

PAPER • OPEN ACCESS

Amino acid and sensory profile of *Kopi Luwak* (Civet Coffee)

To cite this article: M Muzaifa *et al* 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **523** 012028

View the [article online](#) for updates and enhancements.

Amino acid and sensory profile of *Kopi Luwak* (Civet Coffee)

M Muzaifa^{1,2,*}, D Hasni², D Yunita², Febriani³, A Patria², A Abubakar⁴

¹ Doctoral Study Program of Agricultural Science, Syiah Kuala University, Darussalam, Banda Aceh, 23111, Indonesia

² Department of Agricultural Product Technology, Faculty of Agriculture, Syiah Kuala University, Darussalam, Banda Aceh, 23111, Indonesia

³ Department of Chemistry, Faculty of Mathematics and Natural Science, Syiah Kuala University, Darussalam, Banda Aceh, 23111, Indonesia

⁴ Department of Animal Husbandry, Faculty of Agriculture, Syiah Kuala University, Darussalam, Banda Aceh, 23111, Indonesia

E-mail: murnamuzaifa@unsyiah.ac.id

Abstract. *Kopi luwak* (civet coffee) is produced exclusively from coffee beans excreted by Indonesian palm civet or luwak (*Paradoxurus hermaphroditus*) and relatively has a high price. The aim of this research was to identify amino acid compositions of *kopi luwak* (green and roasted beans), its changing forms and its correlation to sensory profile. The coffee beans of *kopi luwak* were collected from six locations in Gayo Highland, Aceh-Indonesia. Amino acid compositions were analyzed by LC-MS and sensory profiles were performed using a cupping test based on Specialty Coffee Association of America (SCAA) procedures. The result showed that green and roasted beans had significant differences on their Alanine, Tyrosine, Cysteine and Arginine contents. Valine, Glutamine, Aspartic acid and Glutamic acid are considered as the main amino acids which have higher percentage in both green and roasted bean. Several amino acids are absence in green bean but presence in roasted bean or vice versa, such as alanine and arginine. Glutamic acid content was the highest among others, both in green bean and roasted bean. The sensory profile of civet coffee showed that all samples from various origins/sources were classified as specialty coffee because the obtained total score of cupping was above 80.

1. Introduction

Coffee is one of the most commercialized agricultural product and considered as the most widely consumed beverage in the world. Coffee is drunk by millions of people every day and has attracted a considerable attention among consumer world-wide mainly because of its health benefits and sensory quality, especially its specific flavor [1-3].

The flavor of brewed coffee is determined by pre and postharvest factors [4], [5]. Numerous investigations with respect to the influence of the “from farm to fork practices” such as species, origin, coffee processing methods which turns out coffee cherries to green coffee bean roasting, and brewing have been published [6-8]. The research has acknowledged that the coffee processing method is

*Corresponding author : murnamuzaifa@unsyiah.ac.id



known as one of the most important aspect that can influence coffee flavor. In coffee bean processing, farmers usually applies three common ways, which include wet fermentation/washed methods, semi dry (washed) and dry fermentation (natural) processing methods [9], [10]. However, there is an uncommon process called 'digestive bio-processing' which is a fermentation step. In some countries like Indonesia, this fermentation of green coffee bean occurs in the gastrointestinal tract of *luwak*, Indonesian name for civet (*Paradoxorus hermaphroditus*), and the product is named as civet coffee or *kopi luwak* [5], [11], [12].

Kopi luwak is known as one of the most expensive coffee available today. This coffee is very hard to find, since its produced only 500-700 kg per year [13]. The price for a single cup of *kopi luwak* is \$35-\$80 and one pound of beans costs \$100 to \$600 (<http://www.most-expensive.coffee>). People have been discussing this rare coffee because of its exclusive harvesting process and originality. The coffee is made from coffee cherries that have been eaten by civet and pass through its digestive system. However, civet is unable to digest the beans and excretes them with its faeces and these excreted beans are collected to make *kopi luwak* [13], [14]. *Kopi luwak* quality is mainly contributed by two factors: selection of the best (the ripest) coffee cherries by civet and chemical changes that occur in the digestive tract of civet which produces specific aromatic chemical compounds [12], [15]. In detail, [16] stated out that caged luwak that regularly fed up with papaya and water melon tends to produce *kopi luwak* with papaya-aroma like, whilst *kopi luwak* from the wild one, tend to have more complex aroma since the wild luwak that able to look up their feeding by themselves.

The diversity of chemical compounds are responsible for the flavor and aroma of coffee. Most chemical studies were carried out on coffee related to chlorogenic acids, caffeine, trigonelline, nicotinic acids, sugar, lipid and volatiles [17]–[20]. The intensive studies on amino acids of green and roasted coffee are yet to be carried out especially for *kopi luwak*. Amino acids have important contributions to beverage quality because they serve as precursors to coffee aroma and flavors. Then during the roasting process these amino acids take part in Maillard reactions resulting various components that contribute to flavour and color of coffee brew [8], [21]–[23]. According to [24], green coffee beans contain a variety of free amino acids where these amino acids are largely transforming during roasting. To our knowledge, there is no study that has investigated the amino acids composition of kopi luwak and its correlation to its sensory profile. Therefore, this study aimed to analyze amino acid compositions of kopi luwak in both green and roasted coffee bean and its correlation to its sensory profile.

2. Materials and Methods

2.1. Samples Preparation

Six samples of *wild kopi luwak* were obtained from six cultivation areas, four from Bener Meriah and two from Central Aceh. The samples are collected in June-July 2017. The detail location for each sample could be seen in Table 1. The coffee species used in this study was *Coffee arabica*.

Collected coffee beans were cleaned and washed from the *luwak* feces with flowing water, and dried to reach moisture content of 12%. Furthermore, the dried coffee beans were manually dehulled to produce green coffee beans. A half of the green beans were roasted at a medium degree based on the Specialty Coffee Association of America (SCAA) cupping protocol standard method [25] one day prior to analysis. Both, the green beans (unroasted beans) and roasted coffee beans, were stored in a vacuum container at ambient temperature prior to analysis. Prior to analysis, both coffee beans (green and roasted) are grounded with coffee grinder where the resulted particle size should able to passing through US standard 20 mesh (approximately 70-75% of the particles).

Table 1. List of coffee samples

Sampels	Origin/source
A	Arul Badak Bener Meriah, Aceh-Indonesia
B	Jejem 1 Central Aceh, Aceh-Indonesia
C	Wih Pongas Bener Meriah, Aceh-Indonesia
D	Blang Panas Bener Meriah, Aceh-Indonesia
E	Jejem 2 Central Aceh, Aceh-Indonesia
F	Kenawat Redelong Bener Meriah, Aceh-Indonesia

2.2. Amino Acid Analysis

The procedure of amino acid analysis referred to [8] with a slightly modification. Coffee sample was first acid hydrolyzed to convert the proteins into their constituent amino acids. About 0.1 g of ground coffee sample was transferred to a 15-mL glass tube with sealer and 10 mL of HCl 6M was then added. The mixture was heated under vacuum at 110°C for 22 h. The solvent was then evaporated until the solution became a thick liquid of extract. The extract then was re-soluted with 3 ml ultrapure water and centrifuged at 13.000 rpm for 10 minutes. The supernatant was ready to be injected to the UPLC-QToF-MS/MS system (Waters, Massachusetts, USA) equipped with Acquity UPLC BEH C18 column to perform amino acid analysis. The mobile phase consisted of a mixture of 0.1% pentadecafluorooctanoic acid (PDFOA) and 0.1% formic acid in H₂O/Acetonitrile (99.5%/0.5%) (A) and 0.1% pentadecafluorooctanoic acid (PDFOA) and 0.1% formic acid in H₂O/Acetonitrile 10%/90% (B). The injector volume was 5 μ L and the flow rate was 0.4 mL/min. The amino acid content of the samples was quantified by comparing their retention times and peak area with these of standard curves. The amino acid content was expressed as milligram amino acid per 100 g DW of coffee beans. All solvents used were HPLC grade.

2.3. Sensory Analysis (Cupping Test)

Evaluation of sensory profile of coffee was carried out using the cupping test, a method published by [25], was commonly used as a standard protocols to measure ten coffee sensory attributes which are fragrance, aroma, flavor, aftertaste, body, acidity, uniformity, clean cup, sweetness and overall characteristics of beverages. Briefly, the procedure started with roasting 100 g kopi luwak (green bean) in medium degree (65 Agtron Scale) at one day prior to cupping test take place. The coffee then grounded and the examination was started by preparing five glass for each samples. Each glass contained 8.25 g of ground coffee and then 150 ml of water at a temperature range of 93-95°C were poured into the uncovered cup of coffee. After 4-5 minutes, the assessment starts with length of time approximately 20-30 minutes.

The steps of the procedure of cupping evaluation were started evaluation of fragrance and aroma. The dry fragrance should be examined during the first 15 minutes. Fragrance score is obtained by lifting the lid to sniff the coffee powder. Then aroma is valued by infusing the coffee grounds with water, the appeared crusts on the surface left unbroken for approximately 3-5 minutes. Then the beverage is stirred three times to break out the crust. Sniffing the aroma is done with spooning the liquor with the back of the spoon, which allows the foam to run down the cup. The score for fragrance is then marked by dry grounds evaluation whilst the aroma by the liquid drinks. Then it continued to flavour, aftertaste, acidity, body, and balance evaluation. It took place when the coffee liquor temperature reached to 160°F (71°C) which is commonly occurred roughly 8-10 minutes from the infusion. The process starts by aspirating the liquor into the mouth in a special way in order to the liquor covers the palatable area as large as possible, especially the tongue and upper palate. By doing so, the retronasal vapors are at its maximum intensity at these elevated temperatures, where flavor and aftertaste are marked at this point. Then as the liquor temperature decreases (160°-140°F), acidity, body, and balance are followed. Then sweetness, uniformity, and cleanliness are evaluated in order as

the coffee liquor reaches room temperature (below 100°F) and should be over when the temperature of the sample down to 70°F (21°C). For this evaluation, one sample is placed on five different cups. Then each coffee grader should examine on each individual cup, the maximum marks for each cup are 2 points (total 10 points for each sample). Overall refers to the holistic reflection of integrated rating of samples as perceived by the coffee grader. The overall score is given after the coffee grader assessed all attributes and combined these as "cupper's points". As similar as sweetness, uniformity, and cleanliness, overall is measured by awarding 2 points per cup where coffee grader is assessed 5 cups or each coffee samples in order to reach 10 points maximum scores. Defects refer as negative or poor flavors, which detect during cupping test. Defects divided into two categories, which is taint and fault. Taint is score -2 meaning that this score is given to sample, which has off-flavor, but not overwhelming. Taint commonly notice in aromatic aspects. Another one is the fault, as an off-flavor which cause unpalatable. Defects mostly found in taste aspects, since it affects all cupping attributes, the score is -4. When the evaluation is over, all the scores are marked on the standard SCAA cupping form. The final score is based on the flavor experience of the individual assessor as single judges.

The panellist, named as Q-grader in this descriptive sensory measurement is three certified and trained panellist who professionally do the cupping test at regular basic. Since all the protocols refers to [25], the test form based on its standard and scale used in this cupping test ranged between 6 and 9 (good to outstanding quality). The protocols stated out that to be classified as specialty coffee, the 6 point should be absent and the total score must exceed 80.

2.4 Data Analysis

The collected data was tabulated and processed with Microsoft Excell Version 2010. All data were expressed as mean $\pm$ standard deviation. For amino acids compositions, statistical significance was assessed using one-way analysis of variance (ANOVA) from two replications for each sample and continued with a Least Significant Different (LSD) Test ($P \leq 0.05$). For correlation between the amino acid contents and cupping test results, the statistical measurement was conducted using 2015 released SmartPLS v.3.2.3. (SmartPLS GmbH).

3. Results and Discussion

3.1. Amino Acid Compounds

From Table 2, it could be seen that from all amino acids presented in coffee bean, only four amino acids were significantly different ($P \leq 0.05$) between green bean and roeasted bean. Accordingly, alanine, cysteine and arginine in green bean were statistically lower than those in roasted bean, whereas tyrosine was significantly lower in roasted bean than green bean.

Table 2 also presents the types of amino acids available in green bean and roasted bean of *kopi luwak*. The amino acids are classified based on their R side chains, which in detail are divided into six groups. Aliphatic amino acids are amino acids which has non polar R group and hydrophobic sides [26]. For *kopi luwak* green bean, glycine and alanine are not detectable, but after roasting process alanine is present in small contents. In this group, Valine has the highest value followed by Proline in green and roasted bean. Isoleucin presents for both green and roasted bean in neglected amount. This group with Proline specifically is responsible for produce pyrolles, pyridines by reacting with Maillard intermediates during roasting [23].

The second explained group is aromatic amino acids, which classified as well as non-polar R-group. The amount for three mentioned amino acids, Phenylalanine, Tyrosine and Tryptophan are closely in range from 0.418 to 0.467 mg/100 g of green bean. However, in this group, the contents of all amino acids are down to half of it and even disappeared after roasting.

Table 2. Amino acids in green and roasted bean of *kopi luwak* (mg/g of bean)

No.	Amino Acids	Green Bean		Roasted Bean	
1	Aliphatic Amino acids with R Non Polar and aliphatic				
	a. Glycine	0	±0.00	0.000	±0.00
	b. L-Alanine*	0	±0.00	0.562	±0.2
	c. L-Proline	0.758	±0.36	1.100	±0.64
	d. L-Valine	1.998	±0.91	2.022	±0.82
	e. L-Leucine	0.773	±0.30	0.588	±0.34
	f. L-Isoleucine	0.230	±0.08	0.168	±0.06
2	Aromatic amino acids				
	a. L-Phenylalanine	0.465	±0.19	0.280	±0.24
	b. L-Tyrosine*	0.418	±0.09	0.000	±0.00
	c. L-Tryptophan	0.467	±0.29	0.237	±0.12
3	Amino acids with ionized R Polar				
	a. Asparagine	0.083	±0.06	0.265	±0.21
	b. L-Glutamine	1.088	±0.37	1.177	±0.61
	c. L-Serine	0.605	±0.21	0.685	±0.16
4	d. Threonine	0.638	±0.27	0.742	±0.26
	Sulfuric amino acids				
	a. Methionine	0.085	±0.06	0.285	±0.27
5	b. Cysteine*	0.133	±0.04	0.815	±0.33
	Amino acids with R negative ion (acid)				
	a. Aspartic acid	1.493	±0.33	1.858	±0.65
6	b. Glutamic acid	2.102	±0.37	2.460	±0.92
	Amino acids with R Positive ion				
	a. L-Arginine*	0.525	±0.26	0.000	±0.00
6	b. L-Lysine	0.162	±0.06	0.190	±0.08
	c. Histidine	0.283	±0.12	0.153	±0.05

Note: Mean values of amino acid followed by an asterick (*) showed significant differences ($P \leq 0.05$).

The third group is amino acids with ionized R group and also has polar side which makes this group hydrophilic [26]. In this group, Glutamine has the highest contains and considers among the major amino acids in coffee bean. In this group, majority of amino acids values are not change even after the roasting process, except for Asparagine. Asparagine presents in trace amounts in green bean (0.083 mg/g) and being higher after roasted (0.265 mg/g). For sulfuric amino acids group, Methionine and Cysteine are belong to this classification. Sulfuric amino acids considers as aromatic amino groups [26]. [23] stated that this group is bound with protein in stage of green bean. Then this amino acid degrades during roasting and interact with reducing sugars and Maillard intermediates to perform intensely aromatic volatiles such as furfurylthiol.

Moreover, amino acids group with positive ion such as Arginine, Lysine and Histidine tend to have less value after roasting process. Even Arginine, which considered as amino acids with highest contains in this group, has zero value after roasting. This finding is similar with [27]. Lastly to discuss is amino acids with negative ion and considers as acid, such as aspartic acid and glutamic acid. Both of these are reported to be the most common amino acids found in coffee and has significantly influenced

during coffee flavor formation. These compounds shares major parts of amino acid compositions, for both green and roasted coffee bean.

3.2. Sensory Quality of Kopi Luwak

The sensory quality of *kopi luwak* is done by cupping test. The protocol system evaluates ten attributes sequentially which are aroma/fragrance, flavor, aftertaste, acidity, body, balance, sweetness, clean cup, uniformity and overall. For sweetness, clean cup, and uniformity, the scores were all 10 meaning that these three sensory attributes were in their best characteristic levels (Data were not shown in Table 3). The cupping test score of *kopi luwak* could be seen in Table 3. In Table 3 only seven attributes are measured its correlation with amino acids composition. For sweetness, clean cup and uniformity attributes, Gayo coffee has specific characteristics which has maximum score in these attributes. Therefore, these three attributes are ignored since all the six samples have similar scores.

Table 3. Cup quality of *kopi luwak*

Sample	Sensory Attributes Measured by SCAA Cupping Test Standard							Cup Score Total
	Fragrance	Flavor	Aftertaste	Acidity	Body	Balance	Overall	
A	7.80	8.00	7.80	7.50	8.00	7.80	7.80	84.50
B	8.00	7.80	7.80	7.50	8.00	8.00	7.80	84,75
C	8.00	7.80	7.50	7.50	8.00	7.80	7.80	84,00
D	8.00	8.00	7.80	7.50	7.80	7.50	8.00	84,75
E	8.00	8.00	8.00	7.80	8.00	7.80	8.00	85,75
F	7.80	7.50	7.80	7.50	8.00	8.00	7.80	83,75
Average	7.93	7.85	7.78	7.55	7.97	7.82	7.87	84.50

Table 3 shows that avarage value of sensory attributes (fragrance, balance, aftertaste, acidity, body, balance and overall) reaches very good quality scale and classified as specialty coffee. Coffee, which categorized the specialty coffee, should have a total score above 80.00. The highest total score belongs to sample E (85.75), whilst the lowest is sample F (83.75) with avarage 84,50. Compared to previous research [16] who reported that wild palm civet coffee has total score 85.25, sampel E considers as excellent quality, whilst the others samples are a coffee specialty with very good classification. Based on Table 3 as well, aftertaste and acidity of E are in excellent quality scale and also the highest among others. Acidity is categorized as aroma and taste sensations, describe as delicate and crisp, lush and rich, powerfully tart but sweet at same time. On the other hand, aftertaste or alternatively named as mouthfeel is flavor that lingers after the liquor is swallowed [25], [28]. Compared to another sample, F has the lowest value of flavor among other samples. Flavor is main quality standard in cupping test, which holistically combination aroma and taste, where aroma perceived by volatile compounds inhale through the nose [29] with basic taste perceived by tongue receptors [30].

3.3. Correlation the amino acids and the cup quality of green bean of kopi luwak (Civet Coffee)

Correlation of amino acids and cup quality of *kopi luwak* is could be seen in Figure 1. The data presents in Table 2 statistically correlated with the amino acids contents in Table 1 and performed by Smart PLS v 3.2.3.

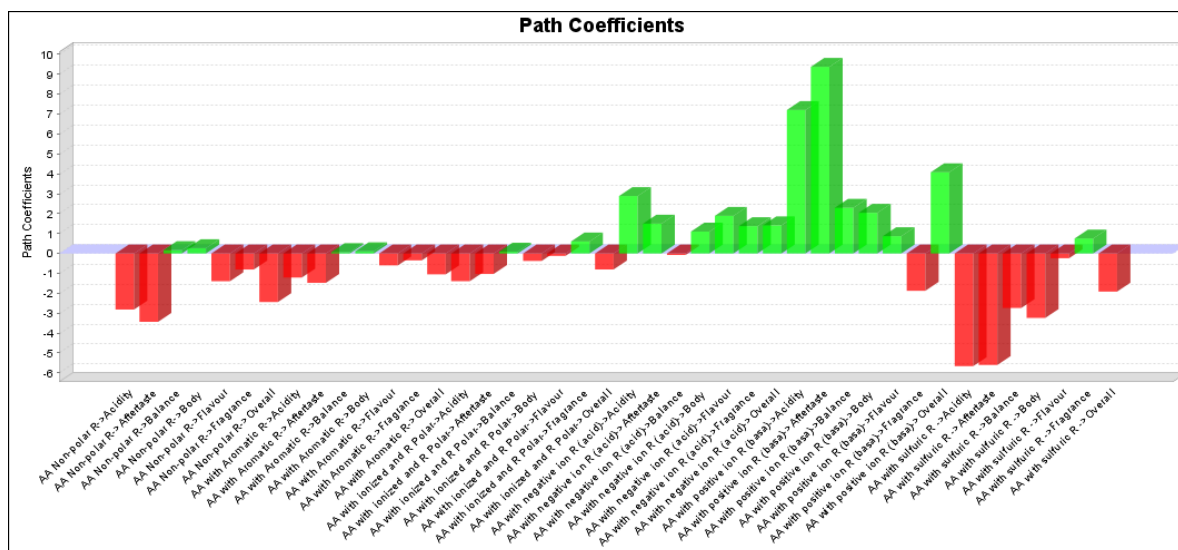


Figure 1. Path Coefficients based on Partial Least Square Result based on contents of amino acids in green bean and the Cupping quality of *kopi luwak*.

In Figure 1, it can be shown the correlation within the amino acids compounds based on its R function with the sensory profile of *kopi luwak*. Even in SCAA cupping protocols, ten attributes are evaluated, here we are only perform the correlation within seven attributes (fragrance, flavor, aftertaste, acidity, body, balance and overall) since the other three presented the similar value over the varied samples. Figure 1 shows that most of amino acid groups performs negative correlation towards the cupping sensory, except amino acids with ionized polar group, amino acids with negative ion R group and positive ion R group which are assumed to have positive correlation based on its bar color and positive value of path coefficients.

First to discuss is correlation within the non-polar group with body and balance attributes. Many reports have been made in terms of the significance of non-polar group which has the hydrophobic sites in their molecules, such as L-Alanine, L-Valine, L-Leucine, and L-Isoleucine towards bitterness [31], [32]. In this cupping test scope, body refers to the combination of bitterness and astringency, whilst balance defines as how well the equilibrium between aftertaste, body and acidity in one cup of coffee. In this study, L-Glycine and L-Alanine are absence in green bean, but L-Valine as the major amino acid presents in this group, shows strong correlation among other amino acid compounds ($R^2=0.984$). Therefore it can be stated that L-Valine, continue with L-Leucine and L-Isoleucine performs the body and balance attributes in coffee. [33] reported that the presence of Valine, Leucine, Glycine, Alanine in peptides also influence the bitter taste since these are considered as binding determinants.

For fermentation product like coffee, the metabolic activity of responsible microorganisms during fermentation will classify food quality, generates taste and flavor as well as the food acceptance [32], [34]. Usually, the presence of glutamate in fermented foods such as meat, soy and cheese product will contribute to the umami taste [32]. However in this study, amino acids with negative ion R group which consists of aspartic acid ($R^2=0.953$) and L-Glutamic acid ($R^2=0.961$), shows the strong and positive correlation towards acidity, aftertaste, body, flavor, fragrance, and overall exclude balance. Acidity refers to the feel of crispiness and sweetness which produces bright and dry sensation that elevates the saliva and coffee taste [5]. This might be occurred due to both of aspartic acid and glutamic acids are having R with acidotic ion. In Figure 1 as well presents the correlation between positive on R groups with acidity, aftertaste, balance, body, flavor attributes. In this groups, Histidine claims its major percentage and showed strong correlation ($R^2=0.952$) than others. Therefore, it can be concluded that histamine has major influenced towards sensory profile of *kopi luwak*.

4. Conclusions

Green and roasted bean of *kopi luwak* had significantly differences for the value compounds of L-alanine, L-Tyrosine, Cysteine and L-Arginine. Valine, L-Glutamine, Aspartic acid and Glutamic acid are considered as the main amino acids that have higher percentage in *kopi luwak*. Several amino acids in *kopi luwak* are absence in green bean which later on enable to track its presence in roasted bean or vice versa. Glycine is not available in both green and roasted bean. Average value of each *kopi luwak*'s sensory attributes (fragrance, balance, aftertaste, acidity, body, balance and overall) reaches very good quality scale and classified as specialty coffee. Certain amino acid contributes to develop the sensory quality of *kopi luwak*. PLS correlation L-Valine which belongs to non polar group positively interferes the body and balance attributes. In group of negative ion R-group, namely aspartic acid and glutamic acid have positive correlation on acidity, body, flavor, fragrance and overall. Lastly mentioned is histamin that belongs to positive acidotic ion group which also has positive correlation towards acidity, aftertaste, balance, body, flavor and overall attributes.

5. Acknowledgements

The authors would like to thank The Ministries of Research and Technology and Higher Education of Indonesia for financial support in project "Strategis Nasional Institusi" with contract number 92/UN.112/PP/SP3/2018.

6. References

- [1] A. Farah, 2012 *Coffee Constituent in Emerging Health Effect and Diseases Prevention*, C. Fang, Ed. (John Wiley and Sons Inc. Blackwell Publishing Ltd.) pp. 21–58.
- [2] S. Alves, R. Dias, M. de T. Benassi, and M. dos S. Scholz 2006 *Quim. Nova*, vol. 29, no. 6, pp. 1164–1168
- [3] A. Maurya 2016 *Int. J. Pure Appl. Res.*, vol. 1, no. 2, pp. 151–156,
- [4] D. Selmar, M. Kleinwachter, and G. Bytof 2015 *Metabolic responses of coffee beans during processing and their impact on coffee flavor,* in *Cocoa and Coffee Fermentations* (Boca Raton: CRC Press).
- [5] W. Sunarharum, D. Williams, and H. Smyth 2014 *Food Res. Int.*, vol. 62, pp. 315–325
- [6] L. Batista and S. Chalfoun 2015 *Quality of coffee bean in Cocoa and Coffee Fermentations* (Boca Raton: CRC Press) pp. 114–124.
- [7] L. Lee, M. Cheong, P. Curran, B. Yu, and S. Liu 2015 *Food Chem.*, vol. 185, pp. 182–191
- [8] W. Dong, L. Tan, J. Zhao, R. Hu, and M. Lu 2015 *Molecules*, vol. 20, no. 9, pp. 16687–16708
- [9] A. Teixeira, C. Brando, R. Thomaziello, and R. Teixeira 2005 *The raw bean in Espresso coffee: the science of quality*, A. Illi and R. Viani, Eds. (London: Elsevier Academic Press), p. 91.
- [10] G. Duarte, A. Pereira, and A. Farah 2010 *Food Chem.*, vol. 118, no. 3, pp. 851–855, 2010.
- [11] M. Marcone, 2004 *Food Res. Int.*, vol. 37, no. 9, pp. 901–912
- [12] U. Jumhawan, S. Putri, Yusianto, T. Bamba, and E. Fukusaki 2016 *J. Biosci. Bioeng.*, vol. 122, no. 1, pp. 79–84
- [13] I. Smith 2014 *Kopi Luwak Coffee-World most expensive coffee beans from civet poop or an urban myth?* (iPACT Limited).
- [14] M. Muzaifa, A. Patria, Febriani, A. Abubakar, D. Hasni, F. Rahmi, and I. Sulaiman 2016 *Kopi luwak: produksi, mutu, dan permasalahannya*. (Syiah Kuala University Press)
- [15] S. P. Putri, U. Jumhawan, and E. Fukusaki 2015 *J. Biosci. Bioeng.*, pp. 33–41
- [16] M. Muzaifa and D. Hasni 2016 *Pakistan J. Nutr.*, vol. 15, no. 5, pp. 486–491
- [17] F. Mayer, M. Czerny, and W. Grosch 2006 *Eur. Food Res. Technol.*, vol. 211, no. 4, pp. 272–276
- [18] S. de Moraes, F. de Aquino, P. do Nascimento, and E. Chang 2009 *Quim. Nova*, vol. 32, no. 2, pp. 327–331
- [19] A. C. L. Amorim, A. M. C. Hovell, A. C. Pinto, M. N. Eberlin, N. P. Arruda, E. J. Pereira, H. R. Bizzo, R. R. Catharino, Z. B. Moraes Filho, and C. M. Rezende 2009 *J. Braz. Chem. Soc.*,

- vol. 20, no. 2, pp. 313–321
- [20] E. A. Nascimento, S. A. L. Morais, R. Chang, F. J. T. Aquino, B. H. P. Alves, and R. J. C. F. Afonso 2015 *Int. J. Appl.*, vol. 5, no. 1, pp. 26–37
- [21] Y. Liu and D. Kitts 2011 *Food Res. Int.*, vol. 44, no. 8, pp. 2418–2424
- [22] H. Czech, C. Schepler, S. Klingbeil, S. Ehlert, J. Howell, and R. Zimmermann 2016 *J. Agric. Food Chem.*, vol. 64, no. 25, pp. 5223–5231.
- [23] O. Janzen 2013 *Chemistry of coffee* (Hamburg, Germany: Elsevier Ltd.)
- [24] M. Murkovic and K. Derler 2006 *J. Biochem. Biophys. Methods*, vol. 69, no. 1–2, pp. 25–32,
- [25] SCAA (Specialty Coffee Association of America) 2009 *Coffee facts and statistics* [Online]. Available: www.scaa.org. [Accessed: 21-Oct-2015].
- [26] U. Satyanarayan and U. Chakrapani 2013 *Biochemistry with Clinical Concepts and Case Studies*, 4th Revise (New Delhi: Elsevier)
- [27] E. Bekedam 2008 *Coffee Brew Melanoidins, Structural and Functional Properties of Brown-coloured Coffee Compounds* (University Wageningen)
- [28] A. Taylor and J. Roozen 1996 *Crit. Rev. Food Sci. Nutr.*, vol. 36, no. 8, pp. 765–784
- [29] M. Petraco, 2001 *Beverage preparation: brewing trends for the new millenium*, in *Coffee: Recent Development*, R. Clarke and O. Vitzthum (Eds. Oxford: Blackwell Science) pp. 140–164.
- [30] N. Rawson and X. Li 2004 *The cellular basis of flavour perception: taste and aroma*, in *Flavour Perception*, 3rd ed., A. Taylor and D. Roberts, (Eds. Oxford: Blackwell Publishing) p. 75.
- [31] H. Akitomi, Y. Tahara, M. Yasuura, Y. Kobayashi, H. Ikezaki, and K. Toko 2013 *Sensors Actuators B Chem.*, vol. 179, pp. 276–281
- [32] C. Zhao, A. Schieber, and M. Ganzle 2016 *Food Res. Int.*, vol. 89, pp. 39–47
- [33] N. Ishibashi, T. Kubo, M. Chino, H. Fukui, I. Shinoda, E. Kikuchi, H. Okai, and S. Fukui, 1998 *Agric. Biol. Chem.*, vol. 52, no. 1, pp. 95–98, 1988.
- [34] R. Hutkins 2006 *Microbiology and technology of fermented foods* (Blackwell Publishing)