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Evaluation of total phenolic content, antioxidant activities, caffeine, and chlorogenic acid content in single-origin green coffee beans grown in Thailand

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Abstract

Third-wave coffee culture has placed emphasis on the quality and origin of coffee beans. Chemical and physical properties as measured scientifically can augment sensory characteristics of single-origin coffee in identifying their geographical identities. This study aimed to assess the properties of 31 green coffee bean samples, comprising 23 Arabica and 8 Robusta species, collected from 12 Thai provinces. The total phenolic content, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) antioxidant activities, and concentrations of chlorogenic acid and caffeine were measured using the Folin-Ciocalteu method, DPPH and FRAP assays, and high-performance liquid chromatography (HPLC). The distributions of measured quantities and their correlations were examined by species and by origins. The results showed that the total phenolic content, antioxidant activity and caffeine concentration in Robusta species were significantly higher than in Arabica species. Additionally, chlorogenic acid content in Robusta was slightly higher than in Arabica but there was no significant difference between species. From the samples measured, Robusta coffee from Tak, Sisaket, Ranong, and Chumphon consistently ranked highest in terms of total phenolic content, FRAP, and DPPH values. For chlorogenic acid and caffeine contents, Chumphon Robusta and Phitsanulok Arabica samples ranked the highest. Overall, across species and regions, strong correlations were found among the five measured quantities as indicated in Pearson's coefficients and principal component analysis. The data can be used as supplementary information to assess the differences and characteristics of green single-origin coffee beans from different parts of Thailand.

Keywords: Arabica, Robusta, Geographical identification, Chemical composition, Analysis

1. Introduction

Coffee is one of the most valuable raw materials, with its production and trade playing a significant role in the global economy. Coffee belongs to the Rubiaceae family, genus Coffea. *Coffea arabica* and *Coffea canephora* var. Robusta, which are commonly known as Arabica and Robusta, respectively, are the two most significant and widely cultivated species of coffee [1]. The proportion of the world's coffee production is approximately 64% Arabica and 35% Robusta [2, 3]. These two species exhibit chemical differences and are characterized by higher levels of minerals, volatile substances, chlorogenic acid and caffeine which make them different flavor profiles after roasting [2]. Consequently, Arabica and Robusta coffees differ not only in their botanical, chemical, and sensory characteristics but also in their commercial value [4]. Arabica is typically more expensive than Robusta. The average prices for Arabica and Robusta coffee worldwide from 2014 to 2023 were approximately 3.86 USD/kg and 2.02 USD/kg, respectively, and are expected to increase in price to 4.40 USD/kg and 2.40 USD/kg in 2024, respectively [5]. However, the fact that Arabica is more popular than Robusta may be due to its superior taste and aroma [6].

Regarding the difference of coffee beans, the identification of the geographical origins of coffee beans plays a crucial role to assuring the quality of coffee, tracing the source of coffee beans, differentiating the price, and certifying the label requirement. With different growing regions having distinct climates, soil compositions and

growing conditions, the flavor profile and quality of coffee beans can be affected, leading to them having unique flavor profiles and characteristics [7, 8]. The identification of the geographical origin of coffee beans can help assure the growers and consumers of the authenticity, consistency, and quality of the product, which can potentially increase its value. Moreover, the traceability of coffee beans enables stakeholders to identify their geographical origin throughout the supply chain, from farm to cup. Coffee beans from certain regions may differ in price due to perceived quality or unique characteristics. Knowledge of the geographical origin of coffee beans can help justify pricing and differentiate the product in the market. Consequently, identifying the origin of beans using scientific methods can assist consumer decision-making and increase confidence, benefiting both coffee growers and sellers.

Numerous previous studies have demonstrated that chemical composition analysis can be used to discriminate the geographical origins of coffee beans. Coffee cultivated in different regions exhibits variations in certain chemical constituents, such as caffeine, chlorogenic acid, polyphenols, elements [9-13]. The fatty acid composition of one hundred samples of Arabica green coffee was determined by gas chromatography coupled with mass spectrometry and the results show that the total fatty acid content varied across the regions and can be used to identify the production regions [14]. The combination of various chemical analyses provides supplementary data for the characterization and geographical identification of green coffee beans from different regions in Thailand. As in other industries, uniqueness adds value to products. Therefore, this study aims to establish a baseline dataset of Thai green coffee beans, without the effect of roasting conditions, using polyphenol content and antioxidant efficacy of green coffee beans, combined with chlorogenic acid and caffeine contents, for differentiation and identification of coffee bean species and origins. The statistical analyses were done in R version 4.3.2 [15].

2. Materials and methods

2.1 Coffee bean samples

Green single-origin coffee beans from 31 different farms (12 provinces) across Thailand were used in this study. Among these, 23 are Arabica green coffee beans from Chiang Mai (5 samples), Chiang Rai (7 samples), Lampang (2 samples), Mae Hong Son (3 samples), Nan (2 samples), Phitsanulok, Phrae, Tak (2 samples) and 8 Robusta green coffee beans from Chumphon, Kanchanaburi, Nan, Phrae, Ranong, Sisaket (2 samples), Tak. Due to the limited number of samples in some regions, the current measurement data should be treated as survey values and not as representatives of the regions. Thailand has a mostly tropical climate. Different regions have unique climates and topographical characteristics. The northern part of the country is mountainous and has large forest areas. Major rivers such as Ping and Nan cut through the region. The northeastern part consists of mainly flat (Khorat plateau) with some rocky hills. It has Mekong and Mun as major rivers. The central and western regions are flat and fertile, dominated by the Chao Phraya River, and have good irrigation systems suitable for farming. The eastern region is a mix of mountains and coastal plains. Like the northeastern region, it has heavy rainfall during the rainy season (June to October). The south region is mostly flat and is split into the Andaman Sea coast (west) and the Gulf of Thailand coast (east). Most coffee bean samples in this study came from Chiang Rai and Chiang Mai, which have high elevations. In the measurement preparation, the coffee bean samples were coarsely ground into powder using a laboratory mill (Kinematica PX-MFC 90 D) and then dried in an oven at 50°C.

2.2 Phenolic compounds and antioxidant activities analysis

The coffee powder samples were prepared into solutions for analysis. For each sample, 0.1 g of ground coffee was weighed and dissolved in 10 ml of hot distilled water and placed in a tube. The tubes were then placed in a temperature-controlled shaker at 70°C and shaken at 70 rpm for 10 min. The solution was then centrifuged at 7,000 rpm for 5 min. Subsequently, the clear supernatant was directly collected using a 0.45-micron syringe filter.

Total phenolic content was analyzed using the Folin-Ciocalteu colorimetric method. The Folin-Ciocalteu reagent, which is originally yellow, changes to purple or blue when reacting with a phenolic compound in the sample extract. A light absorption method was calibrated with gallic acid as a reference [16]. 100 µl of the test sample was added to 750 µl of 10% Folin-Ciocalteau reagent, then shaken well and incubated for 5 minutes, then 750 µl of 6% Na_2CO_3 was added. The solution was incubated at room temperature for 90 minutes, and absorbance was measured at a wavelength of 725 nm. Total phenolic content was calculated by comparing the absorbance with gallic acid standard curves. Values are expressed in terms of milligrams equivalent of gallic acid equivalents (GAE) per gram of sample (mg GAE/g).

Antioxidant activities were measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity assay and the ferric reducing antioxidant power (FRAP) method [17, 18]. When DPPH, a nitrogen radical that acts as an electron receiver, interacts with antioxidants from the extract, it receives electrons from the extract, stabilizing them and causing a color change from purple to yellow. To conduct the test, 100 µl of the sample was combined with 900 µl of a 0.2 mM DPPH methanolic solution, shaken well, and incubated for 15 minutes in the

dark at room temperature. Absorbance was then measured at a wavelength of 517 nm. The resulting values were compared to a standard graph of ascorbic acid, and the results were expressed as milligrams of ascorbic acid equivalents (AAE) per gram of sample (mg AAE/g). The FRAP assay was used to measure the antioxidant capacity of the samples by assessing their ability to transfer free electrons to the iron complex compound Fe^{3+}-TPTZ (ferric tripyridyl triazine), converting it to the Fe^{2+}-TPTZ (ferrous tripyridyl triazine) form, which turns blue. A total of 300 µl of the test sample was added to 2700 µl of the FRAP reagent (a mixture of 300 mM acetate buffer pH 2.0, 10 mM 2,4,6-tripyridyl-s-triazine, and 20 mM $FeCl_3$ in a 10:1:1 ratio). The mixture was thoroughly combined and incubated for 30 minutes at room temperature before measuring absorbance at 615 nm. The values obtained were compared to a standard curve of $FeSO_4$, and the results were expressed in µmol of $FeSO_4$ equivalent per gram of sample (µmol $FeSO_4$/g). The determination of total phenolic content and antioxidant activities was performed in triplicate.

2.3 Caffeine content and chlorogenic acid determination

A standard mixture of caffeine, caffeic acid, and chlorogenic acid (purchased from Sigma Aldrich and used without purification) were separated using a high-performance liquid chromatography (HPLC) system comprising Alliance 2695 separation module and a 2489 UV/Visible detector (Waters), on a Zorbax eclipse XDB C18 column (Agilent 150x4.6 mm), monitored at a wavelength of 275 nm. The gradient system involved increasing the percentage of MeOH (Solvent A: 0.1% formic acid in water; solvent B: 0.1% formic acid in MeOH; gradient method 0-8 min 25-50% B, 8-9 min 50% B constant, 9-11 min 50-25% B). After optimizing the chromatographic resolution of the three standards, the retention time of each standard peak was confirmed by stepwise spiking of each standard into the mixture, followed by re-separation on the HPLC system. Calibration curves of concentration versus absorbance at 275 nm of the three standards were constructed using the optimized gradient system and were subsequently used to analyze all the extracted coffee samples. The HPLC measurements were conducted in triplicate.

3. Results and discussions

Phenolic compounds, antioxidant activities, caffeine, and chlorogenic acid content were determined in green single-origin coffee beans from 31 different farms. The means and standard deviations (SD) of the five measured quantities, grouped by province, are listed in Table 1. Note that the SD values are for each province for both Arabica and Robusta samples. Groupwise comparisons for each quantity were conducted using the Kruskal-Wallis test, a non-parametric equivalent of ANOVA for comparing three or more groups. The *p*-values indicate significant differences across all five quantities. Dunn's test was then used for post-hoc pairwise comparisons. The results showed a statistically significant difference in total phenolic content between Sisaket, Chumphon and Tak when compared to Chiang Rai. Measurements of antioxidant activities using the FRAP method indicated that Chumphon, Ranong and Sisaket samples had the highest values with 680.87 ± 4.45, 605.31 ± 3.50 and 572.11 ± 13.01 µmol $FeSO_4$/g, respectively, similar to the results from DPPH method.

Caffeine and chlorogenic acid are essential constituents of coffee beans which play a pivotal role in determining their taste and aroma [19]. From the chromatogram of 1:1:1 mixed standard of chlorogenic acid, caffeine, and caffeic acid, the peaks were close to one another. From the chromatograms of most samples, caffeic acid amounts were found to be very small, with areas under curve under 0.5% of the total area. From the literature, studies also found the amount of caffeic acid in coffee and tea to be low [19-21]. Caffeic acid is the metabolized form of chlorogenic acid (ester) whenever exposed to heat or light. After the coffee sample was extracted with warm water, its solution was kept under -80°C. It is thus speculated that the chlorogenic acid did not degrade to caffeic acid, so the chromatogram peak of caffeic acid was absent or appeared very low. Therefore, only caffeine and chlorogenic acid concentrations in the coffee samples were measured for analysis. From the HPLC measurements, the caffeine and chlorogenic acid contents of green coffee beans from various regions in Thailand were found to be in the ranges of 8-24 mg/g and 34-68 mg/g, respectively. Phitsanulok and Chumphon samples exhibited the highest values while Chiang Rai samples showed the lowest values. In general, the Robusta beans exhibited higher levels of caffeine than the Arabica beans, which agrees with the findings of a previous study [22].

Table 1 The summary statistics of the total phenolic content, FRAP, DPPH, caffeine, and chlorogenic acid grouped by province (mean ± SD). In all the units, /g refers to per gram crude extract.

Province	Total phenolic (mgGAE/g)	FRAP (μmol $FeSO_4$/g))	DPPH (mgAAE/g)	Chlorogenic acid (mg/g)	Caffeine (mg/g)
Chiang Mai	42.79 ± 8.15 ^{ab}	390.26 ± 108.78 ^{ab}	38.62 ± 8.80 ^{ab}	40.58 ± 8.51 ^{ab}	11.52 ± 2.99 ^{ab}
Chiang Rai	34.50 ± 3.51 ^{b}	311.12 ± 54.75 ^{b}	33.23 ± 9.33 ^{b}	36.29 ± 8.74 ^{b}	11.36 ± 1.88 ^{b}
Chumphon	58.94 ± 2.09 ^{a}	680.87 ± 4.45 ^{a}	67.84 ± 0.82 ^{a}	67.63 ± 3.36 ^{a}	23.56 ± 1.47 ^{a}
Kanchanaburi	46.45 ± 5.58 ^{ab}	393.76 ± 6.83 ^{ab}	41.27 ± 2.91 ^{ab}	41.09 ± 3.68 ^{ab}	16.31 ± 5.38 ^{ab}
Lampang	39.99 ± 4.78 ^{ab}	391.02 ± 51.78 ^{ab}	34.76 ± 6.13 ^{ab}	41.11 ± 10.33 ^{ab}	11.98 ± 0.94 ^{ab}
Mae Hong Son	39.19 ± 4.13 ^{ab}	338.08 ± 23.86 ^{ab}	39.82 ± 8.79 ^{ab}	42.52 ± 7.94 ^{ab}	11.13 ± 0.86 ^{ab}
Nan	44.85 ± 9.78 ^{ab}	390.96 ± 98.84 ^{ab}	43.81 ± 6.27 ^{ab}	42.21 ± 6.35 ^{ab}	13.32 ± 3.85 ^{ab}
Phitsanulok	37.06 ± 0.36 ^{ab}	409.97 ± 0.32 ^{ab}	47.05 ± 1.69 ^{ab}	65.66 ± 1.72 ^{a}	23.61 ± 1.03 ^{a}
Phrae	39.76 ± 3.54 ^{ab}	351.08 ± 40.17 ^{ab}	37.18 ± 2.38 ^{ab}	36.15 ± 4.89 ^{ab}	8.45 ± 1.17 ^{ab}
Ranong	51.72 ± 0.70 ^{ab}	605.31 ± 3.50 ^{a}	62.63 ± 1.30 ^{a}	48.61 ± 2.75 ^{ab}	14.26 ± 0.93 ^{ab}
Sisaket	60.90 ± 3.47 ^{a}	572.11 ± 13.01 ^{a}	51.14 ± 3.54 ^{a}	34.49 ± 4.56 ^{ab}	13.58 ± 1.91 ^{ab}
Tak	49.54 ± 11.53 ^{a}	469.85 ± 163.71 ^{ab}	42.07 ± 8.16 ^{ab}	42.44 ± 8.25 ^{ab}	13.16 ± 3.69 ^{ab}

Different letters (column-wise) indicate statistically significant differences ($p \leq 0.05$)

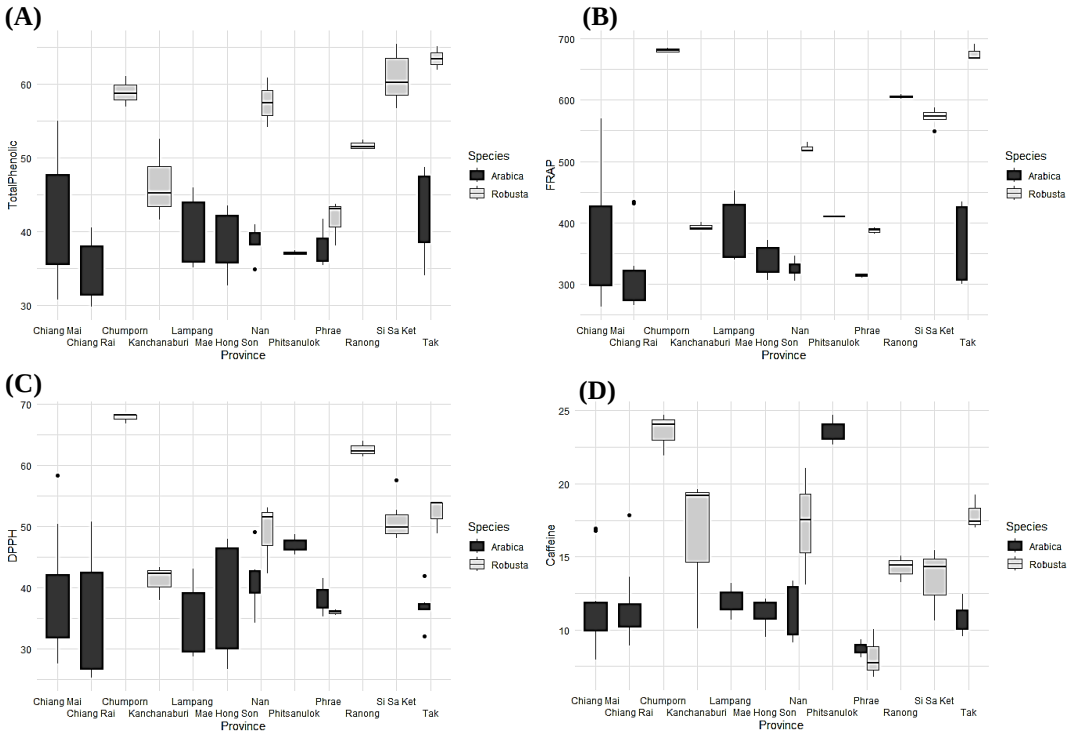
The means and standard deviations of the measured quantities grouped by species and region are reported in Table 2.

Table 2 The summary statistics of the total phenolic content, FRAP, DPPH, caffeine, and chlorogenic acid grouped by species and region (mean ± SD). In all the units, /g refers to per gram crude extract.

Species	Region	count	Total phenolic (mgGAE/g)	FRAP (μmol $FeSO_4$/g))	DPPH (mgAAE/g)	Chlorogenic acid (mg/g)	Caffeine (mg/g)
Arabica	Central	3	37.1±0.4 ^{ab}	410.0±0.3 ^{ab}	47.0±1.7 ^{abc}	65.7±1.7 ^{ab}	23.6±1.0 ^{a}
Arabica	North	60	38.4±6.0 ^{a}	345.0±73.4 ^{a}	36.8±8.6 ^{a}	39.1±8.2 ^{ac}	11.3±2.1 ^{b}
Arabica	West	6	42.6±6.0 ^{ab}	367.0±68.0 ^{ab}	37.0±3.2 ^{ab}	39.8±9.1 ^{abc}	10.8±1.1 ^{ab}
Robusta	North	6	49.6±9.2 ^{ab}	455.0±73.7 ^{ab}	42.5±8.0 ^{abc}	41.7±9.8 ^{abc}	12.7±5.7 ^{ab}
Robusta	Northeast	6	60.9±3.5 ^{b}	572.0±13.0 ^{b}	51.1±3.5 ^{bc}	34.5±4.6 ^{c}	13.6±1.9 ^{ab}
Robusta	South	6	55.3±4.2 ^{b}	643.0±41.5 ^{b}	65.2±3.0 ^{c}	58.1±10.8 ^{b}	18.9±5.2 ^{a}
Robusta	West	6	55.0±10.0 ^{b}	535.0±155.0 ^{b}	46.8±6.5 ^{abc}	44.4±4.5 ^{abc}	17.1±3.6 ^{ab}

Different letters (column-wise) indicate statistically significant differences ($p \leq 0.05$).

Distributions of the measured quantities, grouped by province and species, are shown in Figure 1. Total phenolic content, labelled as TotalPhenolic, is shown in 1A, FRAP in 1B, DPPH in 1C, caffeine in 1D, and chlorogenic acid content in 1E. Some provinces have both Arabica and Robusta coffee samples.



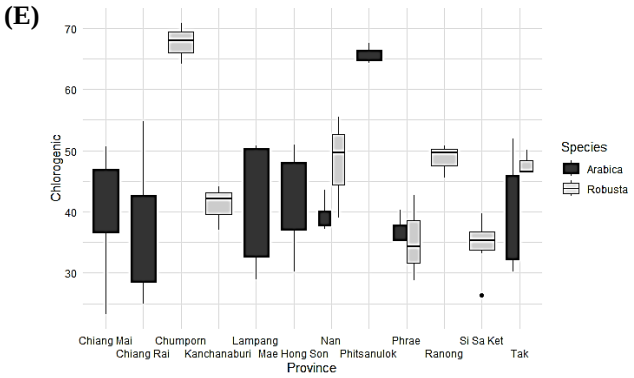


Figure 1 Box plots of total phenolic content, labelled as TotalPhenolic, (A), antioxidant activity (B: FRAP and C: DPPH), caffeine (D) and chlorogenic acid content (E), grouped by province and species, in green coffee bean samples. The Y-axis of the graphs are in the same units as those shown in Table 1.

In these provinces, the total phenolic content, FRAP, DPPH, caffeine, and chlorogenic acid levels of Robusta samples were generally higher than those of Arabica samples, except for Phrae. Robusta samples from Chumphon ranked highest in antioxidant activity, caffeine, and chlorogenic content while Robusta samples from Tak exhibited the highest total phenolic content. Notably, the caffeine and chlorogenic acid content of Arabica samples from Phitsanulok were almost as high as those of the highest-ranking Chumphon Robusta samples.

Scatterplots illustrating the correlations among the five measured quantities are shown in Figure 2. Correlation coefficients were determined to assess the relationship between variables, revealing positive correlations for all pairs. The highest coefficients were observed for FRAP-total phenolic (0.920) and FRAP-DPPH (0.843). This suggests that samples with higher levels of total phenolic compounds exhibit dominant antioxidant activity compared to Arabica. Arabica Specifically, the samples from Chumphon, Ranong and Sisaket, representing Robusta coffee, demonstrated elevated levels of total phenolics, resulting in correspondingly high antioxidant activity. Consistent with prior research comparing phenolic compounds and antioxidant activities in Arabica and Robusta coffee, Robusta bean extracts exhibited high total phenolic content and strong antioxidant activity [23]. The biosynthesis and biodegradation of caffeine in coffee involve numerous steps that influence substrate availability for enzymes linked to gene expression [24, 25].

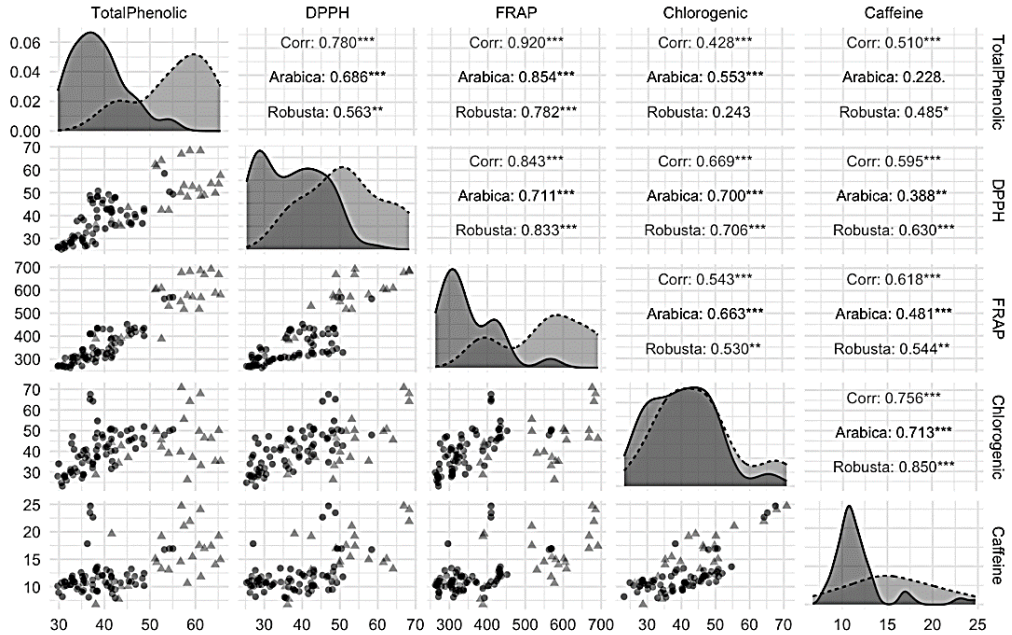


Figure 2 Scatter plots showing correlations among the total phenolic content, DPPH, FRAP, caffeine, and chlorogenic acid. Pearson's correlation coefficients between corresponding pairs are shown in the upper right panels. Asterisks (stars) indicate the level of correlation significance (*** if the p-value is < 0.001, ** if the p-value is < 0.01, * if the p-value is < 0.05). For the scatter plots, the X-axis and Y-axis are in the same units as

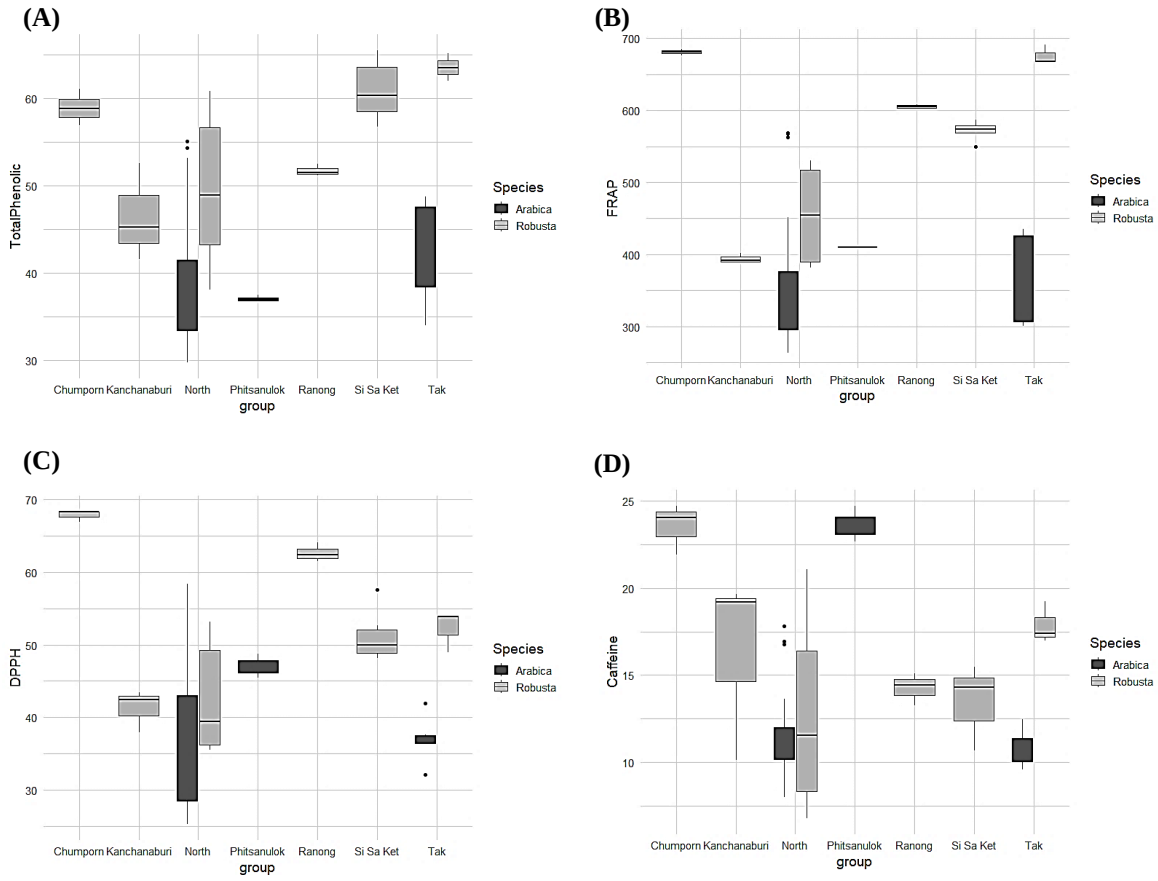
those shown in Table 1. For the density plots (on the diagonal), the Y-axis are normalized so the total area under curve is one.

For regional analysis, green single-origin coffee beans from 31 different farms across Thailand were divided into five distinct regions: Central (Phitsanulok), North (Chiang Rai, Chiang Mai, Mae Hong Son, Lampang, Nan, and Phrae), Northeast (Sisaket), South (Ranong and Chumphon) and West (Tak and Kanchanaburi). The means and standard deviations of the five measured quantities, grouped by region, are listed in Table 3. Distributions of the measured quantities, grouped by region and species, are shown in Figure 3. The total phenolic content is shown in 3A, FRAP in 3B, DPPH in 3C, caffeine in 3D, and chlorogenic acid content in 3E. It is important to note that there is an imbalance in the sample distribution, with the majority of samples acquired from the northern part of the country. Similar to the province analysis, Kruskal-Wallis and Dunn tests were employed for region comparison. In general, samples obtained from the north, predominantly Arabica, exhibited lower values compared to samples from other regions. In contrast, samples from the south region displayed the highest measured quantities compared to samples from other regions.

Table 3 The summary statistics of the total phenolic content, FRAP, DPPH, caffeine, and chlorogenic acid, with the north provinces grouped as one region and other provinces as individual groups. In all the units, /g refers to per gram crude extract.

Region	Total phenolic (mgGAE/g)	FRAP (μmol $FeSO_4$/g))	DPPH (mgAAE/g)	Chlorogenic acid (mg/g)	Caffeine (mg/g)
SiSaKet	60.9±3.5 ^{b}	572.1±13.0 ^{a}	51.1±3.5 ^{a}	34.5±4.6 ^{b}	13.6±1.9 ^{ab}
Chumporn	58.9±2.1 ^{ab}	680.9±4.4 ^{a}	67.8±0.8 ^{a}	67.6±3.4 ^{a}	23.6±1.5 ^{a}
Ranong	51.7±0.7 ^{ab}	605.3±3.5 ^{ab}	62.6±1.3 ^{a}	48.6±2.7 ^{ab}	14.3±0.9 ^{ab}
Tak	49.5±11.5 ^{ab}	469.9±163.7 ^{ab}	42.1±8.2 ^{ab}	42.4±8.3 ^{ab}	13.2±3.7 ^{ab}
Kanchanaburi	46.5±5.6 ^{ab}	393.8±6.8 ^{ab}	41.3±2.9 ^{ab}	41.1±3.7 ^{ab}	16.3±5.4 ^{ab}
North	39.4±7.0 ^{a}	354.6±79.5 ^{b}	37.3±8.6 ^{b}	39.3±8.3 ^{ab}	11.4±2.5 ^{b}
Phitsanulok	37.1±0.4 ^{ab}	410.0±0.3 ^{ab}	47.0±1.7 ^{ab}	65.7±1.7 ^{a}	23.6±1.0 ^{a}

Different letters (column-wise) indicate statistically significant differences ($p \leq 0.05$)



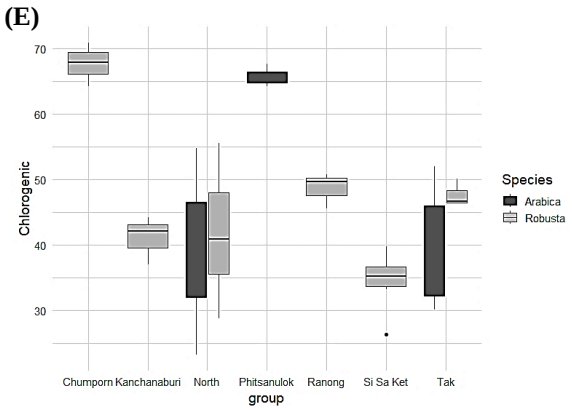


Figure 3 Box plots of total phenolic content (A), antioxidant activity (B: FRAP and C: DPPH), caffeine (D) and chlorogenic acid content (E), with the north provinces grouped as one region and other provinces as individual groups.

Principal component analysis (PCA) was conducted using the first two components derived from the five measured quantities, plotted in Figure 4. The first and second components (PC1 and PC2) explained approximately 73.6% and 16.3% of the variance, respectively. The five loading vectors in the biplot indicate the contribution of each original variable to the principal components. These vectors generally point in similar directions, suggesting the high correlation among the variables, all positively correlated with PC1. Despite the limited number of samples, there appears to be some degree of discrimination between the two coffee species and, to a lesser extent, among the five regions in the two-component PCA plot. Further experiments with more samples will validate whether this distinction holds.

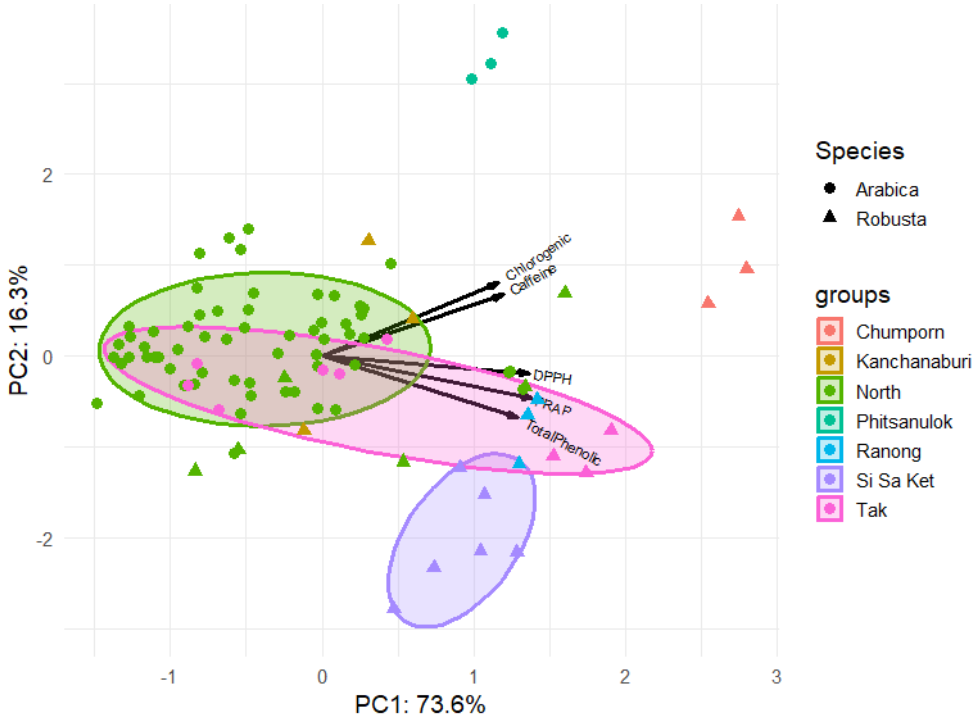


Figure 4 PCA plot showing the clusters of coffee regional origin and species. Ellipses are drawn for groups with more than three data points.

4. Conclusion

In this study, Thai green coffee beans from 12 provinces, primarily from the northern part of the country, were analyzed for their antioxidant properties and chemical compositions. Strong correlations were found among total phenolic content, FRAP, and DPPH, as well as between caffeine and chlorogenic acid. Robusta samples generally exhibited higher values of the measured parameters compared to Arabica samples. The regional and provincial

summary statistics of the measured quantities are reported. However, a higher number of samples will be needed in further research to draw any statistical conclusion. There are positive pairwise correlations among all the pairs of measured quantities. The principal component analysis showed that the first two components explain approximately 90% of the variance in the chemical data and there is a degree of separation in coffee species and region, indicating the potential of using the conventional chemical characteristics data in traceability modeling. The data provides a basis for a chemical characteristic database of green coffee beans obtained from different locations in Thailand. Future plans include an investigation into the effect of roasting conditions, geographical factors such as climate and altitude, and a comparison with coffee beans from outside Thailand.

5. Acknowledgements

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