (5.76 points). On the other hand, The overall acceptability of chocolate produced from beans roasted at 130°C and 25min had the highest score of 5.26 points and was significantly ($p<0.05$) higher than that of chocolate produced under the most light roasting condition (3.63 points). The results showed that the more astringency of chocolate, the less acceptability of the chocolate by consumer. The astringency taste of chocolate is mainly due to the presence of PAC, which are not only responsible for the astringency taste of chocolate, but are also beneficial to human health [11,34]. The amount of PAC concentration in the chocolate produced from beans roasted by different conditions were shown in Table 2. The results indicated that chocolate manufactured from beans roasted at 90°C and 15min had the highest PAC content among the others. This could be an appropriate explanation for the high rating score in the assessment of the astringency strength. Even though chocolate manufactured from beans roasted at 150°C and 35min had the lowest PAC content among the others, its overall acceptability rating (4.37 points) was lower than that of chocolate produced from beans roasted at 130°C and 25min (5.26 points). As a result, a proper adjustment of the roasting conditions of cocoa bean could be beneficial to flavor properties and overall acceptability of chocolate product by consumer.

Table 5. Principal component factor loadings for aroma compounds of the six types of chocolate

Compound	PC1	PC2	PC3
1-Butene	0.26	-0.041	-0.022
Ethanol	0.254	-0.075	0.019
Acetaldehyde	0.253	-0.065	0.035
tert-Butylmethylether	0.249	-0.127	-0.031
Trimethylpyrazine	0.244	0.051	-0.09
2-Methylbutane	0.244	0.157	0.152
Pentane	0.242	-0.162	-0.126
2-Methylbutanal	0.241	0.147	-0.16
2,3-Butanediol	0.239	0.212	-0.022
cis-3-Hexenol	0.238	0.226	-0.016
Nonan-2-one	0.235	-0.024	0.021
Acetic acid	0.234	0.186	0.2
2-Methyl-2-cyclopenten-1-one	0.234	-0.074	-0.061
Propanal	0.197	0.337	0.17
Methyl 2-methylbutanoate	0.185	-0.076	-0.247
Ethylbenzene	0.174	-0.332	0.075
Ethenyl-dimethylpyrazine	0.165	-0.135	0.019
Ethyl 2-methylbutyrate	0.161	-0.357	0.175
Propyl acetate	-0.158	0.231	0.12
Ethyl isobutyrate	0.153	-0.111	-0.343
2-Methylfuran	0.144	0.426	0.167
1S-(-)-alpha-pinene	0.046	-0.372	0.452
Homofuraneol	-0.032	0.008	-0.623

4. Conclusions

Cocoa bean roasting plays a decisive role in chocolate manufacturing and results in the generation of desirable

flavor and odor compounds contributing to high consumer acceptability. Six types of chocolate were manufactured from cocoa beans under different roasting degree. The results showed that the total amount of FMO and PAC in the chocolate both decrease with the intensity of the roasting degree of cocoa beans. Twenty-three important volatile aroma compounds were identified by electronic nose analysis in the six types of chocolate. The influence of roasting intensity is mainly on their levels of the volatile aroma compounds, not on the types and numbers of aroma compounds. This is probably due to the beans with the same genotype and through the same fermentation process. From sensory point of view, chocolates produced from cocoa beans roasted under roasting condition (130°C and 25min) rated the highest score of 5.63 points of overall acceptability among the six types of chocolates by the sensory panel. However, chocolate produced from cocoa beans under light roasting degree was retained more content of the bioactive compounds and could be a potent candidate of health functional food. PCA was successful to distinguish the flavor profiles among the six types of chocolate. It permits a better visualization of the divided groups in the six types of chocolate. Therefore, PCA is useful to discriminate the flavor profiles of chocolate produced from cocoa beans under different roasting degree and could be used as a tool to recognize the origin of the chocolate product. These results of this study could provide an important approach for producing chocolate products with desired flavor properties and bioactive compounds that have positive benefits for human health.

Acknowledgements

This work was funded by the Ministry of Science and Technology of the Republic of China [MOST 107-2637-E-041-001].

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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