**Special Issue:** Novel Advances in Agricultural Science and Technology for Sustainable Farming in Tropical Region

# Characterization of Amino Acids in Coffee Cherry Flour from Different Coffee Cultivation Areas: As Potential Functional Food

**Roy Hendroko Setyobudi[1], Shazma Anwar[2], Mohammed Ali Wedyan[3], Damat Damat[1]*, Yogo Adhi Nugroho[4], Tony Liwang[4], Praptiningsih Gamawati Adinurani[5], Satriyo Krido Wahono[6], Evika Sandi Savitri[7], Bayu Agung Prahardika[7], Irma Rahmaita Utarid[8], Iswahyudi Iswahyudi[9] and Hemalia Agustin Rachmawati[1]**

*[1]University of Muhammadiyah Malang, Malang 65144, East Java, Indonesia; [2]University of Agriculture, Peshawar 25130, Khyber Pakhtunkhwa, Pakistan; [3]The Hashemite University, PO Box 330127, 13133 Zarqa, Jordan; [4]Plant Production and Biotechnology Division, PT Smart Tbk., Bogor 16810, West Java, Indonesia; [5]Merdeka University of Madiun, Madiun 63133, East Java, Indonesia; [6]Research Center for Food Technology and Processing (PRTPP), National Research and Innovation Agency (BRIN) Special Region of Yogyakarta 55281, Indonesia; [7]State Islamic University of Maulana Malik Ibrahim, Malang 65145, East Java, Indonesia; [8]Central Research and Diagnostic "Satwa Sehat" Indonesia, Malang 65146, East Java, Indonesia; [9]Universitas Islam Madura, Pamekasan 69317, East Java, Indonesia.*

**Abstract** | Coffee is one of the most popular drinks in the world. However, during processing coffee a substantial quantity of waste is produced. This study analyzed the amino acid composition of coffee cherry flour (CCF) from four farms: Ijen Farm, Karang Ploso Farm, Mengani Farm and La Boitê. The results depicted that the amino acid composition of CCF varied among different sources. Serine, histidine, threonine, isoleucine and cysteine were noted higher in CCF from La Boitê (0.80±0.01), (1.03 ± 0.01), (1.05 ± 0.02), (0.72 ± 0.00) and (0.61 ± 0.04) while lower in Mengani Farm (0.16 ± 0.00), (0.02 ± 0.01), (0.27 ± 0.15) and (0.43± 0.08) respectively. Glutamic acid and proline were found more in Karang Ploso Farm (1.83 ± 0.01) and (0.92 ± 0.00) while less in Mengani Farm (1.09 ± 0.13) and (0.31 ± 0.06) respectively. Glycine and arginine were maximum in CCF from Ijen Farm (1.54 ± 0.02) and (0.52 ± 0.00) while minimum in Mengani Farm (0.29 ± 0.14) and (0.02 ± 0.01) respectively. Lysine, tyrosine and valine were more in CCF from Karang Ploso Farm (0.64 ± 0.00), (0.31 ± 0.01) and (1.38 ± 0.01) while lower in La Boitê (0.26 ± 0.01), (0.09 ± 0.00) and (0.27 ± 0.01) respectively. Methionine was maximum in La Boitê (0.96 ± 0.01) and minimum in Ijen Farm (0.05 ± 0.03). The total amino acid content was higher in Karang Ploso (12.70 ± 0.00) and lower in Mengani Farm (7.34 ± 0.89).

**Received** | May 12, 2024; **Accepted** | June 11, 2024; **Published** | July 05, 2024
***Correspondence** | Damat Damat, Department of Food Science, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, Jl. Raya Tlogo Mas No. 246, Malang 65144, East Java, Indonesia; **Email:** damatumm@gmail.com
**Citation** | Setyobudi, R.H., S. Anwar, M.A. Wedyan, D. Damat, Y.A. Nugroho, T. Liwang, P.G. Adinurani, S.K. Wahono, E.S. Savitri, B.A. Prahardika, I.R. Utarid, I. Iswahyudi and H.A. Rachmawati. 2024. Characterization of amino acids in coffee cherry flour from different coffee cultivation areas: As potential functional food. *Sarhad Journal of Agriculture,* 39 (Special issue 1): 36-46.
**DOI** | https://dx.doi.org/10.17582/journal.sja/2023/39/s1.36.46
**Keywords** | Cascara, Circular economy, Coffee pulp, Environment friendly, Sustainable resource management, Waste to functional food

## Introduction

Coffee is one of the most widely consumed beverages worldwide, known for its stimulating effects and rich flavor. However, coffee processors generate a significant amount of waste material (Ballesteros *et al*., 2014; Corro *et al*., 2014; Das and Venkatachalapathy, 2016; Genanaw *et al*., 2021; Geremu *et al*., 2016; Li *et al*., 2023; Novita, 2016; Setyobudi *et al*., 2018, 2021, 2022), including solid waste, *i.e*., pulp (CP – from wet coffee method), or husk (CH – from dry coffee method). In recent years, efforts have been made to utilize this solid waste to create value-added products. CP and CH may employ coffee cherry tea or cascara (from Spanish cáscara, meaning husk or peel and pulp of a coffee cherry) with simple technology, especially for functional food. According to Ota (2018), cascara is a traditional beverage in Yemen (named Qishr), Ethiopia (Hashana), Bolivia (Sultana), and Costa Rica (Cáscara). Several researchers have also published on cascara studies (Ariva *et al*., 2020; Arpi *et al*., 2018; Heeger *et al*., 2017; Muzaifa *et al*., 2021; Nafisah and Widyaningsih, 2018; Novita *et al*., 2021; Pua *et al*., 2021; Rohaya *et al*., 2023; Zeckel *et al*., 2020). However, Setyobudi *et al*. (2019, 2021, 2022, 2023) concluded that it is more efficient and effective to recycle CP and CH to coffee cherry flour (CCF).

CCF is gaining attention as a potential functional food ingredient due to its high content of bioactive compounds, including polyphenols and dietary fiber (Corro *et al*., 2014; Damat *et al*., 2019; Reguengo *et al*., 2022; Setyobudi *et al*., 2019, 2022, 2023; Rohaya *et al*., 2023), which reported to possess various health benefits, such as antioxidant, anti-inflammatory and antimicrobial properties (Ballesteros *et al*., 2014; de Melo Pereira *et al*., 2020; de la Rosa *et al*., 2019; Dorsey and Jones, 2017; Geremu *et al*., 2016; Ifadah *et al*., 2021; Lestari *et al*., 2022) a attributed to the presence of phytochemicals, such as chlorogenic acids and melanoidins (Campos *et al*., 2021; Dorsey and Jones 2017; Janissen and Huynh, 2018).

Setyobudi *et al*. (2019, 2021, 2022) recommend using CCF as an iron (Fe) booster. Anemia is reported in high rankings, especially among adolescent girls and mothers in worldwide (Milman, 2011; Nurbadriyah, 2019) and in Indonesia (Bukhari *et al*., 2020; Ellie *et al*., 2012). Fe as a hemoglobin booster is also needed by people for auto-immunity and COVID-19 to increase their immune system (Das and Venkatachalapathy, 2016; Khadim and Al-Fartusie, 2023; Petek *et al*., 2020; Talebi *et al*., 2020). Several publications reported that the number of people with auto-immune diseases tends to grow globally (Illescas-Montes *et al*., 2019; Lerner *et al*., 2022; Miller, 2023). CCF will be of double benefit because it is a source of non-heme Fe, antioksidan, and gluten-free; a substance that auto-immune sufferers should avoid (Hansen *et al*., 2022; Illescas-Montes *et al*., 2019; Passali *et al*., 2020).

Several experts (Singh and Prasad, 2023; Shubham *et al*., 2020) stated that the absorption of non-heme Fe in the digestive tract of mammals is influenced by the presence of enhancing agents (also called boosters or promoters). One is an amino acid, which improves iron absorption by forming dissolved chelate (Fernández-Lázaro *et al*., 2020; Kanimozhi and Sukuma, 2023; Shubham *et al*., 2020). There are three types of amino acids, namely essential amino acids, semi-essential amino acids, and non-essential amino acids. Non-essential amino acids are amino acids that can be made in the body, also known as endogenous amino acids. In contrast, essential amino acids (indispensable amino acids) are amino acids that cannot be made in the body and can only be obtained by consuming foods that contain protein (Hubli *et al*., 2023; Winarno, 2008). There are 20 kinds of amino acids (Hubli *et al*., 2023; Trakalo *et al*., 2019) and several researchers (Bailey-Shaw *et al*., 2009; Bouafou *et al*., 2011; Penaloza *et al*., 1985; Tadese and Mebratu, 2017) stated that CP dry contains 17 various amino acids, with eight to nine are essential amino acids. Sera *et al*. (2000) stated that the amino acid composition in CP in the pulp was the same as in soybean (*Glycine max* (L.) Merr.) or Kapok (*Ceiba pentandra* (L.) Gaertn.) flour.

Setyobudi *et al*. (2021) compared amino acid levels of CCF Mengani, Bali, Indonesia to CCF-La Boite, a commercial product from Brazil. However, the manuscript reports the total amino acid levels, which needs to be more accurate because the important for health is essential amino acids, especially sulfuric amino acids, which act as boosters in the absorption of non-heme Fe (Andika *et al*., 2021; Trakalo *et al*., 2019; Kurniawati *et al*., 2022; Yuniastuti, 2014). Therefore, a comparative analysis of coffee flour from different sources is essential to understand the potential variations in its nutritional profile and health-promoting effects. This study aims to investigate the amino acid composition of coffee flour obtained from different coffee farms, including Ijen Farm at the side of Mount Ijen, Bondowoso, East Java; Karang Ploso Farm at the downhill Mount Arjuno, Malang, East Java; Mengani Farm at the slope of Mount Batur, Mengani, Bali and La Boitê, a commercial product created in Brazil - purchased from La Boite, a Manhattan business located at 724 11th Avenue, New York, NY 10019. Furthermore, this comparative analysis can contribute to the development of standardized production methods and quality control measures for CCF, ensuring consistency and reliability in its applications as a functional food ingredient.

## Materials and Methods

*Sample collection*

Samples of Arabica coffee cherry were collected from three different sources: 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), as CCF. Coffee cherries from three locations were dried to become cascara, then turned into powder with an electric coffee grinder K-500N (China). The powder is filtered by sieve size No. 80 mesh (local product). The filtered CCF is stored in a 7 cm × 10 cm CTIK plastic clip. Each sample was obtained in triplicate to ensure data reproducibility.

*Sample preparation and analysis*

**Sample preparation:** 1 g of CCF sample was weighed using an analytical balance laboratory- JA-3003 (China) and transferred it to a clean, dry container. The samples were properly labeled for identification.

**Sample extraction:** 10 mL of 0.1 M HCl (extraction solvent) was added to each container. The containers were sealed tightly and were vortexed for few minutes to ensure thorough mixing. The extraction was done according to method highlighted by Arpi *et al.* (2018).

**Filtration and derivatization:** The extracted sample solution was transferred to the filtration apparatus and filtered to remove any particulate matter or debris. The filtered sample solution was collected in a clean vial for further analysis. To derivatize, 10 µL of the sample and 10 µL of the internal standard were buffered to pH 8.8 in a total volume of 80 µL using borate buffer. The derivatization was initiated by addition of 20 µL reagent solution (6-amino-quinolyl-N-hydroxysuccinimidyl carbamate, AccQ. Flour, 3 mg mL$^{-1}$ in acetonitrile). All chemical using pro analysis grade, Merck.

**High-performance liquid chromatography (HPLC) analysis:** The samples were heated to 55 °C for 10 min and then analyzed by HPLC (PerkinElmer® LC 300, USA) with fluorescence detection. The HPLC system consisted of a quaternary pump, a vacuum degasser, a thermostated auto sampler, a thermostated column department and a fluorescence detector. A small volume (20 µL) of the derivatized sample solution was injected into the HPLC column. The HPLC system was ran using the appropriate mobile phase and gradient conditions suitable for amino acid separation. The elution of amino acids was monitored using UV detector and recorded the chromatographic data.

**Data analysis:** The HPLC data was analyzed to determine the retention times and peak areas of individual amino acids. The amino acid concentrations were quantified by comparing the peak areas of the samples with standard calibration curves prepared using known concentrations of amino acid standards. The concentration of individual amino acids were calculated in the CCF samples based on the peak areas and calibration curves. Analysis of variance (ANOVA) was performed to assess the significance of differences in amino acid levels among the CCF samples (Adinurani, 2016).

## Results and Discussion

Table 1 supports several previous researches (Bailey-Shaw *et al.*, 2009; Bouafou *et al.*, 2011; Penaloza *et al.*, 1985; Tadese and Mebratu, 2017), there are 17 kinds of amino acids in CP which are processed into CCF. Aspartic acid, serine, glutamic, glycine, histidine, arginine, threonine, alanine, proline, cysteine, tyrosine, valine, methionine, lysine, isoleucine, leucine, and phenylalanine were among the amino acids identified. Eight categories of essential amino acids, two categories of semi-essential, and seven categories of non-essential. Two types of sulfuric amino acids (cysteine and methionine) which play an important role in the absorption of non-heme Fe (Andika *et al.*, 2021; Trakalo *et al.*, 2019; Kurniawati *et al.*, 2022; Yuniastuti, 2014) were also found. A further study of ANOVA found the following findings:

**Table 1:** *Concentrations of different amino acids in Coffee Cherry Fluor from different sources.*

| Parameters | Source of coffee cherry fluor - CCF | | | | Anova test | |
|---|---|---|---|---|---|---|
| | Ijen farm | Karang ploso farm | Mengani farm | La Boitê | F value | p value |
| Asp | 0.61 ± 0.46 | 1.29 ± 0.01 | 0.56 ± 0.07 | 0.58 ± 0.01 | 2.36 | 0.213 ns |
| Ser | 0.33 ± 0.00 b | 0.26 ± 0.01 c | 0.16 ± 0.00 d | 0.80 ± 0.01 a | 2589.53 | 0.000 ** |
| Glu | 1.56 ± 0.01 ab | 1.83 ± 0.01 a | 1.09 ± 0.13 c | 1.37 ± 0.00 bc | 22.78 | 0.006 ** |
| Gly | 1.54 ± 0.02 a | 1.46 ± 0.01 a | 0.29 ± 0.14 c | 0.78 ± 0.01 b | 68.01 | 0.001 ** |
| His | 0.03 ± 0.03 b | 0.03 ± 0.03 b | 0.02 ± 0.01 b | 1.03 ± 0.01 a | 385.67 | 0.000 ** |
| Arg | 0.52 ± 0.00 a | 0.46 ± 0.01 ab | 0.02 ± 0.01 b | 0.03 ± 0.01 a | 7.11 | 0.044 * |
| Thr | 0.58 ± 0.00 b | 0.65 ± 0.02 ab | 0.27 ± 0.15 b | 1.05 ± 0.02 a | 17.55 | 0.009 ** |
| Ala | 0.63 ± 0.00 b | 0.65 ± 0.02 ab | 0.27 ± 0.15 b | 1.05 ± 0.02 a | 1.50 | 0.343 ns |
| Pro | 0.57 ± 0.01 b | 0.92 ± 0.00 a | 0.31 ± 0.06 c | 0.49 ± 0.00 b | 72.28 | 0.001 ** |
| Cys | 0.01 ± 0.00 b | 0.00 ± 0.00 b | 0.00 ± 0.00 b | 0.61 ± 0.04 a | 283.82 | 0.000 ** |
| Tyr | 0.31 ± 0.01 a | 0.31 ± 0.01 a | 0.24 ± 0.05 a | 0.09 ± 0.00 b | 19.8 | 0.007 ** |
| Val | 1.18 ± 0.19 ab | 1.38 ± 0.01 a | 0.67 ± 0.09 bc | 0.27 ± 0.01 c | 21.40 | 0.006 ** |
| Met | 0.05 ± 0.03 b | 0.08 ± 0.00 b | 0.32 ± 0.11 b | 0.96 ± 0.01 a | 55.72 | 0.001 ** |
| Lys | 0.55 ± 0.00 a | 0.64 ± 0.00 a | 0.31 ± 0.06 b | 0.26 ± 0.01 b | 37.97 | 0.002 ** |
| Ile | 0.54 ± 0.00 ab | 0.54 ± 0.01 ab | 0.43± 0.08 b | 0.72 ± 0.00 a | 9.02 | 0.030 * |
| Leu | 1.14 ± 0.05 | 1.59 ± 0.00 | 1.33 ± 0.04 | 0.75 ± 0.01 | 2.01 | 0.255 ns |
| Phe | 0.54 ± 0.00 a | 0.53 ± 0.00 a | 0.25 ± 0.03 b | 0.34 ± 0.01 b | 90.05 | 0.000 ** |
| Total AA | 10.65 ± 0.25 a | 12.70 ± 0.00 a | 7.34 ± 0.89 b | 10.73 ± 0.09 a | 23.14 | 0.005 ** |

*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. * Significantly different (α = 5 %). ** significantly different (α = 1 %). Ns, not significantly different.*

Table 1 provides data on various amino acids present in CCF obtained from different sources: Ijen Farm, Karang Ploso Farm, Mengani Farm and La Boitê. The analysis of the data showed that there is a significant 1 % in the 12 observed variables, a significant 5 % in the two observed variables, and a non-significant effect in the three observed variables.

Aspartic acid (Asp) did not show significant differences among the different sources of CCF. Non-essential amino acid aspartic acid is used in the production of proteins and the metabolism of other amino acids. The lack of variation in aspartic acid content among the sources could be attributed to the stability and uniformity in the processing methods used for CCF production across the farms (Clifford and Ramirez-Martinez, 1990).

Serine (Ser) content varied significantly, with the highest concentration found in La Boitê and the lowest in Mengani Farm. Serine is a metabolic amino acid that is conditionally necessary for a number of metabolic processes, including the generation of neurotransmitters and proteins (Dorsey and Jones, 2017). The observed variations in serine content could be attributed to factors such as genetic differences in coffee plant varieties, environmental conditions and post-harvest processing techniques used at different farms (Farah *et al*., 2006).

Glutamic (Glu) acid content differed significantly among the CCF with more concentration in Karang Ploso Farm and less in Mengani Farm. Glutamic acid is an important neurotransmitter and plays a crucial role in taste perception (Montavon *et al*., 2018). The differences in glutamic acid content may be influenced by coffee plant genetics, environmental conditions, and post-harvest processing, including drying methods (Pushpa *et al*., 2012), which may affected the amino acid composition of CCF.

Glycine (Gly) content showed significant differences among the sources, with maximum concentration in Ijen Farm and minimum in Mengani Farm. A non-essential amino acid called glycine is used in the production of proteins and a number of metabolic activities. The variations in glycine content may be affected by elements such as coffee plant genetics (Sunarharum *et al*., 2014), soil composition, and agricultural practices used at different farms

(Montavon *et al*., 2018).

Histidine (His) content exhibited significant altrations among the sources, with the highest concentration in La Boitê and the lowest in Mengani Farm. Histidine is an essential amino acid that plays a crucial role in protein synthesis, tissue repair, and the production of histamine and carnosine (Ariva *et al*., 2020). Variations in histidine content can be attributed to coffee plant genetics, soil composition (Cheng *et al*., 2016) and agricultural practices.

Arginine (Arg) content differed significantly among the different sources of coffee flour, with more concentration in Ijen Farm and less in Mengani Farm. An amino acid known as arginine is a semi-essential component of several physiological functions, including immune system operation, hormone control, and cardiovascular health (Muzaifa *et al*., 2021). Variations in Arginine content may be influenced by factors such as coffee plant genetics, soil composition, and agricultural practices.

Threonine (Thr) content varied significantly, with the maximum concentration in La Boitê and the minimum in Mengani Farm. Threonine is an essential amino acid necessary for protein synthesis and the maintenance of healthy skin and connective tissues (Ciaramelli *et al*., 2019). Differences in threonine content may be due to variation in coffee plant genetics, environmental conditions, and post-harvest processing methods (Farah *et al*., 2006).

Alanine (Ala) did not show significant differences among the sources of coffee flour. A non-essential amino acid called alanine is important in the production of glucose and energy metabolism (Winarno, 2008). The similarity of alanine level in all sources could be attributed to the common metabolic pathways and stability of this amino acid during CCF production (Clifford and Ramirez-Martinez, 1990).

Proline (Pro) content exhibited significant variations among the sources, with more concentration in Karang Ploso Farm and less in Mengani Farm. Proline is a non-essential amino acid that is used to make proteins and aids in cellular defense and stress response (Ginz and Engelhardt, 2000). Variations in Proline content can be ascribed to coffee plant genetics, environmental conditions, and post-harvest processing techniques (Farah *et al*., 2006).

Cysteine (Cys) content altered significantly among the sources of coffee flour, with maximum concentration in La Boitê and minimum in Karang Ploso Farm and Mengani Farm. Cysteine is a semi-essential amino acid and a precursor for the antioxidant compound glutathione. Variations in cysteine content can be influenced by elements such as coffee plant genetics, environmental conditions, and processing methods, including drying (Ludwig *et al*., 2000).

Tyrosine (Tyr) content differed significantly among the sources, with the highest concentration observed in Ijen Farm and Karang Ploso Farm, while the lowest concentration was found in La Boitê. A non-essential amino acid called tyrosine is used to make hormones and neurotransmitters (Clifford and Knight, 2004). Variations in tyrosine content of coffee sources may be due to differences in coffee plant genetics, environmental conditions, and post-harvest processing methods.

Valine (Val) content showed significant variations among the sources of CCF, with more concentration in Karang Ploso Farm and less in La Boitê. Valine is an essential amino acid used in the synthesis of proteins and the production of energy (Machado *et al*., 2020). Variations in valine content can be influenced by factors such as coffee plant genetics, soil composition, and agricultural practices.

Methionine (Met) content varied significantly among different CCF sources with the maximum concentration in La Boitê and the minimum in Ijen Farm. Methionine is an essential amino acid involved in the production of proteins and other metabolic activities. Differences in methionine content may be affected by elements such as coffee plant genetics, environmental conditions (Romano *et al*., 2021) and post-harvest processing techniques.

Lysine (Lys) content differed significantly among the sources of coffee flour, with the highest concentration in Karang Ploso Farm and the lowest in La Boitê. Lysine is an essential amino acid that is crucial for protein synthesis, immune system health, and collagen formation (Liu *et al*., 2021). The causes of variations in Lysine content may be coffee plant genetics, environmental conditions and agricultural practices.

Isoleucine (Ile) content showed significant variations among the different sources of coffee flour, with maximum concentration in La Boitê and minimum in Mengani Farm. Isoleucine is an essential amino acid that is involved in the production of proteins and the control of energy. Variations in isoleucine content may be the result of variation in coffee plant genetics, soil composition and agricultural practices (Tadese and Mebratu, 2017). Coffee plant genetics play an important role, as coffee varieties possess unique genetic traits that affect amino acid biosynthesis pathways. Additionally, soil composition influences nutrient availability, impacting the plant's ability to produce isoleucine. Agricultural practices, including fertilization techniques and pest management, can also influence the overall health and metabolic activity of coffee plants, consequently affecting isoleucine production (Machado *et al*., 2020).

Leucine (Leu) did not show significant differences among the different sources of CCF. Leucine is an essential amino acid that is involved in protein synthesis and is important for both muscle development and repair. The similar content of Leucine among the sources could be attributed to the common metabolic pathways and stability of this amino acid during CCF production (Clifford and Ramirez-Martinez, 1990). The stability of leucine during coffee production processes may contribute to its uniformity. Leucine resistance to degradation during roasting and other processing steps could result in its consistent levels (Rohaya *et al*., 2023). Furthermore, leucine shares metabolic pathways with other amino acids that might experience fluctuations due to environmental and processing factors. While coffee varieties and growth conditions influence amino acid compositions, leucine's essential role in biological processes might necessitate a more regulated synthesis, maintaining its levels across diverse sources (Jo *et al*., 2023).

Phenylalanine (Phe) content differed significantly among the sources of coffee flour, with the highest concentration in Ijen Farm and the lowest in Mengani Farm. Phenylalanine is an essential amino acid and a precursor for various neurotransmitters and hormones (Clifford and Knight, 2004). Variations in Phenylalanine content may be due to coffee plant genetics, environmental conditions and post-harvest processing techniques. The genetic variation can influence the enzymatic pathways responsible for phenylalanine biosynthesis (Cheng *et al*., 2016). Genetic traits can affect the expression of enzymes involved in the shikimate pathway, which is a metabolic pathway leading to the synthesis of phenylalanine and other aromatic compounds.

Total Amino Acids (Total AA) content varied significantly among the sources of coffee flour, with more concentration in Karang Ploso Farm and less in Mengani Farm. The total amino acid content reflects the overall composition of amino acids in CCF and is affected by a combination of factors such as coffee plant genetics, environmental conditions, agricultural practices, and post-harvest processing methods (Reguengo *et al*., 2022). Genetic variations can lead to differences in the abundance and composition of amino acids in coffee beans (Ciaramelli *et al*., 2019). The significant variation in the amino acid composition could also be attributed to the nutrient availability in the soil that directly affects amino acid synthesis as proper fertilization practices can provide the necessary nutrients for optimal growth and amino acid production. Girma *et al*. (2020) reported that the biochemical composition of the coffee varies with coffee varieties and growing altitudes. Furthermore, different coffee varieties have distinct genetic makeup, influencing their response to microclimate (Murkovic and Derle, 2006). Some varieties might have genetic traits that lead to higher biochemical production, better adaptation to altitude-related stress, or different pathways for producing secondary metabolites.

## Conclusions and Recommendations

The research work concludes that CCF from La Boite performed better as compared to CCF from other three locations of Indonesian (Ijen Farm, Karang Ploso Farm, and Mengani Farm). This finding must be followed up by studying why CCF from La Boite farm outperforms the others. Indonesia, being the fourth-largest coffee-exporting country in the world, primarily relies on smallholder coffee production. By learning the correct CCF preparation method will (i) minimize environmental pollution, (ii) provide gluten-free CCF with a low glycemic index and as a source of non-heme iron, (iii) reduces export of wheat flour (iv) boost the income of farmers as well as coffee stakeholders and (v) stimulate economic circulation.

## Acknowledgments

With sadness, the authors report Yogo Adhi Nugroho, one of the authors of this article, passed away after a fight against COVID-19. We sincerely appreciate his enthusiasm and dedication to the writing of this manuscript, especially the statistical analysis. The authors are grateful to Ricky Hendarto Setyobudi and Fitri Ramli, Arabica Coffee Factory at Mengani, Kintamani, Bali, Soni Sisbudi Harsono, University of Jember, and Pandu Prabowo, Kopi Carlos, Malang for supplying the CCF as research material. Also, thank you to Yohannes Kohar Whisnu Wardhana, which has brought to Indonesia six CCF packages from La Boite, a store at Manhattan, 724 11th Avenue New York, NY 10019. The authors thank the Directorate of Research and Community Service, University of Muhammadiyah Malang (DPPM – UMM), which funded this research with letter No. E.2a/811/BAA-UMM/viii/2023.

## Novelty Statement

Prior studies (Bailey-Shaw et al., 2009; Bouafou et al., 2011; Penaloza et al., 1985; Tadese and Mebratu, 2017) have documented the presence of amino acids in coffee pulp (CP). However, these earlier investigations focused solely on CP from a single coffee cultivation area. This current research shows novelty because it compares amino acids in CP coffee cherry fluor (CCF) from three distinct coffee cultivation areas of Indonesia with commercially available CCF from Brazil. Furthermore, this research establishes a link between the presence of amino acids and their potential role in boosting hemoglobin levels to combat anemia.

## Author's Contribution

**Roy Hendroko Setyobudi:** Conceptualized and designed the study, elaborated the intellectual content, performed literature search, manuscript preparation, and manuscript revision.
**Shazma Anwar:** Performed literature search, manuscript preparation, and manuscript revision.
**Damat Damat:** Research supervision, and elaborated the intellectual content.
**Tonny Liwang and Irma Rahmaita Utarid:** Amino acid analysis.
**Yogo Adhi Nugroho and Praptingsih Gamawati Adinurani:** Statistical analysis.
**Satriyo Krido Wahono, Mohammed Ali Wedyan, Evika Sandi Savitri, and Bayu Agung Prahardika:** Performed the literature search and manuscript review.
**Iswahyudi Iswahyudi and Hemalia Agustin Rachmawati:** Administration, Turnitin check, Grammar check, rewrite.

*Conflict of interest*
The authors have declared no conflict of interest.

## References

Adinurani, P.G., 2016. Design and analysis of agrotrial data: Manual and SPSS. Plantaxia, Yogyakarta, Indonesia

Adriana F. and J. de Paula Lima. 2019. Consumption of chlorogenic acids through coffee and health implications. Beverages, 5 (11): 1–29. https://doi.org/10.3390/beverages5010011

Andika, A., F. Kusnandar and S. Budijanto. 2021. Physicochemical and sensoric characteristics of high protein multigrain analog rice. J. Teknol. Ind. Pangan, 32(1): 60–71.

Ariva A.N., A. Widyasanti and S. Nurjanah. 2020. The effect of rying temperature to the quality of cascara tea from Arabica pulp (*Coffea arabica*). J. Teknol. Ind. Pertan. Indonesia, 12(1): 21–28. https://doi.org/10.17969/jtipi.v12i1.15744

Arpi, N., H.P. Rasdiansyah, Widayat and R.F. Foenna. 2018. Utilization of Arabica coffee (*Coffea arabica* L.) pulp waste into coffee pulp juice with the addition of lime (*Citrus aurantifolia*) and lemon (*Citrus limon*) juice: A feasibility study. Jurnal Teknologi dan Industri Pertanian Indonesia., 10(02): 33–39. https://doi.org/10.17969/jtipi.v10i2.12593

Bailey-Shaw, Y.A., K.D. Golden, A.G.M. Pearson and R.B.R. Porter. 2009. Characterization of Jamaican agro-industrial wastes. Part I: Characterization of amino acids using HPLC: Pre-column derivatization with phenylisothiocyanate. J. Chromatogr. Sci., 47(8): 674–680. https://doi.org/10.1093/chromsci/47.8.674

Ballesteros, L.F., J.A. Teixeira and S.I. Mussatto, 2014. Chemical, functional, and structural properties of spent coffee grounds and coffee silverskin. Food Bioprocess. Technol., 7: 3493–3503. https://doi.org/10.1007/s11947-014-1349-z

Bouafou, K.G.M., A.B. Konan, V. Zannou-Tchoko and Kati-Coulibally. 2011. Potential food waste and by-products of coffee in animal. Feed J. Biol., 7(4): 74–80.

Bukhari, A., H. Firdaus, M. Rahmawati, S.N.

Sheryl and M.C. Prisilia. 2020. Non-nutritional and disease-related anemia in Indonesia: A systematic review. Asia Pac. J. Clin. Nutr. (Suppl.). 29: S.41–S.54.

Campos, R.C., V.R.A. Pinto, L.F. Melo, S.J.S. da Rocha and J.S. Coimbra. 2021. New sustainable perspectives for coffee wastewater and other by-products: A critical review. Future Foods 4(100058): 1–10. https://doi.org/10.1016/j.fufo.2021.100058

Cheng, B., A. Furtado, H.E. Smyth and R.J. Henry. 2016. Influence of genotype and environment on coffee quality. Trends Food Sci. Technol., 57: 20–30. https://doi.org/10.1016/j.tifs.2016.09.003

Ciaramelli, C., A. Palmioli and C. Airoldi. 2019. Coffee variety, origin and extraction procedure: implications for coffee beneficial effects on human health. Food Chem., 278: 47–55. https://doi.org/10.1016/j.foodchem.2018.11.063

Clifford, M.N. and J.R. Ramirez-Martinez. 1990. Chlorogenic acids and their derivatives in coffee. In coffee: Botany, Biochemistry, and Production of Beans and Beverage, pp. 145-171.

Clifford, M.N. and S. Knight. 2004. The cinnamoyl–amino acid conjugates of green robusta coffee beans. Food Chem., 87(3): 457–463. https://doi.org/10.1016/j.foodchem.2003.12.020

Corro, G., U. Pal and S. Cebada. 2014. Enhanced biogas production from coffee pulp through deligninocellulosic photocatalytic pretreatment. Energy Sci. Eng., 2(4): 177–187. https://doi.org/10.1002/ese3.44

Damat, D., R. Angriani, R.H. Setyobudi and P. Soni. 2019. Dietary fiber and antioxidant activity of gluten-free cookies with coffee cherry flour addition. Coffee Sci., 14(4): 493–500. https://doi.org/10.25186/cs.v14i4.1625

Das, A. and N. Venkatachalapathy. 2016. Profitable exploitation of coffee pulp. A review. Int. J. Appl. Nat. Sci., 5(1): 75–82

de la Rosa, L.A., J.O. Moreno-Escamilla, J. Rodrigo-Garcia and E. Alvarez-Parrilla. 2019. Phenolic compounds. In: Yahia, E. and Carrillo-Lopez, A., (Eds.). Chapter 12. Postharvest Physiology and Biochemistry of Fruits and Vegetables, 1st edition. Elsevier, Netherland. pp. 253–271. https://doi.org/10.1016/B978-0-12-813278-4.00012-9

de Melo Pereira, G.V., D.P.C. Neto, A.I. Magalhães Júnior, F.G. Prado, M.G.B. Pagnoncelli, S.G. Karp and C.R. Soccol. 2020. In: Toldrá F (Ed.). Chapter 3 - chemical composition and health properties of coffee and coffee by-products. Adv. Food Nutr. Res., 91: 65–96. https://doi.org/10.1016/bs.afnr.2019.10.002

Dorsey, B.M. and M.A. Jones. 2017. Healthy components of coffee processing by-products. Chapter 2. In: Galanakis CM (Ed.). Handbook of Coffee Processing By-Products Sustainable Applications. Academic Press, Cambridge, Massachusetts, USA. pp. 27–62. https://doi.org/10.1016/B978-0-12-811290-8.00002-5

Ellie S.S., K. Sun, S. de Pee, K. Kraemer, R. Jee-Hyun, R. Moench-Pfanner, M. Sari, M.W. Bloem and R.D. Semba. 2012. Relationship of maternal knowledge of anemia with maternal and child anemia and health-related behaviors targeted at anemia among families in Indonesia. Matern. Child Health J., 16: 1913–1925. https://doi.org/10.1007/s10995-011-0938-y

Farah, A., M.C. Monteiro, V. Calado, A.S. Franca, and L.C. Trugo. 2006. Correlation between cup quality and chemical attributes of Brazilian coffee. Food Chem., 98(2): 373–380. https://doi.org/10.1016/j.foodchem.2005.07.032

Fernández-Lázaro, D., J. Mielgo-Ayuso, A. Córdova Martínez and J. Seco-Calvo. 2020. Iron and physical activity: Bioavailability enhancers, properties of black pepper (Bioperine®) and potential applications. Nutrients, 12(1886): 1–12. https://doi.org/10.3390/nu12061886

Genanaw, W., G.G. Kanno, D. Derese and M.B. Aregu. 2021. Effect of wastewater discharge from coffee processing plant onriver water quality, Sidama region, south Ethiopia. Environ. Health Insights, 15: 1–12. https://doi.org/10.1177/11786302211061047

Geremu M., Y.B. Tola and A. Sualeh. 2016. Extraction and determination of total polyphenols and antioxidant capacity of red coffee (*Coffea arabica* L.) pulp of wet processing plants. Chem. Biol. Technol., 3(25): 1–6. https://doi.org/10.1186/s40538-016-0077-1

Ginz, M. and U.H. Engelhardt, 2000. Identification of proline-based diketopiperazines in roasted coffee. J. Agric. Food Chem., 48(8): 3528–3532. https://doi.org/10.1021/jf991256v.

Girma, B., A. Gure and F. Wedajo, 2020. Influence of altitude on caffeine, 5-caffeoylquinic acid, and nicotinic acid contents of Arabica coffee varieties. J. Chem. Article ID 3904761: 1–7.

https://doi.org/10.1155/2020/3904761

Hansen, C.H.F., C.S. Larsen, L.F. Zachariassen, C.M.J. Mentzel, A. Laigaard, L. Krych, D.S. Nielsen, A. Gobbi, M. Haupt-Jorgensen, K. Buschard and A.K. Hansen. 2022. Gluten-free diet reduces autoimmune diabetes mellitus in mice across multiple generations in a microbiota-independent manner. J. Autoimmun., 127: 102795. https://doi.org/10.1016/j.jaut.2022.102795

Heeger, A., A. Kosińska-Cagnazzo, E. Cantergiani and W. Andlauer. 2017. Bioactives of coffee cherry pulp and its utilisation for production of cascara beverage. Food Chem., 221: 969–975. https://doi.org/10.1016/j.foodchem.2016.11.067

Hubli, G.B., S. Banerjee, and A.S. Rathore. 2023. Near-infrared spectroscopy-based monitoring of all 20 amino acids in mammalian cell culture broth. Talanta, 254: 124187. https://doi.org/10.1016/j.talanta.2022.124187

Ifadah, R.A., P.R.W. Wiratara and C.A. Afgani. 2021. Scientific review: Anthocyanins and their health benefits. J, Teknol. Pengolahan Pertan., 3 (2): 11–21. https://doi.org/10.35308/jtpp.v3i2.4450

Illescas-Montes, R., Melguizo-Rodríguez, L., Ruiz, C. and Costela-Ruiz, V.J., 2019. Vitamin D and autoimmune diseases. Life Sci., 233: 116744. https://doi.org/10.1016/j.lfs.2019.116744

Janissen, B. and T. Huynh. 2018. Chemical composition and value-adding applications of coffee industry by-products: A review. Resour. Conserv. Recycl., 128: 110–117. https://doi.org/10.1016/j.resconrec.2017.10.001

Jo, A., H. Park, S. Lee and K.G. Lee. 2023. Improvement of Robusta coffee aroma with l-leucine powder. J. Sci. Food Agric., 103(7): 3501–3509. https://doi.org/10.1002/jsfa.12485

Kanimozhi, N.V. and M. Sukumar. 2023. Stability and bio accessibility of heme iron from *Glycine max* and *Vigna unguiculata* for the enhancement strategies in yoghurt fortification. J. Agric. Food Res., 12(100596): 1–7. https://doi.org/10.1016/j.jafr.2023.100596

Khadim, R.M. and F.S. Al-Fartusie. 2023. Evaluation of some trace elements and antioxidants in sera of patients with rheumatoid arthritis: A case–control study. Clin. Rheumatol., 42: 55–65. https://doi.org/10.1007/s10067-022-06324-7

Král, E., J.L. Rukov and A.C. Mendes. 2023. Coffee cherry on the top: Disserting valorization of coffee pulp and husk. Food Eng. Rev., https://doi.org/10.1007/s12393-023-09352-4

Kurniawati, I., M. Fitriyya and Wijayanti. 2022. Treating anemia with Moringa leaf flour. Yuma Pustaka, Surakarta, Indonesia.

Lerner, A., J.F. de Carvalho, A. Kotrova and Y. Shoenfeld. 2022. Gluten-free diet can ameliorate the symptoms of non-celiac autoimmune diseases. Nutr. Rev., 80(3): 525–543. https://doi.org/10.1093/nutrit/nuab039

Lestari, W., K. Hasballah, M.Y. Listiawan and S. Sofia. 2022. Coffee byproducts as the source of antioxidants: A systematic review. F1000 Res.. 11(220): 1–12. https://doi.org/10.12688/f1000research.107811.1

Li, Z., B. Zhou, T. Zheng, C. Zhao, Y. Gao, W. Wu, Y. Fan, X. Wang, M. Qiu and J. Fan. 2023. Structural characteristics, rheological properties, and antioxidant and anti-glycosylation activities of pectin polysaccharides from Arabica coffee husks. Foods, 12(423): 1–16. https://doi.org/10.3390/foods12020423

Liu, X., X. Chen, T. Lin, B. Yin, Q. Li, L. Wang, J. Shao and J. Yang. 2021. The level variation of Nε-(carboxymethyl) lysine is correlated with chlorogenic acids in Arabica L. Coffee beans under different process conditions. Food Chem., 343: 128458. https://doi.org/10.1016/j.foodchem.2020.128458

Ludwig, E., U. Lipke, U. Raczek and A. Jäger. 2000. Investigations of peptides and proteases in green coffee beans. Eur. Food Res. Technol. 211: 111–116. https://doi.org/10.1007/PL00005518

Machado, S., A.S. Costa, F. Pimentel, M.B.P. Oliveira and R.C. Alves. 2020. A study on the protein fraction of coffee silverskin: Protein/non-protein nitrogen and free and total amino acid profiles. Food Chem., 326: 126940. https://doi.org/10.1016/j.foodchem.2020.126940

Miller FW. 2023. The increasing prevalence of autoimmunity and autoimmune diseases: an urgent call to action for improved understanding, diagnosis, treatment, and prevention. Curr. Opin. Immunol., 80, 102266. https://doi.org/10.1016/j.coi.2022.102266

Milman N., 2011. Anemia still a major health problem in many parts of the world! Ann Hematol 90: 369–377. https://doi.org/10.1007/s00277-010-1144-5

Montavon, P., E. Duruz, and F.S. Liverani. 2018.

Coffee. In Handbook of Food Chemistry, pp. 669-691.

Murkovic, M. and K. Derler. 2006. Analysis of amino acids and carbohydrates in green coffee. J. Biochem. Biophys. Methods, 69(1–2): 25–32. https://doi.org/10.1016/j.jbbm.2006.02.001

Muzaifa M., F. Rahmi and Syarifudin. 2021. Utilization of coffee by-products as profitable foods - A mini review. IOP Conf. Ser. Earth Environ. Sci., 672(012077): 1–9. https://doi.org/10.1088/1755-1315/672/1/012077

Nafisah, D. and T.D. Widyaningsih. 2018. Study of drying method and brewing ratio in process of making cascara tea from Arabica coffee (*Coffea arabika* L.). J. Pangan Agroindustri. 6(30): 37–47. https://doi.org/10.21776/ub.jpa.2018.006.03.5

Novita, E., D. Khotijah, Purbasari and H.A. Pradana. 2021. The application of cleaner production in Wulan coffee agroindustry, Maesan sub district, Bondowoso regency. J. Teknik Pertan. Lampung, 10(2): 263–273. https://doi.org/10.23960/jtep-l.v10i2.263-273

Novita, E., 2016. Biodegradability simulation of coffee wastewater using instant coffee. Agric. Agric. Sci. Procedia., 9: 217–229. https://doi.org/10.1016/j.aaspro.2016.02.138

Nurbadriyah, W.D., 2019. Iron deficiency anemia. Deepublish publisher, Yogyakarta, Indonesia.

Ota, K., 2018. Coffee as a global beverage before 1700. J. Int. Econ. Stud., 3: 43–55.

Passali, M., K. Josefsen, J.L. Frederiksen and J.C. Antvorskov. 2020. Current evidence on the efficacy of gluten-free diets in multiple sclerosis, psoriasis, type 1 diabetes and autoimmune thyroid diseases. Nutrients, 12(8): 2316. https://doi.org/10.3390/nu12082316

Peñaloza, W., M.R. Molina, R.G. Brenes and R. Bressani. 1985. Solid-state fermentation: An alternative to improve the nutritive value of coffee pulp. Appl. Environ. Microbiol., 49(2): 388–393. https://doi.org/10.1128/aem.49.2.388-393.1985

Petek, E.T., S.A. Gómez-Ochoa, E. Llanaj, P.F. Raguindin, L.Z. Rojas, Z.M. Roa-Díaz, D. Salvador Jr, D. Groothof, B. Minder, D. Kopp-Heim, W.E. Hautz, M.F. Eisenga, O.H. Franco, M. Glisic and T. Muk. 2020. Anemia and iron metabolism in COVID-19: A systematic review and meta-analysis. Eur. J. Epidemiol., 35: 763–773. https://doi.org/10.1007/s10654-020-00678-5

Pua, A., W. Xian, D. Choo, R.M.V. Goh, S.Q. Liu, M. Cornuz, K.H. Ee, J. Sun, B. Lassabliere and B. Yua. 2021. A systematic study of key odourants, non-volatile compounds, and antioxidant capacity of cascara (dried *Coffea arabica* pulp). LWT 138 (110630): 1–13. https://doi.org/10.1016/j.lwt.2020.110630

Pushpa S.M., M.R. Manjunatha, G. Sulochannama and M. Naidu. 2012. Extraction, characterization and bioactivity of coffee anthocyanins. Eur. J. Biol. Sci., 4(1): 13-19.

Reguengo, L.M., M.K. Salgaço, K. Sivieri and M.R.M. Júnior. 2022. Agro-industrial by-products: Valuable sources of bioactive compounds. Food Res. Int., 152 (110871). https://doi.org/10.1016/j.foodres.2021.110871

Rohaya, S., Multahadi and I. Sulaiman. 2023. Improving the quality of kombucha cascara with different varieties and fermentation time in diverse Arabica coffee (*Coffea arabica* L) cultivars. Coffee Sci., 17(e172056): 1–8. https://doi.org/10.25186/.v17i.2056

Romano, N., H. Fischer, V. Kumar, S.A. Francis and A.K. Sinha. 2021. Productivity, conversion ability, and biochemical composition of black soldier fly (*Hermetia illucens*) larvae fed with sweet potato, spent coffee or dough. Int. J. Trop. Insect Sci., 42(1): 1–8. https://doi.org/10.1007/s42690-021-00532-5

Sera, T., C.R. Soccol, A. Pandey and S. Roussos. 2000. Coffee biotechnology and quality. Proceedings of the 3rd International Seminar on Biotechnology in the Coffee Agro-Industry, Londrina, Brazil. Springer. pp. 536. https://doi.org/10.1007/978-94-017-1068-8

Setyobudi RH, S.K. Wahono, P.G. Adinurani, A. Wahyudi, W. Widodo, M. Mel, Y.A. Nugroho, B. Prabowo and T. Liwang. 2018. Characterisation of Arabica coffee pulp - hay from Kintamani - Bali as prospective biogas feedstocks. MATEC Web Conf., 164 (01039): 1–13. https://doi.org/10.1051/matecconf/201816401039

Setyobudi, R.H., L. Zalizar, S.K. Wahono, W. Widodo, A. Wahyudi, M. Mel, B. Prabowo, Y. Jani, Y.A. Nugroho, T. Liwang and A. Zaebudin. 2019. Prospect of Fe non-heme on coffee flour made from solid coffee waste: Mini review. IOP Conf. Ser. Earth Environ. Sci., 293(012035): 1–24. https://doi.org/10.1088/1755-1315/293/1/012035

Setyobudi, R.H., D. Damat, S. Anwar, A. Fauzi, T.Liwang, L. Zalizar, Y.A. Nugroho, M.A. Wedyan, M. Setiawan, S. Husen, D. Hermayanti, T.D.N. Subchi, P.G. Adinurani, E.D. Septia, D. Mariyam, I.R. Utarid, I. Ekawati, R. Tonda, E.D. Purbajanti, S. Suherman, M.S. Susanti, T.A. Pakarti, I. Iswahyudi, B.A. Prahardika and A.R. Farzana. 2023. Amino acid profiles of coffee cherry flour from different origins: A comparative approach. E3S Web Conf., 432 (00032): 1–11. https://doi.org/10.1051/e3sconf/202343200032

Setyobudi, R.H., E. Yandri, Y.A. Nugroho, M.S. Susanti, S.K. Wahono, W. Widodo, E.A. Saati, M. Maftuchah, M.F.M. Atoum, M.I. Massadeh and D. Yono. 2021. Assessment on coffee cherry flour of Mengani Arabica coffee, Bali, Indonesia as iron non-heme source. Sarhad J. Agric., 37(1): 171–183. https://doi.org/10.17582/journal.sja/2022.37.s1.171.183

Setyobudi, R.H., M.F.M. Atoum, D. Damat, E. Yandri, Y.A. Nugroho, M.S. Susanti, S.K. Wahono, W. Widodo, A. Wahyudi, E.A. Saati and M. Maftuchah, 2022. Evaluation of coffee pulp waste from coffee cultivation areas in Indonesia as iron booster. Jordan J. Biol. Sci., 15(3): 475–488. https://doi.org/10.54319/jjbs/150318

Shubham, K., T. Anukiruthika, S. Dutta, A.V. Kashyap, J.A. Moses, and C. Anandharamakrishnan. 2020. Iron deficiency anemia: A comprehensive review on iron absorption, bioavailability and emerging food fortification approaches. Trends in Food Sci. Technol., 99: 58–75. https://doi.org/10.1016/j.tifs.2020.02.021

Singh, P. and S. Prasad. 2023. A review on iron, zinc and calcium biological significance and factors affecting their absorption and bioavailability. J. Food Compos. Anal.123, 105529. https://doi.org/10.1016/j.jfca.2023.105529

Sunarharum, W.B., D.J. Williams and H.E. Smyth. 2014. Complexity of coffee flavor: A compositional and sensory perspective. Food Res. Int. 62: 315–325. https://doi.org/10.1016/j.foodres.2014.02.030

Tadesse, M., and M. Adamu. 2017. Design and development of biogas production system from waste coffee pulp and its waste water around Tepi. Dev. Eng. Technol., 6(1): 18–30

Talebi, S., Ghaedi, E., Sadeghi, E., Mohammadi, H., Hadi, A., Clark, C.C.T. and Askari, G. 2020. Trace element status and hypothyroidism: A systematic review and meta-analysis. Biol. Trace Elem. Res., 197: 1–14. https://doi.org/10.1007/s12011-019-01963-5

Trakalo, T., O. Shapovalenko and T. Yaniuk. 2019. Amino acid content in extruded feed mixtures. Ukr. Food J. Sci., 7(1): 92–99. https://doi.org/10.24263/2310-1008-2019-7-1-11

Winarno, F.G., 2008. Food chemistry and nutrition. Gramedia Pustaka Utama, Jakarta, Indonesia. pp. 112

Yuniastuti, A., 2014. Micromineral nutrition and health. Unnes Press, Semarang, Indonesia.

Zeckel, S., P.C. Susanto and N.M.D. Erfiani. 2020. Market potential of cascara tea from Catur village Kintamani Bali. Proceeding I-CFAR (International Conference on Fundamental and Applied Research. Bali, Indonesia, 7 October 2019. pp. 331–338.