KnE Life Sciences

PGPR 2021
First Asian PGPR Indonesian Chapter International e-Conference 2021
Volume 2022

Research article

The Fermentation of Robusta Coffee With Termite Enzymes to Improve Quality and Taste

I Wayan Sudarma*, and A.A.N.Badung S.D

Assessment Institute for Agricultural Technology of Bali, Bypass Ngurah Rai, Indonesia

ORCID
I Wayan Sudarma https://orcid.org/0000-0002-0772-4699

Abstract.
This research involved the fermentation of robusta coffee with termite enzymes in Subak Pura Pengulu, Bongancina village, Busungbiu–Buleleng between May and December 2019. The raw materials included red pickled coffee, termite microbes, water, pH meter molasses and plastic. 100 kg of the coffee sample was added in each wet processing treatment. Methods included sample preparation, field observations, coffee harvesting, sorting, and post-harvest processing. A completely randomized design with four treatments and five replications was used to create high-quality products with distinct flavors and aromas. Physical quality, taste, caffeine content and amino acids were reported. According to the findings, the yields of wet coffee beans and dry samples without skin (ose) were 51.73% and 19.36%, respectively. In addition, in the specialty categories, the three-day fermentation period produced the best taste, with a score of 83.50. Furthermore, the defect value was specified as 13.4, water content as 12.5, caffeine content as 3.40% w/w, and the acids, including oleic and α-linoleic, were 51.147 and 0.587 ppm, respectively. According to the economic estimations, the three-day fermented coffee would produce an optimal profit of IDR 8,090,000.

Keywords: robusta coffee, termite, fermentation

Corresponding Author: I Wayan Sudarma; email: swayan41@yahoo.com

Published 07 June 2022

Publishing services provided by Knowledge E

© I Wayan Sudarma, and A.A.N.Badung S.D. This article is distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use and redistribution provided that the original author and source are credited.

Selection and Peer-review under the responsibility of the PGPR 2021 Conference Committee.

OPEN ACCESS

1. Introduction

Coffee plant (*Coffea sp.)* is a major cash crop, with a leading role in foreign exchange earnings. This observation is supported by several data on production, exports, and cultivation regions. Indonesian coffee production has been ranked in the 3rd position globally after Brazil and Vietnam [1] with exports of approximately 0.353 million tons. Meanwhile, the total plantation extended to 1.2 million ha, with dominant smallholder farms, where private and state ownership are 96 and 4%, respectively [2]. Robusta coffee develops at an altitude of 400-700 masl, predominately in mountainous regions, with temperatures between 21-24^{0}C. The cultivations in Bali are centralized around Pupuan and Busungbiu sub-districts. These two locations are within Tabanan and Buleleng

regencies, covering 7,740.13 and 7,260.16 ha, respectively, as well as production rates of 15,521 and 14,830 tons, correspondingly [3]. Generally, coffee appears as the most commonly consumed beverage, probably due to its unique taste and aroma. These properties are influenced by certain factors, including the bean quality, maximum water content of 12 %, odorless nature, and mold absence. Specifically, the coffee taste is also affected by the acidity and pleasant aroma [4]. Various processed samples are widely distributed and relatively popular for connoisseurs, e.g civet coffee. However, with the increasing rate of modern biotechnology, the popularity gradually tends to suffer a decline. Therefore, the need for new and innovative discoveries appears significant, in terms of boosting the unique taste and aroma, as well as expanding the added value of coffee commodities. This new coffee product with fermentation technology uses microbiological termite enzymes. Furthermore, the existence of termites has been disrupting human existence and causing considerable economic losses, worth approximately 2.8 trillion rupiahs per year. Conversely, the insects play an important role with potentials in processing coffee products, due to the fiber content.

Coffee plants including the beans, are comprised of fibers in the form of cellulose, hemicellulose and lignin [5]. Cellulose is a structural polysaccharide that provides protection, shape, and support for cells or tissues. The substance does not exist in a pure state, but combines with other polysaccharides, including lignin, pectin, hemicellulose and xylan [6]. Most cellulose are fused with lignin and are referred as lignocellulose, but are arranged in plants in the form of fibrils, with several parallel molecules connected by strong glycosidic bonds [6]. Lignin is a polymer of N-acetyl glucosamine with -1,4 glycoside bonds β-1.4, a known polysaccharide constituent of coffee beans. These polysaccharides consist of 2 types of sugars, termed N-acetyl muramate and N-acetyl glucosamine, with β-1,4 glycoside bonds. In terms of enzyme functionality, cellulase hydrolyzes various compounds in the form of carbohydrates, fats and proteins, by possibly disintegrating the available bonds [7]. Particular microorganisms, termed bacteria (cellulolytic termites) and fungi, also demonstrate the ability to hydrolyze cellulose into an energy source. Termites are commonly employed in fermented coffee manufacture using enzymes (*Coptotermes formosanus*) from the worker caste. This source accumulates various microorganisms in large quantities, including protozoa, bacteria and fungi [7]. Previous studies showed the ability of termites to digest lignocellulose between 74-99 %, with bodies comprising a variety of beneficial fatty acids, including palmitic, oleic, stearic, and α –linoleic [8]. Furthermore, the termites are potentially applied in the health sector to treat various diseases, termed asthma, diabetes mellitus, skin disorder, stroke, gout, hypertension, male vitality enhancer, and

also serve as antimicrobial agent, antitoxin and termite protein with amino acid contents in the form of lysine, tryptophan and threonine [9].

This study is aimed at developing quality coffee products with distinctive tastes and aromas, using technological innovation and termite enzymes.

2. Materials and methods

2.1. Time and Location

The research was conducted in Subak Abian Pura Pengulu, Bongancina village, Busungbiu sub-district, Buleleng regency, Bali, between May-December, 2019. Materials utilized were red-picked robusta coffee, termite microbial inoculants, molasses, water, pH meters and plastics. Various methods were also applied, including sample preparation, field observations, coffee harvesting, sorting and post-harvest (peeling, fermentation, drying and cleaning the horn skin), in addition to several tests across the yield, physical property, quality, taste, caffeine and fatty acid analysis. Consequently, coffee harvesting and selection were performed at the research location, while peel residue cleaning, fermentation, and drying were achieved at the Assessment Institute for Agricultural Technology Denpasar of Bali. Furthermore, physical property, caffeine content, and taste were analyzed at the Jember Coffee and Cocoa Research Center Laboratory, while the fatty acid content was assessed at the Bogor Post Harvest Center Laboratory.

2.2. Research methods

The fermentation process encompassed the entire coffee, both with or without termites. 100 kg of the red picked robusta coffee was applied in each wet processing treatment with strict selection.

Wet coffee beans were fermented with termites, at least 2 hours after peel removal. The treatment includes:

1. R0: Wet coffee beans fermented for 1 day without using termite microbes
2. R2: Wet coffee beans fermented for 2 days with termites
3. R3: Wet coffee beans fermented for 3 days with termites
4. R4: Wet coffee beans fermented for 4 days with termites microbes

Therefore, at the end of the fermentation, based on the time interval, the samples were sun-dried to remove water content + (11-12 %), followed by the removal of the epidermis (ose) to produce dry coffee beans.

2.3. Yield Measurement

The yield of wet coffee beans was measured to determine the volume of expended inoculant liquid. This was followed by testing the HS samples (with epidermis), where the dry portions were separated from the epidermis (green beans).

2.4. Fatty acid content test

These series of tests were conducted in the laboratory of the Research Center for Post-Harvest Agricultural Products – Bogor. The objectives were to determine the fatty acid types and levels, including palmitic, oleic, stearic, and α-linoleic, contained in coffee beans with termite enzyme fermentation.

2.5. Physical Quality Test, Taste and Caffeine Level.

Various yield assessments were performed on the dry coffee beans, physical quality, and taste including water content, defect value, aroma, flavor, viscosity (body), bitterness, sour taste (acidity), and the caffeine content of each treatment. Apart from conducting the taste analysis at the Coffee and Cocoa Research Center – Jember, direct evaluations were obtained from coffee connoisseurs / drinkers, although only in terms of related qualitative and subjective values. This yield research employed a completely randomized design (CRD) with 4 treatments and 5 replications to measure the output of coffee beans (green) from the wet samples. Furthermore, the parameters observed in the physical test, caffeine content, and taste analysis include physical quality, e.g dirt content, defect value, as well as coffee tastes termed flavor, body, acidity and aroma. In addition, the caffeine content of each fermentation period varied for the coffee beans.

3. Results and Discussion

Termite coffee is a major fermented yield obtainable by chemical processes, and is beneficial in the formation of flavour precursor compounds, including organic and amino acids, as well as reducing sugars [10].

3.1. Robusta Coffee Physical Quality Test

Visual observations showed a darker appearance of the coffee beans color with increasing fermentation time, indicating a stronger termite microbial penetration, in addition to the extensive fermentation period known to increase dissolved protein levels. This result is in line with [11], where the original civet sample observed a color change to become darker after the digestion. The water content for the treatment R0, R2, R3, and R4 were 12.3, 12.4, 12.5, and 12.6 %, respectively. However, the value in dried robusta coffee beans is very high (> 12%). This indicates an extensive drying period, (above 11 days), due to the greater and thicker dimensions, with increasing water content. The use of a 6.5 mm sieve to prevent particle entry, indicated a relatively uniform grain size from R0-R4, and was entirely categorized as large and consistent. In addition, the magnitude of the bean is an important characteristic in determining coffee quality, with great influence on the selling price [12]. Treatments R0, R2, R3, and R4 showed 0 % dirt in the coffee beans, with defect values obtained at 13.6, 13.8, 13.4 and 13.9, respectively. These estimates are due to stripping of the horn skin, with uneven sample sizes or divergent machine settings, causing the materials to disintegrate. Similarly, the entire coffee beans were without a bad smell and mold. Typically, a bad smell emanates from a slightly damp storage area, where the water content is high and mold (fungi) easily grows on the bean surface.

Table 1: Robusta Coffee Physical Quality Test Results.

No	Parameter	R0	R2	R3	R4
1	Impurity Level	0 %	0 %	0 %	0 %
2	Water content	12.3	12.4	12.5	12.6
3	Passed 6.5 mm sieve	0 %	0 %	0 %	0 %
4	Defect Value	13.6	13.8	13.4	13.9

Source: Koka Jember Research Center, 2019.

3.2. Termite Robusta Coffee Caffeine Content Test

In testing the caffeine content of robusta coffee, a bitter or unpleasant taste similar to quinine is expected [13]. This is a common characteristic, due to the high caffeine and aroma, but not as strong as Arabica. Caffeine possesses a bitter taste, white coloration and serves as an important alkaloid or stimulant in drugs. Generally, a lower proportion is known to improve the taste quality. Based on the laboratory results of Jember Pasuruan Coffee and Cocoa Research Center, the caffeine in roasted robusta samples tends to decline, under an extensive fermentation period. This outcome matches the statement of [14], where the general standard of the caffeine content, according to SNI 01-2983-1992 ranged between 2-8 % w/w. However, the results from PUSLIT KOKA obtained the estimates of 3.42, 3.40 and 3.30 % w/w at a fermentation period of 2, 3, and 4 days, respectively, in samples without termite enzymes. This indicates that increasing the process interval possibly reduces the caffeine levels, leading to the production of a safe termite coffee.

[14] agreed that the caffeine decrease is also due to termite bacteria activity known to produce cellulase enzymes as a high protein substrate source. This exact report also stated the protein breakdown was responsible for the declining levels of caffeine. Furthermore, the ability of lignocellulose termite bacteria to hydrolyze lignin and cellulose contents in coffee beans disintegrated the sugar particles influencing the organic acids. [15] described the outer portion of the sample to resemble a gel / mucus, comprising 80 % pectin and 20 % sugar. The mucilage layer of coffee beans contains sugar, and are available to microbes as a substrate, while the reduced mucus film causes water to penetrate more easily through the pores [11]. Also, the water influx triggers the caffeine dissolution, due to the easily soluble nature, in line with [16], where the solubility is attributed to the presence of a single water molecule.

3.3. Wet Coffee Bean Yield

The use of red pickings generated wet coffee beans by 50.40, 51.35, 52.20, and 53.00 %, with an average number of 51.73 %. This indicates the influence of several factors, including the yield type and period. The harvest of robusta coffee is conducted simultaneously during the rainy season, where the Busungbiu region experiences a significant precipitation. Therefore, the water content in coffee cherries appears relatively high and excess deposit in the flesh leads to a minimal bean composition, compared to dry season harvest.

3.4. Dried Seed Yield

The yield of dry coffee beans (post-treatment) was influenced by the fermentation period, as extensive duration instigates lesser output, although the difference was not substantial. This occurrence is probably due to the decomposition of certain food substances in coffee beans into simpler compounds and are more susceptible to evaporation during the fermentation process. Table 2 shows a higher composition of the skin and the water content in the fruit flesh and peel. This triggers an easier fermentation and a massive bean weight, although there is a tendency for lower yield.

Table 2: Yield of Fermented Robusta Coffee Beans.

		Yield logs	(%)			
No	Treatment	Wet Beans	Dried Beans with peel (HS)	Dry Beans without peel (ose)	Roasted dry beans	Coffee powder
1	R0	50.40	26.32 [a]	19.31 [a]	15.16 [a]	13.14 [a]
2	R2	51.35	26.40 [a]	19.38 [a]	15.33 [a]	13.21 [a]
3	R3	52.20	26.44 [a]	19.43 [a]	15.99 [a]	13.29 [a]
4	R4	53.00	26.31 [a]	19.31 [a]	15.23 [a]	13.15 [a]

3.5. Termite Robusta Coffee Taste Quality

Based on taste analysis results, a 3-day termite fermentation showed the optimal total score, in comparison to other samples or control (RO). In addition, the RO value was specified at 81.60, although an overall value of 83.50 was generated, below the interval. This observation is in line with [17] concerning specialty coffee limitation, including the total taste score of cupping test results above 80.00, where the coffee beans exhibited high quality similar to the specialty sample. Meanwhile, the total scores for a 2 and 4-day yields were estimated at 78.99 and 79.10, respectively. This indicates that with increasing fermentation period, the taste quality tends to decline and the score value below 80.00 is not classified as a specialty coffee. The results showed the flavour of the 3-day fermented steeping as the maximum value, and also concludes the optimal fermentation period with termites at 3 days. Each treatment obtained a pH of 6.0, similar to [18], with a temperature range of 40-45 ^{0}C. Therefore, termites are potential enzyme sources, particularly cellulose, in boosting the value of food ingredients. However, robusta coffee was fermented between 12-36 hours, due to lower sugar in Arabica species [14]. During the product presentation, 50 coffee connoisseurs reported a unique variation from other samples and when served cold, the termite coffee appeared more delicious and

aroused taste buds . Termite coffee roasting is performed traditionally using firewood, fried ground and manual processing, in line with [19], where these methods significantly influenced the quality and taste. Furthermore, robusta coffee tends to produce a fruity aroma, due to the presence of aldehyde, acetaldehyde and propanal compounds, believed to enhance the taste and quality [19]. Based on the results of the lobe test by panelists at Koka Jember Research Center, the termite coffee with a 3-day fermentation reported the aroma of chocolate, pandanus, astringent, coffee beans, caramel, natural process and fruit, while a 2-day interval showed chocolate, coffee beans, fruit, caramel, cereal, astringent, and natural process. Also, 4-day period recorded chocolate, sour, cereal, and the use of natural process, but samples without termite enzymes perceived chocolate, sour, floral aroma and pandanus fragrance. Based on these findings, the coffee from 3, 2 and 4 days fermentation, demonstrated a superior taste, in comparison to the absence of termite enzymes (R0), specifically in terms of aroma, flavor, aftertaste and sweetness. The advantages of 3-day fermented coffee taste over the 4-day interval was based on the characteristics of flavor, aftertaste, balance and overall performance. Therefore, it is very natural that the termite robusta coffee in the present research possesses a strong taste and is more prominent than samples without termite enzymes. This is in line with [20] where probiotic coffee (fermented) showed a stronger aroma, compared to civet species (without fermentation).

According to [21], a minimal portion of caffeine evaporates during roasting to form other components, including acetone, furfural, ammonia, trimethylamine, formic and acetic acids. This is followed by the decomposition of chlorogenic acid into volatile aromas and melanoidins, released as CO_2. Essentially, caffeine in coffee beans exists as a free compound or combines with chlorogenic as a potassium chlorogenic substance, leading to a change in product taste. During the roasting process, extensive amino acid compounds needed to react with reducing sugars in the Maillard reaction, equally tend to generate additional flavour and aroma compounds [22]. Furthermore, the content of flavour precursor substances results in a higher quality of roasting and brewing coffee containing sufficient flavor compounds, and therefore produces an improved taste and aroma [23].

Description: R0 : Fermented coffee a day without termite enzymes

R2 : 2 days fermented coffee with termite enzymes

R3 : 3 days fermented coffee with termite enzymes

R4 : 4 days fermented coffee with termite enzymes

Based on the results of the taste test panelists at PUSLIT KOKA JEMBER, the 3-day fermented coffee obtained the maximum value, followed by R0, R4 and R2. Similarly, the

Table 3: Results of Termite Fermented Robusta Coffee Taste Test.

No	Characteristics	R0	R2	R3	R4
1	Scent	7.92	7.33	7.87	7.50
2	Flavor	7.60	7.12	8.12	7.26
3	Aftertaste	7.75	7.20	7.75	7.33
4	Acidity	7.85	7.15	7.17	7.01
5	Body / viscosity	7.52	8.00	8.00	8.00
6	Uniformity	10.00	10.00	10.00	10.00
7	Balance	7.30	7.19	8.31	7.50
8	Clean Cup	10.00	10.00	10.00	10.00
9	Sweetness	7.83	7.75	7.90	7.00
10	Overall	7.83	7.25	8.38	7.50
11	Stain / Defect	0.00	0.00	0.00	0.00
	Final Score	81.60	78.99	83.50	79.10

Source: Koka Jember Research Center, 2019.

taste and aroma were actually appreciated by subjective coffee connoisseurs, possibly due to the resemblance to Arabica, with a distinctive aroma. In addition, the lighter and delicious taste was attributed to the ability of the termites to digest the lignocellulose in coffee beans up to 74-99 % [8]. Therefore, several repetitions are necessary to ensure a sufficient quality level for suitable termite coffee taste, as no standard previously existed.

3.6. Organic Acid Content

Robusta coffee beans contain organic acids with the role of precursor compounds contributing to the taste quality, particularly for the acidity components [24]. These constituents are also significant in ester formation, also described as volatile content in the coffee aroma [21]. The analysis results showed the occurrence of several fatty and organic acids types in the termite robusta coffee, including palmitic, stearic, oleic and α -linoleic. Overall, the most prominent fatty acid content was oleic at 52.147 ppm, with a 3-day fermentation. This was followed by R0, R2 and R4 at 46.142, 46.076 and 41.884 ppm, respectively. However, the results are preferred in the 3-day interval, where the α-linoleic acid content appears higher at 0.587 ppm. The linoleic acid is believed to complement the taste and aroma.

Apart from the nutritive function, fat serves as a food component and flavour enhancer, with a fused taste combination of soft, delicious, shape and aroma. Fat is also a carrier of lipophilic flavour compounds caused by the presence of precursors in flavour formation [25]. These results indicate that the shorter fermentation period

tends to increase the linoleic acid (omega 3), but moderate consumption is believed to prevent the constriction risk of blood vessels or coronary heart diseases [26]. Furthermore, coffee beans also contain palmitic and stearic acids, although there is a declining tendency after a certain fermentation period. Equally, the linoleic acid content is also influenced by the composition during the process.

Table 4: Organic Acid Content of Termite Robusta Coffee Beans.

No	Types of Organic Acid		Content	(ppm)	
		R0	R2	R3	R4
1	Palmitate	34.047	34.743	37.855	35.076
2	Stearate	15.153	16.112	17.745	15.641
3	Oleate	46.142	46.076	52.147	41.884
4	Linoleate	0.457	0.560	0.587	0.180

Source: Bogor Post Harvest Center Lab, 2019

Interestingly, the 3-day fermented coffee contains palmitic acid with a fairly high content (37.855 ppm), compared to other treatments. This acid is very useful as an anti-oxidant, with the ability to inhibit intestinal inflammation and suppress stress. Therefore, the presence of the substance demonstrates a significant positive value. Palmitic acid is formed from the breakdown of carbohydrates by microbes during fermentation, specifically by the flavobacterium species [27], commonly present in the digestive tract of animals, including termites (cellulase). In addition to several benefits, colon cancer prevention is also possible [28]. Also, the low composition of palmitic acid in the 2-day fermentation was due to the relatively short interval. Similar to oleic acid, the minimal period (2 days) was known to increase the content of palmitic, stearic, oleic and linoleic acids. However, above 3 days actually caused a decrease in the acid content. This tendency, especially in oleic and α – linoleic, appeared relatively lower, in comparison to control (R0) in a 4-day fermented coffee. Linoleic acid is an essential mono unsaturated fatty acid that functions in lowering low-density lipoprotein (LDL) and increasing high-density lipoprotein HDL. A decrease in the LDL/HDL ratio will prevent atherosclerosis. A high serum LDL-c/HDL-c ratio was found to be independently associated with an increased risk of Sudden cardiac death [29]. Further research is needed to ascertain the fermentation effect on the fatty acids. Potentially, the outcome is expected to provide a more relevant argument.

3.7. Economic Analysis of Quality Improvement and Quality of Termite Coffee Assumptions on a Scale of 100 kg of Red Wet Beans

The economic analysis of fermented coffee with termite enzyme application was strictly selected using red picks of 100 kg scale, in a wet processed single harvest period. This evaluation was conducted by considering the neglected coffee husk waste, single-use plastics for fermentation, as well as disposable enzyme inoculants. The table below shows the quality and taste of robusta coffee with termite fermentation. However, the bitter taste as a characteristic of robusta coffee (high) decreased, and the samples observed a resemblance to the taste of Arabica species, subsequently appreciated by the coffee connoisseurs. Furthermore, by economic evaluation, termite coffee provides a relatively high profit, in comparison to products without termite enzymes.

The Benefit-Cost Ratio of termite coffee in each treatment was obtained (B/C) > 1.0, indicating the material benefits from the total costs incurred during production. This is in line with [30] where B/C 1.0 showed lesser profitability in processing the samples without termite enzymes.

Table 5: Economic Analysis.

No	Description	Treatment			
		R0	R2	R3	R4
A.	In-Put				
1	Wet coffee beans / pick red (Rp/kg)	700.000	700.000	700. 000	700.000
2	Peel (Rp)	75.000	75.000	75.000	75.000
3	Coffee Processing (Rp)	200.000	200.000	200.000	200.000
4	Termite Inoculant (Rp)	-	35.000	35.000	35.000
5	Plastic (Rp)	60.000	60.000	60.000	60.000
6	Drying Coffee (Rp)	150.000	150.000	150.000	150.000
7	Drying Place (Rp)	100.000	100.000	100.000	100.000
8	Molasses (Rp)	-	50.000	50.000	50.000
9	Huller (Rp)	100.000	100.000	100.000	100.000
10	Water for washing coffee (Rp)	100.000	100.000	100.000	100.000
	Total :	1.460.000	1.510.000	1.510.000	1.510.000
B	Out-Put				
1	Sales of Powdered Coffee (Rp)	6.000.000	8.800.000	9.600.00	8.000.000
2	Advantage	4.540.000	7.290.000	8.090.000	6.490.000
3	B/C Ratio :	1,0	> 1,0	> 1,0	> 1,0

4. Conclusion

The fermentation process slightly influenced the yields of OC and ground coffee beans, although the color appeared darker with an extensive duration. Also, the robusta species generated very substantial green beans, due to the composition of the coffee fruit flesh and high water content. Furthermore, the harvest period also contributes to the output, as peak dry seasons tend to obtain sufficient products. Subsequently, the maximum score for a 3-day fermented coffee was incorporated in the specialty category, indicating a high quality R3 samples. Under this interval, the optimal formation of palmitic acid content was observed. Considering that palmitic acid exhibits antioxidant and anti-carcinogenic properties, termite coffee demonstrated an adequate functional value, compared to ordinary coffee (without fermentation). As a consequence, the acids appear very beneficial in preventing digestive-related diseases and other critical health challenges. However, the fermentation treatment didn't instigate any significant change in the fatty acids. Therefore, by economic evaluation, a 3-day fermented coffee provides the maximum profit of IDR. 8.090.000, compared to other intervals, including the control. Further research on coffee fermentation using termite enzymes to obtain the quality and taste of Arabica species appears very necessary. Therefore, additional results are obtained for the farmers to explore and develop sustainable cultivation practices.

References

[1] Hartono. Produksi kopi nusantara ketiga terbesar di dunia. Kemenperin.go.id; 2013 Nov 3. Available from: http://www.kemenperin.go.id/artikel/6611

[2] Asosiasi Eksportir dan Industri Kopi Indonesia. Industri kopi Indonesia. AEKI; 2014. Available from: http://www.aeki-aice.org/page/industri-kopi/id

[3] Badan Pusat Statistik Bali. Provinsi Bali dalam angka. Denpasar : Badan Pusat Statistik Provinsi Bali 2019.

[4] Simanjuntak R. Artikel ilmu bahan makanann bahan penyegar. Semarang: Universitas Diponegoro 2011.

[5] Rahardjo P. Panduan budidaya dan pengolahan kopi arabika dan robusta. Jakarta (ID): Penebar Swadaya; 2012

[6] Brinchi L. Production of nanocrystalline cellulose from lignocellulosic biomass. Journal Carbohydrate Polimer. 2013;94:154-159.

[7] Diba F, Khotimah S, Febriyana U. Isolation and identification cellulolytic bacteria from the termite *Coptotermes curvignatus holmgren* and *Macrotermes gilvus hagen* from

secondary forest in West Kalimantan Indonesia. Paper presented at: Procedings of the 9^{th} Passific Rim Termite Research Group Conference 27-28 February 2012.

[8] Gupta P, Samant T, Sahu A. Isolation of cellulose-degrading termite bacteria and determination of their cellulolityc potential. International Journal of Microbiology. 2012;10(115):1-5.

[9] Poudyal H, Panchal SK, Diwan V, Brown L. Omega-3 fatty acids and metabolic syndrome: Effects and emerging mechanisms of action. Progress in Lipid Research. 2011;50:372-387.

[10] Lin CC. Approach of improving coffee industry in Taiwan promote quality of coffee bean by fermentation. The Journal of International Management Studies. 2010;5:154–159.

[11] Hadipernata M, Nugraha S. Identifikasi fisik, kimia dan mikrobiologi biji kopi luwak sebagai acuan teknologi proses kopi luwak artificial. Paper presented at: Prosiding Seminar Nasional Insentif Riset Sinas; 2012 Nov 29–30; Bandung, Indonesia.

[12] Nugroho D, Mawardi S, Yusianto, Arimersetiowati R. Karakterisasi mutu fisik dan citarasa biji kopi arabika varietas maragogip (*Coffea arabica* L. var. *Maragogype* Hort. ex. Froehner) dan seleksi pohon induk di Jawa Timur. Pelita Perkebunan. 2012;28:1–13.

[13] Budiman H. Prospek tinggi bertanam kopi pedoman meningkatkan kualitas perkebunan kopi. Yogyakarta: Pustaka Baru Press; 2012.

[14] Ana F, Evi R, Kumoro AC. Penurunan kadar kafein dan asam total pada biji kopi robusta menggunakan teknologi fermentasi anaerob fakultatif dengan mikroba nopkor MZ-15. Jurnal Teknologi Kimia dan Industri. 2013;2(3):70-7.

[15] Muchtadi TR, Ayustaningwarno F. Ilmu pengetahuan bahan pangan. Bandung: Alfabeta; 2010.

[16] Oktadina FD, Bambang DA, Hermanto MB. Pemanfaatan nanas (*Anannas comosus* L. merr) untuk penurunan kadar kafein dan perbaikan cita rasa kopi dalam pembuatan kopi bubuk. Jurnal Keteknikan Pertanian Tropis dan Biosistem. 2013;3(1):265-268.

[17] Speciality Coffee Association of America. Coffee term from the Speciality Coffee Association of America. Boston: The Event Specialty Coffee of The Year; 2013.

[18] Saputra B. Analisis pengaruh pH dan lama fermentasi terhadap kadar etanol hasil hidrolisis jerami padi [Skripsi]. Malang: Universitas Brawijaya 2015.

[19] Febriliyani YR. Pengaruh teknik penyeduhan dan ukuran partikel kopi bubuk terhadap atribut sensori seduhan kopi robusta dampit menggunakan metode Rate-All-That-Apply (RATA) [Skripsi]. Malang: Universitas Brawijaya; 2016.

[20] Guntoro S. Proses memproduksi kopi luwak probiotik. Proposal paten. Denpasar: Balai Pengkajian Teknologi Pertanian (BPTP) Bali; 2010

[21] Somporn C, Kamtuo A, Theerakulpisur P, Siriamompun S. Effects of roasting degree on radical scavenging activity, phenolics and volatile compounds of Arabica coffee beans. International Journal of Food Science and Technology. 2011;46:2287–2296.

[22] Wang N. Physicochemical changes of coffee beans during roasting [Master of Science Thesis]. Ontario: University of Guelph; 2012.

[23] Arruda NP, Hovell AMC, Rezende CM, Freitas SP, Couri S, Bizzo HR. Arabica coffee discrimination between maturation stages and post-harvesting processing types using solid phase microextraction coupled to gas charomatography and principal components analysis. Quimica Nova. 2011;34(5):819-824.

[24] Taba J. Coffee taste analysis of an espresso coffee using nuclear magnetic spectroscopy [Bachelor Thesis]. Eindhoven, Holland: Central Ostrobothnia University of Applied Sciences; 2012.

[25] Correa E C, Jiménez-Ariza T, Díaz-Barcos V, Barreiro P, Diezma B, Oteros R, Acheverri C, Arranz F J, Ruiz-Altisent M. Advanced characterisation of a coffee fermenting tank by multi-distributed wireless sensors: Spatial interpolation and phase space graphs. Food and Bioprocess Technology. 2014;2:46–54.

[26] Vaseji N, Mojgani N, Amirinia C, Iranmanesh M. Comparison of butyric acid concentration in ordinary and probiotic yogurt samples in Iran. Iranian Journal of Microbilogy. 2012;2:87–93.

[27] Ni J, Tokuda G. Lignocellulose-degrading enzymes from termites and their symbiotic microbiota. Biotechnology Advances. 2013;(31):838–850.

[28] Santikunaporn M and Danphitak S. Hydrodeoxygenation of linoleic acid on ni-mo catalyst. Thammasat International Journal of Science and Technology. 2010: 15(5): 1-6

[29] Setor K K, Zaccardi F, Karppi J, Kurl S and Laukkanenls J A. High Serum LDL/HDL Cholesterol Ratio an Emerging Risk Factor for Sudden Cardiac Death? Findings from the KIHD Study. Journal of Atherosclerosis and Thrombosis. 2016: 1-9

[30] Nuraeni I, Hidayat H. Manajemen usaha tani. Jakarta: Universitas Terbuka; 2011.