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Amino Acid Profiles of Coffee Cherry Flour from Different Origins: A Comparative Approach

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Abstract. A popular beverage globally attributable to its energizing properties and distinctive flavor, coffee is one of the majorly produced agricultural merchandise. With the reputation, nonetheless, comes a sizeable waste in its production process. Reduce, reuse, recycle, and improve circular economy —coffee pulp and husk waste into functional food, *i.e.*, coffee cherry flour. This study examined the amino acid composition and contents originating from four locations: Ijen Farm, Karang Ploso Farm, Mengani Farm, and La Boitê. In triplication, samples were filtrated, derivated, and analyzed through high-performance liquid chromatography with fluorescence detection. The obtained data were then computed to determine the amino acid retention times and peak areas to facilitate the quantification of amino acid concentrations within the samples. ANOVA was involved in evaluating the significance of amino acid level variations, and then non-parametric correlation and cluster tests were conducted for a dendrogram presentation. The result stated that Serine, Histidine, Threonine, Alanine, Cysteine, Methionine, and Isoleucine are positively correlated to the area characteristics, while Aspartic acid, Glutamic acid, Glycine, Arginine, Proline, Tyrosine, Valine, Lysine, Leucine, and Phenylalanine are of negative association. This finding suggests that locally-grown coffee cherry flour should be feasible for functional food beneficial to health.

Keywords: Circular economy, enviromentally friendly, iron booster, reduce-reuse-recycle, waste to fuctional food

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1 Introduction

Popular for its invigorating attributes and flavorful richness, coffee stands as one of the world's most extensively enjoyed beverages. Nevertheless, the coffee processing sector generates a substantial volume of waste material [1–9] – solid leftover of pulp (from wet coffee method) or husk (from dry coffee method) is the major one. Initiatives have been currently undertaken to transform this solid waste into value-enhanced products. Particularly for functional food applications, simple technology can be employed to utilize Coffee Pulp (CP) and Coffee Husk (CH) in the production of coffee cherry tea (also known as cascara from Spanish "cáscara," meaning husk or skin and pulp of coffee cherry). Ota [10] described that cascara is consumed as traditional beverages in Ethiopia (Hashana), Yemen (Qishr), Costa Rica (Cáscara) and Bolivia (Sultana). While quite a number of researches on cascara have been carried out [11–20, 8, 9] highlight Coffee Cherry Flour (CCF) as a more efficient and effective approach in recycling CP and CH.

Fascination towards CCF as a promising functional food ingredient owes to the presence of abundant bioactive compounds, notably polyphenols and dietary fiber [20–22, 2, 9, 18] acting as antioxidant, anti-inflammatory and antimicrobial properties [23–27, 1, 5]. Phytochemicals such as chlorogenic acids and melanoidins are also discovered in it [28, 29, 25]. The aforementioned contents are beneficial for health.

Setyobudi *et al*. [20, 8, 9] pointed out that CCF can be used as a supplement to enhance iron (Fe) levels. Anemia is a prevalent concern particularly affecting adolescent girls and mothers worldwide [30, 31], and Indonesia is not an exception [32, 33]. Serving as a hemoglobin enhancer, iron is also essential in dealing with autoimmune conditions and COVID-19 effects to bolster their immune system [34–37]. Numerous publications have indicated a global trend of increasing numbers of individuals with autoimmune diseases [38–40]. Being gluten-free, CCF is a friendly source of non-heme iron and antioxidants for people with autoimmune issues [41, 42, 38].

Several researchers [43, 44] have asserted that the absorption of non-heme iron in mammal digestive system is impacted by the presence of enhancing agents (also called boosters or promoters). Amino acid enhances iron absorption by creating a soluble chelate [44–46]. Amino acids come in three categories: essential amino acids, semi-essential amino acids, and non-essential amino acids. While non-essential amino acids – also referred to as endogenous amino acids –can be synthesized internally, essential amino acids – often called indispensable amino acids – cannot be produced by the body and must be acquired through protein-containing food consumption [47, 48]. As there are twenty types of amino acids [49, 47], dry CP comprises 17 amino acids and eight to nine out of them are of essential amino acids [50–53]. It is also identified that the amino acid composition in CP is similar to one in soybean (*Glycine max* (L.) Merr.) or kapok flour (*Ceiba pentandra* (L.) Gaertn.) [54].

Setyobudi *et al*. [8] examined the amino acid profiles of CCF Mengani from Bali, Indonesia, in comparison to CCF-La Boite, a commercial product sourced from Brazil. As the manuscript presents data on total amino acid content, further detail pertaining to essential amino acids – particularly sulfur-containing amino acids for its ability to enhance the absorption of non-heme iron – is called for to cover a crucial aspect for health [55–57, 49]. Consequently, conducting a comparative assessment of coffee flour derived from diverse origins becomes crucial in order to comprehend the possible divergences in its nutritional composition and its potential to promote health.

The objective of this study is to explore the amino acid composition in coffee flour sourced from distinct coffee farms, specifically Ijen Farm (located adjacent to Mount Ijen in

Bondowoso, East Java), Karangploso Farm (situated downhill Mount Arjuno in Malang, East Java), Mengani Farm (on the slopes of Mount Batur in Mengani, Bali), and La Boitê (a commercially available product originating from Brazil obtained from La Boite, a store in Manhattan, situated at 724 11th Avenue, New York, NY 10019). Additionally, this comparative investigation has the potential to play a pivotal role in shaping standardized production techniques and quality assurance protocols for CCF, thereby guaranteeing uniformity and reliability in its utilization as a functional food component.

2 Materials and methods

2.1 Materials

CCF samples of Arabica coffee cherries were gathered from four locations: Ijen Farm (7°57'59.55"S, 114°01'14.37"E), Karang Ploso Farm (7°52'13.80"S, 112°34'54.44"E), Mengani Farm (8°17'16.63"S, 115°15'0.61"E), and La Boitê (40°45'59.6088"N, 73°59'37.5792"W). Coffee cherries were subjected to a drying process to produce cascara, which was subsequently transformed into powder using an electric coffee grinder (K-500N, China). The powder was then sifted through a No. 80 mesh sieve (locally sourced) and carefully stored in CTIK plastic clips measuring 7 cm × 10 cm. To ensure the reproducibility of data, each sample was acquired in triplicate.

2.2 Method

Preparing for each sample, 1 g of CCF was meticulously weighed using an analytical laboratory balance (JA-3003, China) and transferred to a clean, dry container. Each sample was appropriately labeled for identification. Employing the method outlined by Nollet [58] for extraction, 10 mL of 0.1 M HCl acting as the extraction solvent was added to each container and sealed, then subjected to vortex mixing (VM500Pro, China) for several minutes to ensure complete homogenization. The solution was carefully transferred to a filtration apparatus to eliminate any particulate matter or impurities, and the resultant was collected in a pristine vial for subsequent analysis. For derivatization process, 10 µL sample was mixed with 10 µL internal standard, buffered to pH 8.8 using borate buffer to a total volume of 80 µL, and then added with 20 µL of a reagent solution (6-amino-quinolyl-N-hydroxysuccinimidyl carbamate, AccQ. Flour, 3 mg mL^{-1} in acetonitrile). All chemicals utilized were of pro-analysis grade and sourced from Merck.

The samples were heated at 55 °C for a duration of 10 min and subsequently subjected to analysis using HPLC (PerkinElmer® LC 300, USA) equipped with fluorescence detection. The HPLC setup encompassed a quaternary pump, a vacuum degasser, a temperature-controlled auto-sampler, a temperature-controlled column compartment, and a fluorescence detector. An aliquot of the derivatized sample solution, measuring 20 µL, was introduced into the HPLC column through injection. The HPLC system was operated with the pertinent mobile phase and gradient conditions tailored for the separation of amino acids. A UV detector was employed to track the elution of amino acids, and the resulting chromatographic data was meticulously documented.

The HPLC data was scrutinized to ascertain the retention times and peak areas associated with individual amino acids. Quantification of amino acid concentrations was achieved by juxtaposing the peak areas of the samples against standard calibration curves that were constructed using predetermined concentrations of amino acid standards. The concentrations of individual amino acids in the CCF samples were determined by utilizing the peak areas and calibration curves. To evaluate the significance of variations in amino acid levels among

the CCF samples, an Analysis of Variance (ANOVA) was executed [59, 60]. In addition to the ANOVA analysis, non-parametric correlation tests and cluster tests were conducted, and the results were presented in the form of a dendrogram [61, 62].

3 Results and discussion

3.1 Results

To optimally identify CCF as alternative food, correlation between sources and amino acid contents was analyzed. As per Spearman Rho correlation analysis laid out in Table 1, significant correlation exists ($P < 0.05$) in varied levels of direction, implication, and intensity.

Table 1. Correlation coefficients, *P* value, and R-square for amino acid contents in coffee cherry flour of different sources.

Amino acid content of CCF (sourced from Ijen Farm, Karang Ploso Farm, Mengani Farm, and La Boite)	Correlation coefficients (r)	*P* value	R-square
Asp	-0.420	0.007	17.6 %
Ser	0.409	0.009	16.7 %
Glu	-0.581	0.000	33.8 %
Gly	-0.775	0.000	60.0 %
His	0.477	0.002	22.7 %
Arg	-0.810	0.000	65.6 %
Thr	0.387	0.014	15.0 %
Ala	0.387	0.014	15.0 %
Pro	-0.581	0.000	33.8 %
Cys	0.863	0.000	74.5 %
Tyr	-0.808	0.000	65.2 %
Val	-0.839	0.000	70.4 %
Met	0.949	0.000	90.1 %
Lys	-0.738	0.000	54.5 %
Ile	0.335	0.035	11.2 %
Leu	-0.387	0.014	15.0 %
Phe	-0.775	0.000	60.0 %
Total AA	-0.360	0.022	13.0 %

Asp = Aspartic acid, Ser = Serine, Glu = Glutamic, Gly = Glycine, His = Histidine, Arg = Arginine, Thr = Threonine, Ala = Alanine, Pro = Proline, Cys = Cysteine, Tyr = Tyrosine, Val = Valine, Met = Methionine, Lys = Lysine, Ile = Isoleucine, Leu = Leucine, Phe = Phenylalanine, Total AA = Total Amino Acids.
Note: *P* value < 0.05 = a significant relationship.

A number of amino acids – Ser, His, Thr, Ala, Cys, Met, and Ile – presented positive correlation with CCF sources. Referring to the order, it is clear that La Boite had higher contents of the aforementioned amino acids than Mengani, while Mengani had higher contents than Ijen and Karang Ploso. Further, this study has revealed variations in correlation intensity between certain amino acids and souces. The highest correlation coefficients were of Met (0.949) and Cys (0.863), while the lowest was of Thr and Ala (0.387). With determination coefficient of 90.1 % in Met, it can be inferred that the source affects the content of Met in CCF up to 90.1 % when the remaining 9.9 % is of other factors.

On the other hand, amino acids Asp, Glu, Gly, Arg, Pro, Tyr, Val, Lys, Leu, and Phe showed negative correlation with CCF sources. Based on the order, Ijen contains the most of those amino acids while La Boite the least. As of correlation coefficients, Val (- 0.839), Arg

(-0.810), and Tyr (-0.808) were of the highest while Leu (-0.387) the lowest. With determination coefficient of 70.4 % in Val, it it is postulated that the source affects the content of Val in CCF up to 70.4 % when the remaining 29.6 % is of other factors. With the total amino acid rate as low as -0.360, the source affects only 13.0 % of amino acid contents in CCF.

Cluster analysis – of which results are displayed in Table 2 along with ANOVA test outcome to indicate any occurring difference – follows. The table points out significant differences in every cluster, suggesting significantly different relative scores.

Table 2. Cluster analysis and ANOVA test results.

Variable	Cluster 1	Cluster 2	Cluster 3	F (ANOVA)	Sig
Asp	0.6370	0.5650	1.0075	11.051	0.000
Cys	0.0080	0.6123	0.0010	95 364.523	0.000
Tyr	0.2410	0.0900	0.3079	221.247	0.000
Val	0.6579	0.2726	1.3511	783.283	0.000
Ser	0.2137	0.7950	0.2667	323.180	0.000
Glu	1.0250	1.3700	1.6898	101.339	0.000
Gly	0.2724	0.7806	1.5003	1 084.169	0.000
His	0.0205	1.0294	0.0453	6 618.108	0.000
Arg	0.0163	0.0248	0.4873	1 766.704	0.000
Thr	0.3835	1.0545	0.6168	357.871	0.000
Ala	0.2131	1.0440	0.6386	386.688	0.000
Pro	0.3146	0.4900	0.7450	38.792	0.000
Met	0.3702	0.9637	0.0684	1 057.326	0.000
Lys	0.3195	0.2591	0.5950	281.978	0.000
Ile	0.4069	0.7200	0.5431	180.046	0.000
Leu	1.3403	0.7435	1.3556	44.955	0.000
Phe	0.2539	0.3394	0.5350	1 239.506	0.000
Total AA	7.5041	10.4982	11.6772	81.422	0.000

The dendogram in Figure 1 depicts how each of 40 variables is related to each other and brought to three clusters.

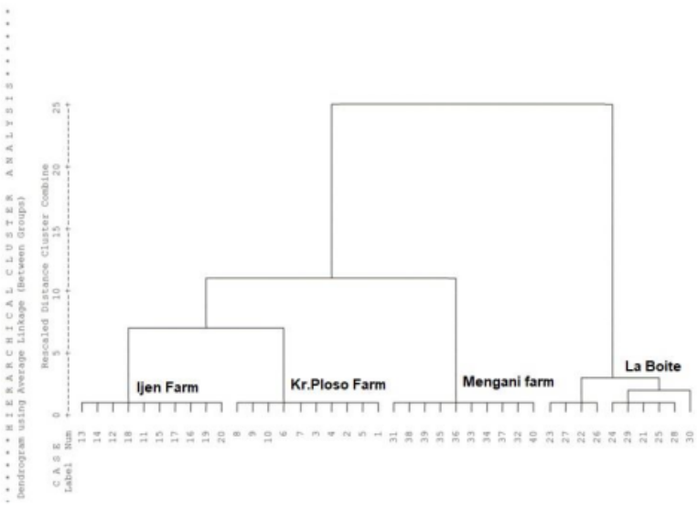

Fig. 1. Dendrogram of amino acid contents in coffee cherry flour from different sources.

Figure 1 puts Mengani, La Boite, and combination of Ijen and Karang Ploso in Cluster 1, Cluster 2, and Cluster 3, respectively. In other words, the contents of 18 amino acid

parameters in Mengani CCF are different from La Boite and neither is the same as Ijen-Karang Ploso. The dendogram also reveals how Ijen-Karang Ploso CCFs are more closely-related to La Boite than Mengani. Sub-clusters are formed specifically in Mengani while such phenomenon does not appear in other variants.

3.2 Discussion

The analysis generally explains that amino acid contents of CCF vary in accordance with their sources. This finding concurs previous studies examining CCF from the same sources on other nutrient contents. In regards of amino acids, some were found at higher concentrations in Indonesia local CCF than in La Boite and some others were of lower ones. Parallel occurrence of other nutrients, such as vitamin C and beta carotene, in Mengani is lower than in La Boite, but the reducing sugar in Mengani is higher [8]. Vitamin C and phenol contents in Ijen and Karang Ploso CCFs are also lower than La Boite [9]. The higher similarity in amino acid contents between Mengani and La Boite compared to the other local CCFs in this study is in sync with previous researches on other nutrient contents [9].

CCF is a potential alternative food containing constructive nutrition [63, 64] including amino acids. The amino acids in coffee pulp protein are of equal quantities to ones in other prominent protein sources like corn and soybean [65]. In association with this study, four amino acids – valine, glutamic acid, aspartic acid, and leucine – are negatively correlated with the sources. These are of the highest contents found in coffee bean [66]. Moreover, aspartic acid, glutamic acid, glycine, alanine, and proline found in coffee pulp are of higher proportions than in corn [67].

However, different treatments may impinge their CCF nutrient profiles including amino acid. Coffee harvesting method [68, 63] and ripeness when harvested [69] as well as production method applied [67] determine amino acid contents in CCF. Setyobudi *et al*. [8] reported that there were indications of lower total amino acids at Mengani farm compared to La Boite because of drying CP to cascara; Mengani used an electric dryer with a temperature of 80 °C. Even though several researchers [11, 12] stated that the best temperature for drying cascara was 40 °C to 50 °C. Mengani Farm using artificial dryers aims to increase the quantity of cascara, even though the amino acids, and vitamin C obtained are lower [8, 9].

Environmental factors like climate, land, and elevation are also influential since temperature, sunlight exposure, and soil composition are key to amino acid biosynthesis in coffee plant. High temperature can form a stressful environment, impending the plant's growth and metabolism [70] since they demand strings of biochemical reactions sensitive to temperature while stress defense may alter amino acid production in the plant. Sunlight intensity can modulate phosphate availability affecting amino acid metabolism in the plant [71]. Soil fertility, nutrient availability, and acidity are pertinent in nutrient absorption and utilization in the plant. Fertile soil supports optimal plant growth by providing nutrients for the plant [72], so coffee plants grown in rich, balanced soil will more likely produce rich, balanced amino acid for CCF. Soil minerals are crucial in controlling essential nutrient availability [73], thus mineral-flushed soil should yield Arabica coffee containing higher rate of amino acid. Conversely, nutrient-deprived soil degrades nutrients contained in the plant [74].

Amino acid is a metabolic product of plant, and synthesizing it calls for certain nutrient sources [75]. Soils rich in nitrogen, phosphor, and kalium maintain optimum growth and development of coffee plant. Availability of diversed nutrients should allow plant to access varied resources for synthesizing different kinds of amino acid, therefore it shapes amino acid profile in CCF.

Soil acidity – considered as soil's main variable towards biological, chemical, and physical characteristics and is crucial in plant growth and plant nutrient content [76] – also

controls amino acid contents in CCF as it affects soil microorganism activities [77, 78] that may be involved in chemical transformation of available nutrients [79, 80]. Soil acidity variation of the CCF sources differs microbiological condition and, in the long run, brings an impact on amino acid production. Soil acidity variation also determines essential nutrient availability [81, 82] – while required by the plant in merely a small amount, the substances are critical in amino acid and protein biosynthesis processes. Furthermore, the plant's ability to absorb and utilize the substances depends on soil acidity level resulting in amino acid composition of CFF.

Another factor that distinguishes amino acid contents in CCF is humidity of each source. Low humidity instigates stress towards the plant, affecting its physiological process [83, 84] including amino acid synthesis, as such condition obstructs water and nutrient absorption necessary for the process. Sufficient humidity, conversely, should support efficient biosynthesis.

4 Conclusion

In comparing and grouping coffee cherry flour produced in a number of locations based on their amino acid contents, it is concluded that Ser, His, Thr, Ala, Cys, Met, and Ile are positively correlated to the area characteristics while Asp, Glu, Gly, Arg, Pro, Tyr, Val, Lys, Leu, and Phe are negatively associated. Out of 17 amino acids analyzed, it is also surmised that Met, Cys, Val, Arg, and Tyr are of the highest correlation rates; meanwhile, Thr, Ala, and Leu are of the lowest coefficient rates. Cluster analysis categorizes the four locations into three main clusters – Mengani, La Boite, and Ijen-Karang Ploso belong to Cluster 1, Cluster 2, and Cluster 3, respectively.

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