

Composition of amino acids and fatty acids on Luwak coffee processing

¹Fitri, ²Laga, A., ³Dwyana, Z. and ^{2,*}Tawali, A.B.

¹Agricultural Science Study Program, Postgraduate School of Hasanuddin University, Perintis Kemerdekaan Street Km. 10 Tamalanrea, Makassar, 90245, Indonesia.

²Food Science and Technology Study Program, Department of Agricultural Technology, Faculty of Agriculture, Hasanuddin University, Perintis Kemerdekaan Street Km. 10 Tamalanrea, Makassar, 90245, Indonesia.

³Department of Biology, Faculty of Math and Science, Hasanuddin University, Perintis Kemerdekaan Street Km. 10 Tamalanrea, Makassar, 90245, Indonesia.

Article history:

Received: 7 November 2020

Received in revised form: 20 December 2020

Accepted: 22 February 2021

Available Online: 10 May 2021

Keywords:

Amino acid,
Fatty acid,
Fermentation,
Luwak coffee,
Roasting

Abstract

The processing carried out on coffee beans such as fermentation and roasting can affect the contents of amino and fatty acids of coffee beans. This study aimed to determine the amount of amino acid and fatty acid content in Luwak coffee bean during processing. The amino acids of coffee were analyzed using HPLC, while fatty acids of coffee were analyzed using GC-MS. The results showed a change in total amino acid content in raw coffee bean (3.04%), green bean coffee (6.93%), and roasted coffee (6.83%). The total fatty acid of raw coffee bean (1199.86 mg/100 g), green bean coffee (3147.56 mg/100 g), and roasted coffee (6282.4 mg/100 g) also experienced significant changes.

DOI:

[https://doi.org/10.26656/fr.2017.5\(3\).637](https://doi.org/10.26656/fr.2017.5(3).637)

1. Introduction

Coffee is one beverage that is popular among people across the world. That drink comes from the processing of coffee plants. Although not an indigenous plant in Indonesia, coffee plants have long been cultivated in Indonesia and become one of the largest sources of foreign exchange earnings. Coffee cultivation and processing are very developed in Indonesia. Various types and varieties of coffee can be produced in Indonesia. Luwak coffee is one Indonesian coffee that is very well known and become one of the most expensive coffees in the world (Marcone, 2011; Jumhawan *et al.*, 2013).

Luwak coffee is a coffee that is obtained by an unusual process. Luwak coffee is produced from various metabolic processes that take place in the palm civet (known as Luwak in Indonesia) digestion. Luwak coffee is favoured by society because of the distinctive taste that other coffee does not have (Panggabean, 2011; Fitri *et al.*, 2019).

Flavour is the main quality of a coffee. All processing carried out on coffee since it was planted

until brewed caused several changes in chemical components that affected the flavour of coffee (Sunarharum *et al.*, 2014). One important component that influences the taste of coffee is amino acids. The quality of the taste or colour of the coffee brewed is strongly influenced by the amount and type of amino acids contained in the coffee. Amino acids are one of the principal flavour precursors in the coffee bean (Poisson *et al.*, 2009). During the processing, the amino acid content in coffee beans can experience an increase or decrease and transform mainly in the process of roasting coffee beans (Homma, 2001). In addition to the amino acid component, the fat component in coffee beans is also an essential component in coffee beans. Components of fat and fatty acids often affect the perceived aroma, taste of food, and mouthfeel on some types of food (McClements and Decker, 2017).

Research to evaluate the quality of Luwak coffee flavour has been carried out. However, information regarding the composition of fatty acids and amino acids as a coffee flavour precursor is still rare. This study was aimed to determine how many changes in the composition of several types of amino acids and fatty

*Corresponding author.

Email: abubakar_tawali@unhas.ac.id

acids in coffee beans during the Luwak coffee processing.

2. Materials and methods

2.1 Materials

The coffee used in this study is a type of Arabica coffee from the Enrekang, South Sulawesi, Indonesia. The moisture content in the raw coffee bean is around 55-60% (Ghosh and Venkatachalapathy, 2014). Luwak coffee beans were obtained from Luwak breeding in Malino, South Sulawesi, Indonesia. Harvested Luwak coffee is washed clean using running water. After that, the coffee beans are dried using sunlight until the moisture content reaches 12-13%, and green bean coffee is obtained. Green bean coffee is then roasted at 180°C for 15 mins to produced roasted coffee.

2.2 Amino acids content analysis

The amino acid in the sample was analyzed by the method used by the Integrated Laboratory of Bogor Agricultural Institute (IK.LP-04.7-LT-1.0). The samples were analyzed using HPLC instrument. The sample to be analyzed is hydrolyzed first. The hydrolyzed sample was dissolved in 5 mL of 0.01 N HCl then filtered with millipore paper. Potassium borate buffer pH 10.4 has been added with a ratio of 1: 1. In a clean empty vial, 50 µL of the sample and 250 µL of OPA reagent was added. The solution was left for 1 min so that the derivatization takes place completely. Next, 5 µL was injected into the HPLC column, and then waited until the separation of all amino acids was complete. After that, the percentage of amino acids in the sample is calculated.

2.3 Fat content analysis

The measurement of fat content was carried out using the extraction method. A total of 10 g of the sample was weighed and placed on cotton-coated paper. The sample was then put into the Soxhlet apparatus and extracted using hexane solvent for 6 hrs. The fat extract was dried in an oven at 105°C. The fat extract has then been cooled and weighed until constant weight was achieved.

2.4 Fatty acid content analysis

Fatty acid levels in the sample were carried out using the AOAC method (2012). Before being injected into chromatography, the sample has been derivatized to obtain fatty acid methyl ester (FAME). As much as 20 mg of fat from the previously extracted coffee beans was put into a teflon-covered tube. As much as 1 mL of 0.5 N methanol NaOH has been added and exhaled with nitrogen gas for 15 s, has been shaken tightly for 20

mins. Then, 2 mL of 20% BF₃ (Boron trifluoride) was added and heated again for 20 mins. After that the tube was cooled. A total of 2 mL of saturated NaCl and 1 mL of hexane was added to the tube and then shaken. After forming the two layers, the hexane layer was transferred to a tube of 0.1 g anhydrous Na₂SO₄ and left for 15 mins. The liquid phase has been separated, and the organic phase has been injected into the gas chromatograph.

The sample has been analyzed using a capillary column. As much as 1 µL of the standard FAME mixture was injected. When all the peaks had been removed, 1 µL of the prepared FAME sample was injected into the column. The retention and peak of each component has been measured. Sample retention times were compared with standards to obtain information about the type of components in the sample. The percentage of fatty acids in the sample was calculated.

2.5 Data processing

The data obtained in the study were processed with one way ANOVA with three replications. Duncan test at a confidence level of 0.05 (p < 0.05) was used to see the level of difference in each sample.

3. Results and discussion

3.1 Amino acids

The amino acid composition is an essential precursor to the aroma's formation of coffee beans. The result showed that Luwak coffee has a quite complete amino acid component (Table 1). There are 15 components of essential amino acids (lysine, histidine, threonine, leucine, methionine, phenylalanine, valine, isoleucine,) and non-essential (glutamic acid, aspartic acid, serine, alanine, glycine, arginine, tyrosine) in Luwak coffee beans. Glutamic acid is the highest amino acid in coffee beans, either on raw coffee bean (0.64%), green bean (1.51%), and roasted coffee bean (1.87%), while the lowest number of amino acid is methionine (0.01% on raw coffee bean, 0.04% on green beans). Where all these amino acid components experienced a significant increase compared to raw coffee beans except methionine.

Changes in the total amino acid content of Luwak coffee at each stage of the processing can be seen in Figure 1. The total amino acid content in coffee beans generally increases during wet processing. This increase occurred due to the fermentation process in coffee beans (Zhang *et al.*, 2019). Based on Figure 1, the fermentation process of Luwak coffee can also increase the total amino acid, such as the wet process of coffee bean in general. The total amino acid content of raw coffee beans

Table 1. Amino acids content of Luwak coffee during the processing

Asam Amino	Raw Bean (%)	Green Bean (%)	Roasted Coffee (%)
Aspartic acid	0.34±0.007 ^a	0.76±0.028 ^b	0.77±0.001 ^b
Glutamic acid	0.64±0.028 ^a	1.51±0.113 ^b	1.87±0.007 ^c
Serine	0.19±0.007 ^a	0.34±0.028 ^b	0.29±0.021 ^b
Histidine	0.08±0.001 ^a	0.19±0.021 ^b	0.16±0.001 ^b
Glycine	0.21±0.014 ^a	0.46±0.014 ^b	0.45±0.042 ^b
Threonine	0.14±0.007 ^a	0.25±0.014 ^b	0.24±0.001 ^b
Arginine	0.18±0.007 ^a	0.41±0.064 ^b	0.08±0.001 ^a
Alanine	0.16±0.001 ^a	0.36±0.021 ^b	0.41±0.007 ^c
Tyrosine	0.09±0.014 ^a	0.30±0.071 ^b	0.25±0.007 ^b
Methionine	0.01±0.014 ^a	0.04±0.035 ^a	0 ^a
Valine	0.20±0.007 ^a	0.46±0.021 ^b	0.54±0.001 ^c
Phenylalanine	0.20±0.007 ^a	0.41±0.021 ^b	0.49±0.007 ^c
I-leucine	0.16±0.007 ^a	0.37±0.007 ^b	0.43±0.001 ^c
Leucine	0.29±0.007 ^a	0.67±0.014	0.79±0.014 ^c
Lysine	0.19±0.007 ^a	0.43±0.014 ^b	0.09±0.028 ^c

Values followed by different letter in the same row are significantly different at $p < 0.05$

and green bean coffee resulting from the metabolism of Luwak experienced a significant increase. As long as in digestion, coffee beans experience a variety of degradation in the chemical composition caused by the metabolic process of mongoose animals. Marcone (2004) found that the penetration of proteolytic enzymes found in gastric acids such as pepsin or trypsin into the coffee beans from Luwak metabolism. The proteolytic enzymes then perform the breakdown of proteins into simpler components so that the protein content of fermented beans decreases and its amino acid content increases sharply.

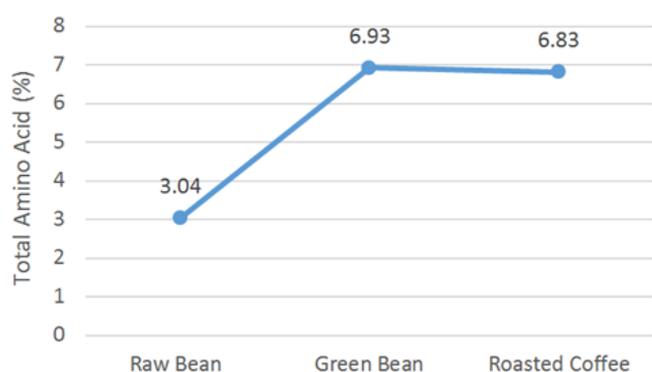


Figure 1. Composition of total amino acids in Luwak coffee beans during the processing

Increasing the number of amino acids in the fermented coffee beans can also be caused by microorganisms found in the digestion of Luwak animals. Research results of Muzaifa *et al.* (2018) found that there were groups of lactic acid bacteria that were proteolytic in the digestion of the civet. The group of lactic acid bacteria is able to break down the protein components contained in coffee beans into simpler component such as amino acids. In the roasting process,

the transformation of amino acid components into flavour components through the Maillard reaction. This reaction can occur if the temperature of roasting coffee beans is right. Hernández *et al.* (2007) suggested that heat transfer is an important factor in the roasting process. Perfect roasting occurs in coffee beans at temperatures above 160°C.

3.2 Fatty acids

The fatty acids content in Luwak coffee beans is very diverse. The amount of Luwak coffee's fatty acid can be seen in Table 2. The highest fatty acid content in Luwak coffee beans is linoleic acid (551.08 mg/100 g on raw coffee bean, 1386.64 mg/100 g on green bean, 2901.65 mg/100 g on roasted coffee), while the lowest is caprylic acid (0.98 mg/100 g on green bean, 2.72 mg/100 g on roasted coffee). Luwak coffee beans contain many components of unsaturated fatty acids such as palmitoleic acid, linoleic acid, arachidonic acid, oleic acid, and linolenic acid where these fatty acids are types of fatty acids that have a good impact on health (Sartika, 2008).

The fat content and total fatty acid composition of raw coffee beans, after fermentation, and roasting experienced a significant increase (Figures 2 and 3). The increase of these content in Luwak fermented coffee beans is expected to be caused by the metabolic processes experienced by coffee beans in the digestion of Luwak animals. The research result of Joët *et al.* (2010) showed that there was an increase in the amount of fat content of coffee beans in wet processing. The increase in fat content is followed by an increase in the content of fatty acids, which are constituents of fat components

Table 2. Fatty acid content of Luwak coffee in the processing (mg/100 g)

Fatty Acid	Raw Bean	Green Bean	Roasted
Caprylic acid, C8:0	0 ^a	0.98±0.043 ^b	2.72±0.046 ^c
Lauric Acid, C12:0	0.29±0.019 ^a	1.06±0.022 ^a	12.12±5.095 ^b
Myristic Acid, C14:0	2.14±0.190 ^a	3.72±0.022 ^a	10.15±1.389 ^b
Pentadecanoic Acid, C15:0	0.71±0.057 ^a	1.33±0.151 ^b	2.03±0.093 ^c
Palmitic Acid, C16:0	430.41±0.948 ^a	1182.54±1.947 ^b	2262.70±0.463 ^c
Palmitoleic Acid, C16:1	1.22±0.019 ^a	1.24±0.022 ^a	2.29±0.463 ^b
Heptadecanoic Acid, C17:0	1.54±0.095 ^a	4.41±0.043 ^b	7.27±0.093 ^c
Stearic Acid, C18:0	72.56±2.179 ^a	203.03±1.515 ^b	365.49±7.410 ^c
Oleic Acid, C18:2n6c	68.68±2.179 ^a	192.78±1.298 ^b	412.98±4.168 ^c
Linoleic Acid, C18:3n3	551.08±4.453 ^a	1386.64±6.708 ^b	2901.65±9.263 ^c
Arachidonic Acid, C20:0	32.70±1.137 ^a	87.36±0.649 ^b	145.41±6.484 ^c
Cis-11-Eicosenoic Acid, C20:1	2.88±0.284 ^a	8.72±0.216 ^b	15.07±1.853 ^c
Linolenic Acid, C18:3n3	19.10±0.663 ^a	28.46±0.433 ^b	78.93±1.389 ^c
Heneicosanoic Acid, C21:0	0.87±0.095 ^a	2.75±0.433 ^b	3.96±0.046 ^c
Cis-11, 14-Eicosadienoic Acid,	0.60±0.095 ^a	1.68±0.216 ^b	2.95±0.463 ^c
Behenic Acid, C22:0	10.32±0.379 ^a	22.95±0.433 ^b	33.08±1.389 ^c
Tricosanoic Acid, C23:0	0.95±0.019 ^a	2.46±0.022 ^b	3.96±0.046 ^c
Lignoceric Acid, C24:0	3.82±0.284 ^a	15.45±3.678 ^b	18.01±3.242 ^b

Values followed by different letter in the same row are significantly different at $p < 0.05$

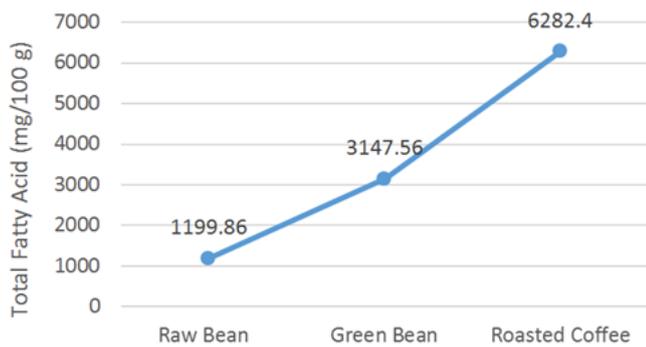


Figure 2. Total fatty acid composition of Luwak coffee beans during the processing

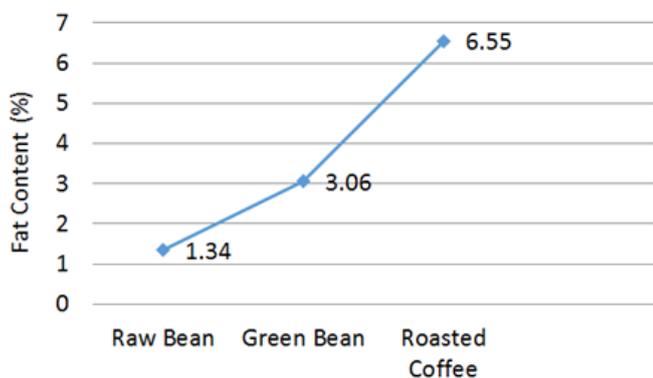


Figure 3. Fat Content of Luwak coffee beans during the processing

Both fat and fatty acids content of Luwak coffee also increase significantly during the roasting process (Figure 2 and Figure 3). During this process, several chemical reactions occur in the coffee beans. Budryn *et al.* (2012) showed there is an increase in fat components in coffee

after roasting. During the roasting process, pyrolysis occurs which affects the texture of the coffee beans. This can facilitate the penetration of solvents into coffee beans and extract fat from the inside (Yuwanti *et al.*, 2016). This causes an increase in the fat and fatty acids content of the roasted coffee.

4. Conclusion

Components of amino acids and fatty acids in coffee beans can change during processing. The fermentation process which takes place in the Luwak animal digestion can increase the composition of amino acids and fatty acids of coffee beans. The appropriate roasting process can cause an increase in the fatty acid composition and decrease the amino acid content due to the Maillard reaction, which is a process that plays a role in the formation of aroma and taste in coffee beans.

Acknowledgements

This research was supported by the Directorate of Research and Community Service, the Ministry of Research and Technology of Indonesia.

References

AOAC (2012). Official Methods of Analysis of AOAC International. 19th ed., p. 27-29. Maryland, USA: AOAC International Press.

- Budryn, G., Nebesny, E., Żyżelewicz, D., Oracz, J., Miśkiewicz, K. and Rosicka-Kaczmarek, J. (2012). Influence of roasting conditions on fatty acids and oxidative changes of Robusta coffee oil. *European Journal of Lipid Science and Technology*, 114(9), 1052–1061. <https://doi.org/10.1002/ejlt.201100324>.
- Fitri, Tawali, A.B. and Laga, A. (2019). Luwak coffee *In Vitro* fermentation: literature review. *IOP Conference Series: Earth and Environmental Science*, 230(1), 012096. <https://doi.org/10.1088/1755-1315/230/1/012096>.
- Hernández, J.A., Heyd, B., Irlles, C., Valdovinos, B. and Trystram, G. (2007). Analysis of the heat and mass transfer during coffee batch roasting. *Journal of Food Engineering*, 78(4), 1141-1148. <https://doi.org/10.1016/j.jfoodeng.2005.12.041>.
- Homma, S. (2001). Non-volatile compounds. In Clarke, R.J. and Vitzthum, O.G. (Eds.). *Coffee recent development*, p. 50-67. London: Blackwell Science.
- Joët, T., Laffargue, A., Descroix, F., Doulebeau, S., Bertrand, B., de Kochko, A. and Dussert, S. (2010). Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. *Food Chemistry*, 118(3), 693-701. <https://doi.org/10.1016/j.foodchem.2009.05.048>.
- Jumhawan, U., Putri, S.P., Yusianto, Y., Marwani, E., Bamba, T. and Fukusaki, E. (2013). Selection of discriminant marker for authentication of Asian palm civet coffee (kopi Luwak): a metabolomics approach. *Journal of Agricultural and Food Chemistry*, 61(33), 7944-8001. <https://doi.org/10.1021/jf401819s>.
- Marcone, M.F. (2004). Composition and properties of Indonesian palm civet coffee (kopi Luwak) and Ethiopian civet coffee. *Food Research International*, 37(9), 901-912. <https://doi.org/10.1016/j.foodres.2004.05.008>.
- Marcone, M.F. (2011). Origins and compositional analysis of novel foods: kopi Luwak coffee and bird's nest soup. In Moo-Young, M. (Ed.). *Comprehensive Biotechnology*. 2nd ed. p. 683-702. Netherlands: Elsevier B.V. <https://doi.org/10.1016/B978-0-08-088504-9.00319-6>
- McClements, D.J. and Decker, E.A. (2017). *Lipids*. Fennema's Food Chemistry. 5th ed., p. 171-234. London: CRC Press.
- Muzaiifa, M., Hasni, D., Patria, A., Febriani, and Abubakar, A. (2018). Sensory and microbial characteristics of civet coffee. *International Journal on Advanced Science, Engineering and Information Technology*, 8, 165–171. <https://doi.org/10.18517/ijaseit.8.1.3092>
- Panggabean, E. (2011). *Dredging the Benefits of the Luwak coffee business*. Jakarta, Indonesia: AgroMedia Pustaka, Jakarta.
- Poisson, L., Schmalzried, F., Davidek, T., Blank, I. and Kerler, J. (2009). Study on the role of precursors in coffee flavour formation using in-bean experiments. *Journal of Agricultural and Food Chemistry*, 57(21), 9923–9931. <https://doi.org/10.1021/jf901683v>
- Sartika, R.A.D. (2008). Effects of saturated, non-saturated, and trans-fatty acid on health (In Indonesian). *Jurnal Kesehatan Masyarakat Nasional*, 2, 154-160. <https://doi.org/10.21109/kesmas.v2i4.258>
- Sunarharum, W.B., Williams, D.J. and Smyth, H.E. (2014). Complexity of coffee flavour: A compositional and sensory perspective. *Food Research International*, 62, 315–325. <https://doi.org/10.1016/j.foodres.2014.02.030>
- Yuwanti, S., Yusianto and Nugraha, T.C. (2016). Characteristics of coffee oil produced from various roasting temperatures. Prosiding Seminar Nasional APTA 26-27 October 2016. Jember, Indonesia.
- Zhang, S.J., De Bruyn, F., Pothakos, V., Torres, J., Falconi, C., Moccand, C., Weckx, S. and De Vuyst, L. (2019). Following coffee production from cherries to cup: Microbiological and metabolomic analysis of wet processing of *Coffea arabica*. *Applied and Environmental Microbiology*, 85(6), 1–22. <https://doi.org/10.1128/AEM.02635-18>