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Effect of Harvest Season on the Physical Properties, Fatty Acid Composition, and Volatile Compounds of Roasted Cacao Beans

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Abstract

The prevailing climate is an important factor affecting the development of the physical quality and flavor of cacao (*Theobroma cacao* L.) beans. The influence of harvest seasons on the physical, chemical, and flavor characteristics of cacao beans from Nakhon Si Thammarat Province, southern Thailand, was assessed. Among cacao beans, those harvested in the rainy season (R) had the highest fresh and dry weights and the lowest bean count per 100 g (87.00 ± 2.83). Although the overall fat content remained relatively constant across harvest seasons, cacao beans harvested in R exhibited a significantly higher fatty acid (C16:0, C18:1, and C18:2) content than those harvested in the dry season (D). Cacao beans harvested in D displayed a more diverse and intense aroma profile than those harvested in R. Therefore, this study proved that harvest seasons significantly influenced the physicochemical characteristics of cacao beans. Cacao beans harvested in D exhibited more complex and distinct postroasting aroma profiles than those harvested in R. Evaluating the seasonal harvesting of cacao beans is crucial for developing cocoa and chocolate with fine and unique flavors in southern Thailand.

Keywords: aroma profile, cacao orchard, fine cocoa, flavor profile, southern Thailand

Introduction

Cacao (*Theobroma cacao* L.) is one of the world's important fruit crops that can be harvested throughout the year. The world's main cacao producers are tropical countries in South America, West Africa, and Asia. Cocoa products (cocoa butter, cacao liquor, or cocoa powder) from different geographic regions possess unique aromatic properties because of different environmental factors [1]. In Thailand, cacao has emerged as a promising economic crop with the potential to boost rural livelihoods and contribute to the country's agricultural diversification. In Thailand, the cacao planting area is currently approximately 900 hectares, and local farmers market cacao from their farms in the form of fresh pods and dried beans. Dried beans are a major marketable form of cacao in the country. A total of 80% of the dried beans produced in Thailand are domestically used, whereas the remaining 20% is exported, earning an export value of more than 70 million USD in 2019 [2]. The overall quality of cacao beans is influenced by various factors, including flavor volatiles, nutritional composition, polyphenolic content, and fermentation quality. Harvesting and postharvest processing are the main factors ensuring good chocolate quality. The quality of cacao beans is determined by size, weight, color, acidity, and flavor [3–4]. Among these factors, flavor volatiles play a crucial role in determining the acceptability and desirability of cacao beans [5–6].

The quality of cacao beans is essential for improving the quality of chocolate processing. Cacao beans contain more than 45%–55% fat, thus affecting the texture of chocolate and resulting in its unique characteristics [7, 8]. Cacao beans contain essential fatty acids, including palmitic (C16:0), stearic (C18:0), and oleic (C18:1) acids, and are commonly used as an ingredient in several confectionery products, particularly chocolate, due to their specific properties [8]. However, the quality of the fatty acids in cacao beans varies with geographical origins and processing conditions [9]. Climate factors also influence fatty acid composition. For example, temperatures and palmitic acid levels are positively correlated [7]. The different geographic sites and planting systems of cacao farms affect the contents of polyamines, phenols, and nutrients in cacao beans [10], resulting in distinctly unique flavor profiles that characterize the cultivating quality of cacao farms [11]. Variations in flavors are caused by different environmental factors in harvest seasons. Geographical location, period, sunlight and rainfall exposure amount, soil, and climatic conditions affect the morphological traits and chemical composition of cacao [6, 12]. Pod maturation and fermentation processes are the factors affecting the flavor development of cacao beans in the postharvest period [4, 11]. The most important flavor properties of cacao products and their precursors develop during the postharvest processing (fermentation and drying), roasting, and storage of cacao beans [13]. In addition, the predominant aroma-active compounds of cacao liquor, such as pyrazines (nutty), aldehydes (aroma), and esters (fruity), remarkably influence cacao flavors [14]. Flavor is the primary criterion for evaluating fine cocoa. The Fine Cacao and Chocolate Institute (FCCI) employs a comprehensive assessment framework that encompasses physical, chemical, and sensory evaluations to evaluate the flavor quality of cacao beans [15].

This study investigated the influence of harvest seasons on the physical and chemical characteristics of cacao beans and the association between the fatty acid composition and aroma characteristics of cacao beans from southern Thailand. Its findings could contribute to enhancing the quality and identity of cacao from southern Thailand. Enhancing our understanding of aroma composition in cacao raw material holds considerable practical value for developing fine cacao products for farmers.

Materials and Methods

Plant material and cultivation conditions. Samples of fresh and dry cacao beans were harvested from the plants of the hybrid cacao variety “Chumphon 1” (Pa7 × Na32) grown as a mixed crop at a coconut plantation in Thung Song District, Nakorn Sri Thammarat Province (8°11′36″N 99°41′00″E). The orchard has an elevation of 62 m above the mean sea level. The area of the orchard experiences an annual average temperature of 24 °C–34 °C and receives 150–600 mm of rainfall per month in the rainy season (R).

Pod harvesting. Cacao pods were harvested in two harvesting periods: the dry season (D) (from January to May 2020) and R (from June to December 2020). Appropriately mature cacao pods were harvested after 150 days of full blooming [16] when their color turned yellow. The ranges of L* (lightness), a* (redness: green to red), b* (yellowness: blue to yellow) values were 64.79–65.53, 3.65–4.28, and 48.07–50.59, respectively, and were recorded by a Chroma meter (CR-400, Konica Minolta, Japan). The sampled pods varied in size from 6.67 cm to 7.13 cm in width and 14.12 to 14.42 cm in length. Their weights ranged between 252.31 and 313.11 g/pod.

Bean sampled preparation. Samples for fermentation were prepared in accordance with the prototype of the FCCI [15]. Fresh samples were kept in a fermentation box (40.0 cm × 56.0 cm × 42.0 cm) for 7 days [17]. Each box could hold approximately 10 kg of fresh beans. The beans were turned around in the box twice (at 24 and 96 h) for aeration. Fermentation was conducted in the shade of a building at 32 °C ± 5 °C and relative humidity of 80% ±10%. Fresh beans were dried in the sun for 14 days to reduce their moisture content to 6% ± 2%. Dried beans were then divided into 300 g subsamples, placed in a zippered polyethylene bag, and stored at room temperature.

External evaluation of cacao beans. External attributes were classified as bean count (bean count per 300 g of beans), bean size, moisture, and raw bean aroma. Six 100 g subsamples of cacao beans from each harvesting season were used. Cacao beans were counted and classified into three sizes: large cacao beans with less than 100 beans per 100 g, medium cacao beans with 100–120 beans per 100 g, and small cacao beans with more than 120 beans per 100 g [18]. The moisture content of cacao beans was analyzed by using the oven method at 105 °C for 24 h. The weights of the beans before and after oven drying were recorded. Raw bean aroma was divided into positive/neutral and defective as described by the assessment protocols of the FCCI. External defects of beans were also classified on the basis of the percentage of beans with blackening, severe molding, germination, and insect damage.

Fermentation levels of cacao beans. The classification value of dry cacao beans was used in the cut-test analysis for fermentation level. The cut-test method was conducted in accordance with FCCI methods [15]. Three subsamples of 100 cacao beans were cut lengthwise through the middle to expose the maximum cut surface of cotyledons. Three distinct color categories were identified: fully brown (fully fermented), partially brown, and violet (under-fermented). All samples were used for the measurement of L*, a*, b*, and hue angle (H°) by using a Chroma meter (CR-400, Konica Minolta, Japan) to assess color categories. Chromaticity coordinates were then used to compare the relationship between fermentation levels for discriminating color variations. Fermentation level was determined by using a method proposed by a previous work [19].

Crude fat extraction and fatty acid composition. Crude fat extraction and cacao bean purification were conducted in accordance with the Soxhlet extraction method described by a previous study [20]. Extracted fat was converted into fatty acid methyl esters (FAMEs) by using official AOAC methods (AOAC 2003.05 & 2003.06 diethyl ether and hexane extraction methods, Foss, SoxtecTM 8000 Extraction Unit). FAME analysis was performed on each sample in triplicate (Agilent Technologies 7890A GC System, the USA). An Agilent 7890A GC system equipped with a Stabilwax capillary column (30 m long, 0.25 mm internal diameter, and 0.25 mm film thickness) was used for compound identification. Eleven FAMEs were used as external standards with helium as the carrier gas. The injection temperature was set at 210 °C, and the oven temperature program was as follows: 120 °C for 3 min, increased by 100 °C/min to 220 °C, held for 30 min, and then increased by 50 °C/min to 240 °C. The split ratio was 100:1, and the detector temperature was set at 280 °C [19]. FAME content was determined on the basis of methyl esters (PORIM test method) [21]. Palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and arachidic (C20:0) acids were used as external standards.

Volatile compounds of cacao beans. Raw beans for aroma evaluation were prepared by following the method proposed by the FCCI. Samples were prepared at the Plant Ecophysiology Laboratory, Prince of Songkla University. A total of 300 g of positive/neutral and defective samples were randomly tested. Five trained tasters were guided to recognize the raw bean aroma described by the FCCI and to follow the flavor wheel chart. A total of 250 g of cacao beans was roasted for 10 min at 180 °C [22] by using a motorized hand roaster heated by a gas burner to distribute heat across cacao beans. In the next step, the beans were cooled, and their shells were removed manually before being ground by using a blender. The separation of volatile compounds in two different cacao liquors was achieved using gas chromatography, with headspace solid-phase microextraction (HS-SPME) employed for sample preparation [23, 14]. The roasted beans were ground to obtain cacao liquor, which was subsequently poured into a capped glass vial. Odor compounds in cacao liquor were separated and detected by using a gas chromatography triple quadrupole spectrometer (GC–MS/MS, Agilent Technologies GC7890B/MS/MS7000D, the USA).

Statistical analysis. Mean differences were determined by using one-way analysis of variance and arranged as a completely randomized design with two harvest seasons. A probability value of $P < 0.05$ was considered statistically significant in the t-test. Statistical analyses were performed by using R software.

Results and Discussion

External attributes of beans. The weights of cacao beans harvested in R were statistically different from those of cacao beans harvested in D. The total fresh bean (83.56 ± 20.84 g/pod), fresh single bean (2.22 ± 0.53 g/bean), and dry single bean (1.14 ± 0.22 g/bean) weights of cacao beans harvested in R were significantly higher than those of cacao beans harvested in D (Table 1).

The fresh beans harvested in R were larger in size and fewer in number per 100 g than those harvested in D; however, the overall size of the beans harvested in R and D was the same (large) (Table 2). At the same time, external defects, such as blackening, severe molding, and germination, were observed. However, none of these symptoms were present in the beans harvested in D or R.

The fresh and dry weights of cacao beans were affected by different seasons. Harvesting in R resulted in cacao beans with better physical properties than harvesting in D. This result shows that climate affects cacao production, which relies mainly on seasonal rainfall [24]. Cacao orchards receiving less than 1200 mm of rainfall per year have low soil moisture and growth potential. Cacao pods harvested in the fourth and fifth months can be considered commercially ready because they have developed good quality [16, 25]. Moreover, rainfall is positively correlated with cacao bean weight during development [26]. Cell proliferation is influenced by appropriate irrigation, which induces sugar accumulation and fat deposition in developing seeds; these processes could affect the weight of seeds in the ripening stages [27]. However, the fruit number and seed weight of cacao beans might vary due to pollination [28]. Pollination also affects the ripening development phase, which might affect the quality characteristics of cacao beans, such as their chemical composition and fat content [29].

Fermented and raw bean aroma. Fermentation levels, including fully brown and partially brown, did not significantly differ between seasons, although differences in violet levels were found (Table 3).

Table 1. Influence of Harvest Season on Cacao Pod and Bean Characteristics

Seasons	Total fresh bean (g/pod)	Fresh single bean (g/bean)	Dry single bean (g/bean)
Dry	76.28 ± 19.18b	1.66 ± 0.57b	1.00 ± 0.18b
Rainy	83.56 ± 20.84a	2.22 ± 0.53a	1.14 ± 0.22a
T-test	*	*	*

Values (mean ± SD) are averages of the samples analyzed individually by t-test ($P \leq 0.05$).

Table 2. External Attributes and External Defects of Cacao Beans by Harvest Season

Season	External attributes			External defects (100 beans, count)			
	Bean count (100 g)	Size	Moisture content (%)	Blackening	Severe molding	Germination	Insect damage
Dry	92.33 ± 2.08	Large	3.66 ± 0.83	1.50 ± 0.71	0.00 ± 0.00	20.50 ± 2.12	0.00 ± 0.00
Rainy	87.00 ± 2.83	Large	3.52 ± 0.75	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3. Effects of Harvest Season on the Fermentation Levels and Colorimetric Indicators of Cacao Beans

Fermentation level	Season	%	Colorimetric indicator			
			L*	a*	b*	H°
Fully brown	Dry	87.00 ± 4.24	27.79 ± 4.94	11.80 ± 1.96ns	15.70 ± 4.68	52.37 ± 3.67
	Rainy	83.50 ± 2.12	27.98 ± 2.06	11.80 ± 1.91	14.20 ± 1.03	50.41 ± 6.18
T-test		**	ns	ns	ns	ns
Partially brown	Dry	5.00 ± 2.94	36.31 ± 8.05	15.53 ± 1.91ns	22.80 ± 2.75	55.74 ± 0.08
	Rainy	6.00 ± 4.08	45.25 ± 3.24	13.15 ± 2.53	19.67 ± 3.29	56.32 ± 2.12
T-test		ns	**	ns	ns	ns
Violet	Dry	0.50 ± 0.71	26.74 ± 3.02	12.35 ± 2.35ns	6.03 ± 2.10	18.45 ± 1.91
	Rainy	5.50 ± 4.95	31.58 ± 2.96	14.01 ± 1.94	10.13 ± 7.76	26.11 ± 5.38
T-test		**	ns	ns	ns	**

Values (mean ± SD) are averages of samples analyzed individually by t-test ($P \leq 0.01$); ns = values were not significantly different from the mean value ($P > 0.05$).

Moreover, this study found that the colorimetric measurement could assess the fermentation level of raw cacao beans. In both seasons, the level of fermentation exhibited higher values in the partly brown cacao samples compared to those exhibiting full brown and violet coloration, especially in terms of L* (36.31 ± 8.05 to 45.25 ± 3.24), a* (13.15 ± 2.53 to 15.53 ± 1.91), b* (19.67 ± 3.29 to 22.80 ± 2.75), and H° (55.74 ± 0.08 to 56.32 ± 2.12). These results imply that color measurement is appropriate for estimating cacao fermentation level and quality parameters [19].

However, colorimetric indicators might increase progressively after roasting [30]. Consequently, the quality of fermentation levels is a critical factor determining the taste, aroma, and texture of chocolate. Good-quality cacao beans are essential for the commercialization of chocolate with good attributes.

The beans harvested in D and R exhibited positive/neutral aromas with cocoa, dried fruit, fresh fruit, nutty, spicy, buttery, dark, and milk chocolate notes (Table 4). Defective aroma, i. e., rancid, medicinal, phenolic, and meaty, was shown only by cacao beans harvested in R. This finding indicates that high humidity, short sunshine periods, and prolonged rainfall in the cacao harvest season might directly contribute to cacao defects [31]. Postharvest drying also remarkably affects cacao bean quality because beans with high moisture content are susceptible to fungal growth, molding, and bacterial contamination during prolonged storage [32, 33]. The flavor mechanism in cacao beans involves a chemical reaction of volatile compounds. The flavor of cacao beans is a characteristic that varies and affects the accumulation of proteins and carbohydrates, which could affect the amount and type of volatiles, especially protein components, polysaccharides, and polyphenols, that lead to the development of flavor characteristics during fermentation [34, 35].

Fatty acid composition. The fat contents of cacao beans harvested in different seasons showed statistically significant differences (Table 5). However, the fatty acid composition of cacao beans remained consistent between harvest seasons.

The average fat content of cacao beans harvested in D was 44.70% ± 4.29% and was higher than that of cacao beans harvested in R (40.47% ± 1.21%). The beans harvested in R showed slight reductions in stearic (C18:0) and arachidic acid (C20:0) contents. Meanwhile, the palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acid contents of beans harvested in R were significantly higher than those of beans harvested in D.

Table 4. Raw Bean Aroma of Cacao Beans Based on Harvest Seasons

Season	Aroma of raw beans	
	Positive/neutral	Defective
Dry	Cocoa, dried fruit, nutty, spicy, dark, and milk chocolate	-
Rainy	Cocoa, fresh fruit, dried fruit, floral, nutty, and buttery	Rancid, medicinal, phenolic, and meaty

Table 5. Fat Content and Fatty Acid Compositions of Cacao Beans Harvested in the Dry and Rainy Seasons

Seasons	Fat content (%)	Fatty acid compositions (%)				
		16:0	18:0	18:1	18:2	20:0
Dry	44.70 ± 4.29	22.12 ± 0.79	42.49 ± 1.09	30.87 ± 0.25	2.42 ± 0.25	1.55 ± 0.07
Rainy	40.47 ± 1.21	26.53 ± 0.38	37.44 ± 0.70	32.42 ± 0.28	3.42 ± 0.08	1.42 ± 0.15
T-test	ns	**	**	**	**	ns

Values (mean ± SD) are averages of the samples analyzed individually by t-test ($P \leq 0.01$); ns = values were not significantly different from the mean value ($P > 0.05$); palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and arachidic (C20:0) acids.

No statistically significant difference was found between the overall fat content of cacao beans harvested in D and R (40.47%–44.70%). However, cacao beans harvested in D exhibited higher fat contents than those harvested in R. Interestingly, the total fat content of cacao beans (30.49%) harvested in D were higher those of cacao beans from northeastern Peru [9]. In comparison to previously reported values, the total fat content of Bahia cacao beans in Brazil ranged from 50.2% to 62.4% [7]. Unroasted cacao beans from Peninsular Malaysia showed significantly higher fat content (42.65%) than roasted cacao beans (30.0%) from the same area [36]. While fatty acid profiles are typically used to identify the geographical origin of cacao beans, this study demonstrates that fatty acid composition can also be influenced by different harvest seasons. The levels of palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and arachidic (C20:0) acids in cacao beans from Indonesia have been reported to range from 25.67% to 27.92%, 34.55% to 36.23%, 32.36% to 33.89%, 2.86% to 3.41%, and 0.85% to 0.99% [37], respectively, which align with the findings of this study. However, several factors, including geographical origin, climatic conditions, agronomic management practices, cultivar type, and harvest timing, remarkably influence the fat content and fatty acid composition of cacao beans [38]. This study shows that climate variations in different harvest seasons can considerably influence the fatty acid composition of cacao beans. Consequently, the fatty acid profile of cacao beans can be influenced by physicochemical characteristics, which vary across different harvesting periods. This finding aligns with previous results showing that climate factors have a remarkable effect on the fatty acid composition of cacao beans, particularly on palmitic, stearic, oleic, and linoleic acid levels [7, 9]. Different planting factors contribute to drastic differences in the content of palmitic, stearic, oleic and linoleic acids in cocoa butter [39].

Aroma profiles of roasted cacao beans. The mean distribution of the main classes of key polar and nonpolar aroma-active compounds (Figure 1) showed large variations in cacao beans harvested in different seasons. A total of 15 key aroma-active compounds in roasted cacao beans were detected. High levels of polar and nonpolar amine, ester, ketone, and alcohol compounds were found in cacao beans. The total concentrations of amines, esters, ketones, and alcohols in the dry samples were 14.08%–22.66%, 14.08%–19.53%, 22.54%–11.72%, and 15.49 to 14.84%, respectively. Meanwhile, the total concentration of amines, esters, ketones, and alcohols in the samples harvested in R showed slight changes and were 11.11%–21.34%, 25.00%–23.01%, 19.44%–11.50%, and 13.89%–13.27%, respectively.

The aroma profiles of cacao beans harvested in D exhibited greater diversity and intensity than those of cacao beans harvested in R. Cacao beans harvested in R had marginally higher intensities of ether, acid halide, ester, and epoxide aromas than those harvested in D. Notably, the predominant aroma-active group in roasted beans was esters, which impart desirable flavors, such as fruity notes [14, 40], as revealed through polar and nonpolar analyses. Interestingly, the aroma profile of raw beans was characterized by fresh and dried fruit notes, largely influenced by R, as evident in Table 4.

Climate conditions during harvest can influence the composition of cacao beans. For example, higher levels of aldehydes (43.07%) and esters (36.13%) were detected in summer than in other seasons, whereas alcohols (7.08%), ketones (11.48%), and other compounds (24.29%) were more prevalent in winter than in other seasons [41]. Moreover, the number of aromatic compounds depends on climate factors in the harvest season, environment, soil conditions [4, 42], and other factors affecting cacao

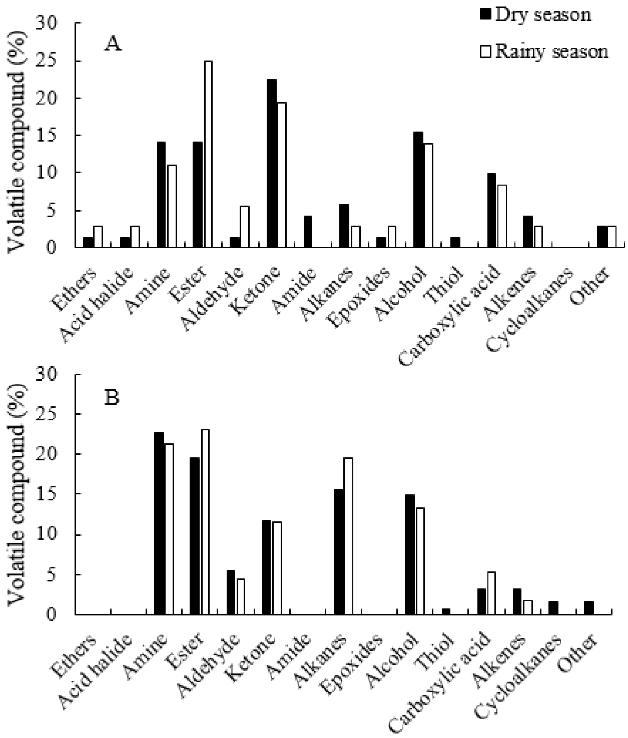

Figure 1. Key Polar (A) and Nonpolar (B) Aroma-Active Compounds in the Volatile Extracts of Roasted Cacao Beans Harvested in the Dry and Rainy Seasons Identified by GC–MS/MS

during maturation [43, 44]. These considerations indicate that protein metabolism may lead to the synthesis of aroma compounds from amino acids, potentially contributing to the intensity of aroma components in the pulp of cacao fruit harvested in different seasons [40, 45]. Thus, Asian and Oceanian beans have a wide range of flavor profiles that range from subtle cocoa and nutty/sweet notes in java beans to intensely acidic, and the total aroma-active compound content of beans from Papua New Guinea is higher than those of beans from other regions [30, 46].

This study demonstrates the influence of harvest seasons on the aroma profiles of cacao beans. In addition to fermentation, weather conditions, such as temperature, relative humidity, and rainfall, influence the aroma development, taste, and quality of beans. Although the flavor development of cacao for fine chocolate production involves various factors from harvesting to processing, distinct harvest seasons considerably affect the composition of cacao beans, resulting in diverse aroma and taste profiles. Interestingly, weather variations in the harvesting period appear to play a crucial role in imparting diverse aroma characteristics to cacao beans, making them indispensable for the production of fine cocoa.

Conclusions

Fine cocoa production in southern Thailand should optimize harvest timing to maximize the quality of raw cacao beans. Harvest season affected the production and quality of cacao beans in terms of aroma profiles, such as raw bean aroma and fatty acid composition. The cacao beans harvested in D had a more complex and different postroasting aroma profiles than those harvested in R. Harvest season could considerably enhance cacao bean quality and contribute to the production identity of chocolate.

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