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Relationship analysis based on phytochemical contents among coffee pulp from three coffee species collected in Southern Thailand and Jambi, Indonesia

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Abstract. *Maxiselly Y, Maulana H, Chumthong A, Chiarawipa R. 2023. Relationship analysis based on phytochemical contents among coffee pulp from three coffee species collected in Southern Thailand and Jambi, Indonesia. Biodiversitas 24: 5439-5445.* Coffee pulp is part of the coffee cherry, except for the seed, and has many benefits for human health. The research aimed to analyze phytochemical contents and the relationship among the coffee pulp of three coffee species: *Coffea arabica* (Arabica), *Coffea canephora* (Robusta), and *Coffea liberica* (Liberica), collected in southern Thailand and Jambi, Indonesia. The coffee pulp of Arabica from Indonesia dominated in three variables: antioxidant activity (67.37±11.83 mg/100g DW), tannin (40.71 ±3.52 mg/100g DW), and phenolic (50.47±5.43 mg/100g DW). Robusta coffee pulp from Thailand contained a lower value of all phytochemical components. However, the coffee pulp of Liberica from Thailand had the highest value for flavonoid content (18.19±0.95 mg/100g DW) and saponin content (0.77±0.09 g/100g DW) among the three species in both countries. Principal Component Analysis (PCA) also supports the result, which shows connectivity among antioxidant activity, tannin, and phenolic to Arabica from Indonesia, with flavonoid and saponin to Liberica from Thailand. These results indicate both the species and the environment influenced the phytochemical compositions of coffee pulp. Thus, Indonesia may benefit from developing more Arabica and Robusta coffee pulp products, while Thailand may benefit from creating the Liberica coffee pulp product.

Keywords: Antioxidant activity, coffee cherry, coffee waste, geographical location, zero waste agriculture

INTRODUCTION

Coffee is one of the perennial beverage plants. Coffee is among the most widely consumed non-alcoholic drinks globally (Silva et al. 2013). An important substance contained in coffee is caffeine (da Silva et al. 2018; Carmen et al. 2020). Caffeine is an alkaloid chemical compound that is also known as trimethylsantin. In addition, coffee also contains many chemical components, including alkaloids, terpenoids, flavonoids, phenolic acids, and others (Socala et al. 2021). This commodity is one of the most important commodities on the world market, with effects including lowering blood sugar and protecting the liver and nerves (Saud and Salamatullah 2021). Thus, coffee plays an important role in human health.

Indonesia is one of the world's largest and best coffee-producing countries. This is because Indonesia has a geographical advantage that strongly supports the growth and development of coffee (Wibowo et al. 2021). In addition, Indonesia also has a wide variety of coffee (Ramadiana et al. 2021). The famous coffee species as a coffee product globally are *Coffea arabica*, *Coffea canephora*, and *Coffea liberica* (Patay et al. 2016); each has strengths and weaknesses. Arabica has a pleasing aroma and favorable taste, contains less caffeine, and is vulnerable to some coffee diseases. In contrast, *Coffea canephora*, famous as Robusta, has lower quality and contains high caffeine but is more unaffected by disease than Arabica (Mathur et al. 2014; Wibowo et al. 2021). On the other hand, *C. liberica* is an African native species with large fruit but gives a more pungent bitter taste than others (Lambot et al. 2017). This species is also highly diverse in varieties, resistant in drought places, and contains large pulp quantities caused by fruit size (Davis et al. 2022; Wibowo et al. 2021).

Many products, beverages, food, and cosmetics use the coffee bean as a raw material. Coffee holds many valuable compounds for humans, mainly used at precise doses. Coffee comprises polyphenols and several antioxidants for human health (Blinová et al. 2017). These antioxidant activities work as a natural antibacterial, antiviral, and anti-inflammatory material.

Part of the beans of coffee are used for the beverage industry; conversely, the other parts are being wasted. Gouvea et al. (2009) and Orrego et al. (2018) reported that around 50% of coffee cherries are wasted after coffee beans are processed. Additionally, it was reported that processing 1 kg of coffee beans would produce 1 kg of waste (43.2% skin and pulp, parchment 6.1%, and 11.8%

mucilage and soluble sugar). It is also indicated that the production of coffee pulp can be calculated from the number of coffee beans produced (Orrego et al. 2018; Rahmah et al. 2023).

Nevertheless, the coffee waste, such as coffee pulp, comprises useful compositions such as caffeine, tannins, and phenolics and is also rich in nutrition such as carbohydrates, protein, and other organic components (pectin, cellulose, and hemicellulose) (Al-Yousef and Amina 2018; Carmen et al. 2020). Specifically, Klingel et al. (2020) found that coffee pulp contains 4%-12% of protein, 1%-2% of lipids, 6%-10% of minerals, and 45%-89% of total carbohydrates, and phenolic and caffeine compounds around 1.3%. The coffee waste has recently been used for various purposes, i.e., drinks (tea), animal feeds, organic fertilizers, and raw materials for biofuel production (Echeverria and Nuti 2017). On the other hand, coffee pulp is commonly utilized as jam, juice, concentrate, jelly, and bakery ingredient (Klingel et al. 2020). As the coffee pulp is enormous and potentially beneficial, it is essential to identify its phytochemical composition to improve the benefits and further develop coffee pulp-based products.

Patay et al. signified that the phytochemical content of coffee pulp was affected by the species and the planting location (Patay et al. 2016). In the Southeast Asia regions, some countries are known as large coffee producers (Vietnam and Indonesia), and other countries (Thailand, Laos, and the Philippines) are small contributors (International Coffee Organization 2018). This study analyzed coffee pulp's antioxidant activity and phytochemical contents from the three coffee species cultivated in Thailand and Indonesia. This information should help both countries in developing their coffee industry.

MATERIALS AND METHODS

Three species of coffee fruits (Arabica, Robusta, and Liberica) were taken from Songkhla (latitude 7°00'17.3 N, longitude 100°30'15.1 E, and altitude 32 m above sea level/masl) and Trang Provinces (latitude: 7° 33′ 27″ N, longitude 99° 36′ 37″ E, and altitude 29 masl) of southern Thailand as well as Jambi Province in Sumatra Island of Indonesia (latitude 01°81'92.5 longitude 101°25893 E and altitude 1,449 masl). The weather conditions of each place are shown in Figure 1 (BPS 2019; World Weather Online 2020). All of the fruits were harvested at the ripe period. The beans were peeled using wet coffee processing (Lani 2015). The coffee pulp was then dried using an oven at 60°C for 72 hrs, ground, and extracted with 95% ethanol (Merck, India) at a 1:10 ratio (1 sample: 10 ethanol. The extracts were put in dark storage for no more than 7 days. The extract was centrifuged (Cryste multi centrifuge-Varispin 6, Korea) for 15 min at 4,000 rpm. A rotary evaporator (Buchi, USA) was used to evaporate the extract's ethanol contents, as explained by Siatka and Kašparová (Siatka and Kašparová 2010). The final samples were stored in a glass bottle. Three aliquots of 0.025 g final sample were diverse with ethanol and used for 3 replications of chemical analysis procedures.

Phytochemical analysis

Antioxidant activity (AA)

Ferric ion-reducing antioxidant power (FRAP) is operated to determine the total antioxidant activity (Panda 2012). FRAP reagent confined 10 portions of 300 mM sodium acetate, 1 portion of 10 mM TPTZ solvent, and 1 portion of 20 mM $FeCl_3.6H_2O$ solvent, and this mixture was incubated at 37°C for 30 min. Every 0.1 mL sample was mixed with 4.5 mL FRAP reagent and then shaken for 10 min at a dark place at room temperature. The mixture was then run for absorbance identification using a UV-Vis series Evolution 200 spectrophotometer (Thermo Scientific, USA) at 593 nm.

Total phenolic (TPC) and total tannin content (TTC)

The minor modifications of the FC (Folin-Ciocalteau) method from McDonald et al. were used to identify total phenolic and tannin content (McDonald et al. 2001). Phenolic standard or a solution extract (0 and 1 mL of 1:20, v/v) was diluted with Folin Ciocalteau reagent (2 mL, 1:5 diluted with distilled water) and 7.5% $NaCO_3$ (1.5 mL) shaken and stored in the dark place for 2 hours. The gallic acid graph was developed as a standard. The mixture absorbance was identified at 751 nm using the spectrophotometer. For total tannin, the tannin standard or diluted extract (0.1 mL of 1:20, v/v) was mixed with Folin Ciocalteau reagent (1.6 mL, 1:5 mixed with distilled water) and 7.0% $NaCO_3$ 2 mL shaken and stored in the dark place as long as 1.5 hrs. The standard graph was set from tannic acid. The solvent absorbance was analyzed at 760 nm using the spectrophotometer.

Total flavonoid content (TFC)

Each extract's total flavonoid content was observed using aluminum chloride colorimetry (Panda 2012). A sample (0.4 mL) was diluted with distilled water (4 mL) and 5% $NaNO_2$ solvent (0.3 mL), then incubated for 5 minutes and further diluted with 10% $AlCl_3$ solvent (0.3 mL). After 6 minutes, 2 mL of 1 $molL^{-1}$ NaOH solution was added to this mixture, and the final volume was diluted with double-distilled water into 10 mL. The mixture was allowed to stand for 15 minutes, and absorbance was measured at 510 nm. The calibration graph using catechin as the standard was used to calculate the total flavonoid content articulated as mg routine equivalent per gram dry weight.

Saponin content (TSC)

The procedure of saponin content measurement was modified by (Corciova and Ivanescu 2017). The dried coffee cherry was treated with 4% vanillin (mixed by ethanol 95%) and 72% H_2SO_4 and then soaked in the water bath at 60°C for 15 minutes. Continued by cooling processing on ice to room temperature for 5 minutes, the absorbance of the solution was analyzed by spectrophotometry at 560 nm. The standards were prepared on a scale of 1-10 mg/L with the stock solution, aescin 0.03 g mixed with 50 mL distilled water.

Statistical analysis

The mean values and standard deviations were used to present the data, and all analyses were conducted in triplicate. The R statistic programming with one-way analysis of variance was operated to analyze the data when P values ≤0.05 (considered significant). Further analysis using the Duncan Multiple Rank Test was implemented.

Analyses of the relationship among coffee species from two countries and phytochemical contents used principal components analysis (PCA), and the trait data were graphically analyzed across the three species from Indonesia and Thailand. In addition, the mean values were used for this analysis. PCA biplots made by XLSTAT examine the association and opposition between phytochemical contents and species variation on a multivariate scale.

RESULTS AND DISCUSSION

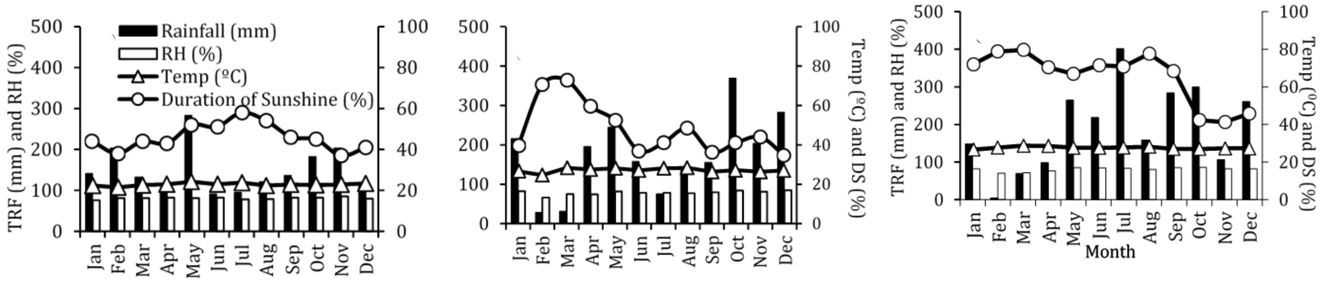
Environmental condition

The coffee cherries in this study were taken from locations with different weather conditions. Almost all Jambi areas had more than 100 mm daily rainfall except for two months in June and July. Other parameters (RH and temperature) were almost constant throughout the year. Additionally, sunshine duration decreased slightly from April to July and gradually decreased; as Figure 1C. The second and third locations of coffee cherries were from Thailand (Songkhla and Trang). Figures 1B and 1C show fluctuating rainfall in both areas. In contrast to Jambi-Indonesia, there were months of extreme rain in Trang and Songkhla Province-Thailand. While the monthly rainfall in Songkhla in February and March was under 50 mm, it peaked at 350 mm in October.

Similarly, Trang Province had limited precipitation in February but very heavy precipitation in July (>400 mm), indicating that coffee plants' water supply in Thailand varies more per month than in Indonesia. The percentage of sunshine duration in Songkhla and Trang was high and variable. In Songkhla, sunshine duration increased from January to March and gradually declined until June after that erratic pattern. On the other hand, Trang province described steady sunshine conditions until August, which started to drop significantly in October. These tree locations were relatively stable for additional weather elements such as temperature and RH.

Phytochemical contents

Table 1 shows the phytochemical compositions of coffee pulp among coffee species from the two countries. The difference of coffee beans from each species is presented in Figure 2. The highest AA content was represented by *C. arabica* from Jambi, Indonesia, followed by *C. liberica* from southern Thailand. *C. arabica* from Jambi, Indonesia, also had the highest TPC and TTC value. The Arabica from Indonesia and Liberica pulp from Thailand had the highest TSC content. Furthermore, for TFC content, *C. liberica* from southern Thailand had the highest value, followed by *C. arabica* from Jambi, Indonesia. Arabica species from Jambi, Indonesia, had the most phytochemical content in that location. Liberica from southern Thailand had a dominant composition compared to other species. The different coffees showed a variation of phytochemical contents in the coffee pulp.



A B C

Figure 1. Weather information (Total rainfall (TRF), Relative humidity (RH), Temperature (Temp.), and Duration of sunshine (DS)) of: A. Jambi Province, Indonesia by Meteorology Station of Depati Parbo Kerinci. B. Songkhla Province, Thailand by Agrometeorological Station of Songkhla. C. Trang Province, Thailand by Trang Meteorological Station

Table 1. Composition of coffee pulp phytochemical on three species origins from southern Thailand and Jambi, Indonesia

Species	Origin	Antioxidant activity content (mg Fe (II) equivalent/ 100 g DW)	Total phenolic content (mg gallic acid equivalent/ 100 g DW)	Total flavonoid content (mg catechin equivalent/ 100 g DW)	Total tannin content (mg tannic acid equivalent/ 100 g DW)	Total saponin content (g vanillin equivalent/100g DW)
C. arabica	TH	3.99 ± 0.52^{a}	10.22 ± 1.03^{ab}	9.99 ± 0.28^{c}	5.57 ± 1.34^{ab}	0.67 ± 0.03^{bc}
	IDE	67.37 ± 11.83^{d}	50.47 ± 5.43^{d}	15.26 ± 0.25^{d}	40.71 ± 3.52^{e}	0.74 ± 0.09^{c}
C. canephora	TH	3.75 ± 0.25^{a}	7.50 ± 0.50^{a}	4.37 ± 0.22^{a}	4.00 ± 0.27^{a}	0.61 ± 0.15^{b}
	ID	6.85 ± 1.18^{ab}	12.64 ± 1.77^{b}	6.38 ± 0.45^{b}	10.45 ± 0.77^{c}	0.50 ± 0.02^{a}
C. liberica	TH	27.30 ± 2.51^{c}	19.25 ± 0.16^{c}	18.19 ± 0.95^{e}	17.35 ± 0.69^{d}	0.77 ± 0.09^{c}
	ID	13.43 ± 1.87^{b}	8.40 ± 0.57^{ab}	5.67 ± 0.21^{b}	7.15 ± 0.86^{b}	0.59 ± 0.06^{ab}

Note: The averages of three samples being a value of each sample (mean ± SD), each analysis used triplicate (n = 1x3x3), (P≤0.05); [1]DW: dry weight; Superscript letters (a-e) within the one column indicate significant (P≤0.05) differences of means within the sample of coffee pulp

Analysis relationship among species and phytochemical contents by principal component analysis (PCA)

This study estimated the relationship between the phytochemical traits of three coffee species from Indonesia and Thailand using principal component analysis (PCA). Table 2 presents the Eigenvalues of all the properties tested. The measurement results show that only PC1 shows an eigenvalue >1 with a variation value of 81.49% (Table 2). This shows that genetic factors very dominantly control the resulting variation.

The first two principal components (PC) accounted for 97.18% of the variation among the three coffee species (Table 2). PCA measurements of three coffee species from Indonesia and Thailand tested based on phytochemical traits showed two axes with Eigenvalues between 4.074 and 0.784 (Table 2). PCA measurements for the diversity of coffee species tested showed a variation of the first component (PC1) of 81.49%, with all traits showing the maximum contribution. In the second component (PC2), the variation contribution is 15.69%. However, in PC2, there are no traits that show maximum contribution. This is because PC2 has an eigenvalue <1, indicating no significant effect on the resulting variation. In addition, this also shows that environmental impacts do not affect the trait content analyzed. The trait values that affect the coffee species diversity are presented in Table 3, which shows all traits have a dominant value (>0.5) in PC1.

PCA biplots generated from the first two PCs were used to identify the relationship between the phytochemical properties and the coffee species analyzed (Figure 3). It is observed that TSC forms a sharp angle with TFC and has a strong and positive relationship. Meanwhile, AA forms an acute angle with TTC and TPC; hence, these three compounds have a strong and positive relationship. The distribution of coffee species tested also varies, where LTH is closer to the TFC trait, meaning that these species are superior in that trait. AID also shows closeness to the AA trait, meaning that AID has a very high AA trait value compared to other species. In contrast to the two, the other four species (ATH, CTH, LID, and CID) were in the opposite direction with the traits observed. That suggests they have little value for the characteristics observed.

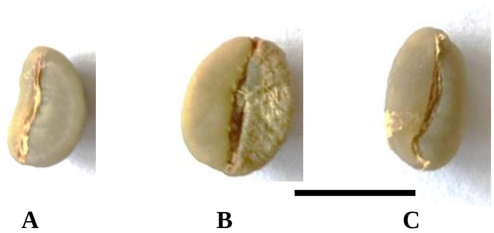


Figure 2. The difference of coffee beans from each species: A. *C. arabica*. B. *C. canephora*. C. *C. liberica*. Bar: 1 cm

Table 2. Eigenvalue, percentage, and cumulative of phytochemical traits from three species of coffee pulp from Indonesia and Thailand

	PC1	**PC2**
Eigenvalue	4.074	0.784
Variability (%)	81.487	15.688
Cumulative (%)	81.487	97.175

Note: PC: Principal Component

Table 3. Trait values that influenced the diversity of three species of coffee pulp

	PC1	**PC2**
AA	0.921	0.064
TPC	0.886	0.104
TFC	0.730	0.205
TTC	0.905	0.092
TSC	0.632	0.319

Note: Numbers in bold indicate a discriminant of >0.5 or <−0.5 that contributed to variance (Jolliffe 2002); PC: Principal Component; AA: Antioxidant activity; TPC: Total phenolic; TTC: Total tannin content; TFC: Total flavonoid content; TSC: Saponin content

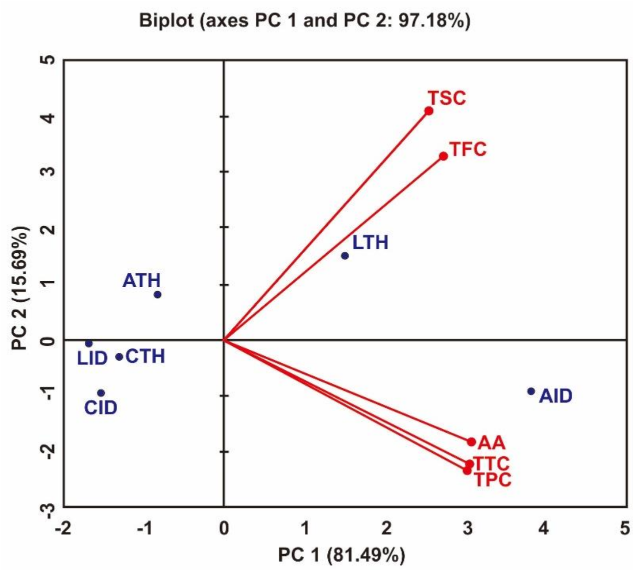


Figure 3. Relationship of phytochemical traits from three species coffee pulp based on Principal component analysis (PCA) biplot. The blue point indicates the position of the species of coffee pulp from Indonesia and Thailand; The red point indicates the position of the tested phytochemical traits. AA: Antioxidant activity; TPC: Total phenolic; TTC: Total tannin content; TFC: Total flavonoid content; TSC: Saponin content; CID: *C. canephora* Indonesia; CTH: *C. canephora* Thailand; AID: *C. arabica* Indonesia; ATH: *C. arabica* Thailand; LID: *C. liberica* Indonesia; LTH: *C. liberica* Thailand

Discussion

A suitable environment is needed for developing coffee trees during the vegetative and reproductive stages until harvesting. Robusta coffee trees need an annual rainfall of 1,200-2,500 mm, while it is 1,100-2,000 mm for Arabica with few dry months (Lambot et al. 2017). Coffee trees need a minimum of one dry month for unvarying flowering. If a dry period is not obtainable, it causes extended flowering time, and the harvesting process will be more sophisticated (Gomez et al. 2016). Regarding temperature, coffee cultivation grows well from 18°C to 21°C for Arabica and 22°C to 26°C for Robusta; it could tolerate up to 30°C. Marginal environments boost the vegetative phase and decrease fruit quantities (Bote and Jan 2017; Lambot et al. 2017; Venancio et al. 2019). One part of the coffee fruit is pulp. The various components of coffee pulp, such as protein, fiber, carbohydrate, and fat, depend on factors such as variety or species, location, and agricultural practices (Wibowo et al. 2021; Machado et al. 2023). One factor that has a high effect on the maturing of the coffee cherry is sunshine. The intensity of sunshine duration in the coffee field strongly influenced the coffee bean's size and composition (Wale Mengistu et al. 2020; Getachew et al. 2022).

The shading technique is an agricultural practice that effectively manipulates sunshine intensity. Based on Vaast et al. (2016), shade management decreases tree stress and productivity through delayed fruit ripening and flavoring of coffee cherry pulp. While the coffee load productivity is low, it is compensated by the improvement in size and quality. The shade benefits the coffee plant in suboptimal and high-temperature areas (Biruk 2018; Sarmiento-Soler et al. 2019). It maintains coffee production in marginal places such as low altitudes and heated-stress conditions. Although the low intensity of sunlight will delay the coffee cherry's maturity, it increases the weight and quality of the fruit (Lambot et al. 2017).

According to Figure 1 (A, B, C), some climate aspects in Jambi Indonesia, such as rainfall and temperature, are more appropriate for coffee growth than those in southern Thailand. It also showed that all environmental factors in Jambi, Indonesia, suit Robusta and Arabica coffee. Meanwhile, both provinces in Thailand (Songkhla and Trang) had higher than the coffee plant's optimal temperature. Therefore, the farmers in these areas can apply the shading technique to minimize this drawback.

The coffee pulp of immature fruit in wild species (Bengal) and local African coffee (Liberica) had higher polyphenol and total phenolic composition than *C. arabica*. That also reported that the tannin composition and total antioxidant capacity of C. *benghalensis* were more significant than Arabica in immature and mature coffee fruit (Patay et al. 2016). Several volatile components in coffee were observed while coffee fruits were harvested at low temperatures; however, the high temperature could reduce their aromatic quality (Cotter and Hopfer 2018; Yu et al. 2021). The biochemical aspect of coffee species will be one of the quality parameters influenced by species and environmental factors (Herrera and Lambot 2017).

The relationship between coffee species and phytochemical properties was analyzed using principal component analysis (PCA). This method can reduce the number of data varians to assist the selection process (Placide et al. 2015), includes grouping genotypes into clear groups (Jolliffe 2002), determining genotypes or species for certain traits, as well as the relationship between each trait being tested (Maulana et al. 2023). In the main component (PC), the eigenvalues can explain the cumulative factor and the diversity, in which the result is more than or equal to one (Jolliffe 2002; Maulana et al. 2023). Therefore, more than one eigenvalue determined the number of PCs used; the measurement results show that only PC1 has an eigenvalue >1 with a variation value of 81.49% (Table 2). This shows that PC1 already represents information about the variation and dominant traits of the species tested. In addition, the resulting contribution value also indicates that genetic factors more dominantly control the traits analyzed. It means that each type of coffee grounds from Indonesia and Thailand has different abilities in terms of phytochemical properties. That is also supported by the value of each trait tested in the first component (PC1), which has a value of >0.5, which indicates the maximum contribution (Table 3) (Jolliffe 2002; Maulana et al. 2023). Several researchers also revealed that the condition of PC 1, which has a contribution value of >0.5, indicates that genetic factors more dominantly control most of the traits tested (Bouargalne et al. 2022; Maulana et al. 2023).

PCA graphs can be used to identify correlations between tested traits from vector positions. This graph was generated from the first two PCs. In Figure 3, TSC forms an acute angle with TFC, which has a strong and positive relationship. Meanwhile, AA forms an acute angle with TTC and TPC, so the three samples have a strong and positive relationship. The angle formed between vectors can estimate the correlation between traits (Maulana et al. 2023). Trait vectors that form an acute angle (closer to 0°) indicate these traits are strongly and positively correlated. In contrast, those with an obtuse angle (closer to 180°) indicate that these traits are strongly and negatively correlated (Solihin et al. 2023).

The distribution of the coffee species tested varied, where LTH was closer to the TFC trait, meaning that this species was superior in that trait. The position of the trait vector and the coffee species points indicate the size of the trait in the species tested. Species that approach a certain trait show its high value on that trait (Jolliffe 2002). AID also indicates a closeness to the AA trait. It means AID has a very high AA trait value compared to other species. In contrast to these coffee species, the other four species (ATH, CTH, LID, and CID) were in the opposite direction with the traits tested. This suggests that they have little value for the characteristics tested. Thus, it can be determined that LTH has the highest TFC value compared to other species, while AID has the highest AA value. Therefore, this information will benefit future coffee research and development programs.

In conclusion, the pulp of coffee fruit originates from southern Thailand and Jambi, Indonesia, has a different value of phytochemical compositions. *C. arabica* and *C. canephora* from Indonesia dominate in antioxidant, phenolic, flavonoid, and tannin. *C. liberica* from Thailand has the highest levels of all parameters (antioxidant, phenolic, flavonoid, tannin, and saponin). Due to different environmental and climate characteristics and also supported by phytochemical content, it is advantageous to Indonesia, specifically Jambi, for developing Arabica and Robusta coffee. At the same time, southern Thailand gets more benefits by developing Liberica species.

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