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Effect of Caffeine-Utilizing Civet Digestive Microbiota on The Physicochemical Properties of Fermented Arabica and Robusta Coffee Beans

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ABSTRACT

The study aims to assess and evaluate the use of a microbial mixed culture derived from civet digestive microbes, selectively enriched for caffeine utilization, on the physicochemical properties of fermented coffee beans. The varieties of coffee used in this study include Arabica and Robusta coffee cherries. The specific objective of this study is to decaffeinate the coffee beans through the fermentation process using civet digestive microbes selectively enriched. The fermentation process was performed at 37±0.5°C for 48 hours. Results revealed that the decaffeination rate of fermented arabica coffee (0.08% per hour) was twice that of fermented robusta coffee (0.04% per hour). After 48 hours of incubation, the caffeine content of the fermented robusta coffee (1.7%) was slightly higher than that of the arabica coffee (1.5%). This finding is significant as the decaffeination method offers a practical, sustainable alternative to chemical decaffeination. The volatile fatty acids (VFA) generated in the fermented arabica coffee peaked at 4 hours of incubation with a concentration of 3.45 g/L VFA. On the other hand, the VFA production in the fermented robusta coffee reached a peak at 20 hours of incubation (3.50 g/L). The caffeine content of both arabica (1.46%) and robusta coffee (1.65%) beans was somewhat higher than that of the original civet coffee (1.32%). Results revealed that prolonged acidic conditions (pH<5.0) during the coffee fermentation not only restrict the degradation of caffeine but also inhibit the formation of VFA.

1. INTRODUCTION

Coffee is a non-alcoholic beverage that is widely consumed by people worldwide. With millions of cups drunk every day, coffee has been one of the most widely traded products in global markets [1]. As one of the most popular drinks in the world, it has had a significant role in consumer culture recently [2]. The largest coffee-producing countries in the world, leading a major part in fulfilling the world's demand for coffee beans, include Brazil, Vietnam, Colombia, Indonesia, and Ethiopia. Most people consume coffee typically to feel relaxed due to its certain aromas and tastes [3]. Besides obtaining nutritional advantages such as antioxidants [4], coffee also provides some positive effects in consumers' physiology and psychology [1, 2]. The study added that the consumption of coffee may potentially have positive effects on the gut microbiota as well as the gastrointestinal tract [5].

Current research and development on coffee production have remarkably produced specialty coffees providing splendid flavors and special aromas [6]. These properties are related to their genotype and the location where they are planted [7, 8]. Some studies reported that the quality of coffees, including their tastes and aromas, could also be improved by introducing various methods in coffee processing, such as fermentation and drying processes [9-11]. The authors added that various processing practices may stimulate the formation of metabolites that could influence the physicochemical properties of the coffee beans, and thereby enhance their level of cupping [11].

Introducing fermentation to coffee processing is a crucial step to produce high-quality coffee beans [12]. Coffee fermentation is normally carried out to remove mucilage from the coffee skin. The mucilage in coffee cherries typically contains carbohydrates, including polysaccharides, cellulose, pectin, and starch [13]. The fermentation process is accelerated by enzymes that are naturally acquired in the coffee cherries and also released by microbes. When the coffee cherries are fermented, carbohydrates in the coffee pulp and mucilage are converted into metabolites, such as organic acids and alcohol [14, 15]. These fermentation end-products specifically would diffuse into the beans and thereby improve the tastes and aromas of the coffee [1].

Some typical microbes used in coffee fermentation include bacteria, fungi, and yeast. Those microbes have a significant role in converting mucilage by generating some enzymes and metabolites [16]. A recent study has reported that coffee fermentation has been more attractive among coffee producers as they could enhance the quality of coffee beans and generate

specialty coffee [1, 17]. Some studies have revealed that the fermentation process may alter the physiological attributes of the coffee beans, including a reduction in water content and simple soluble carbohydrates and improvement of flavor and aroma precursor [18, 19].

Many studies have been reported regarding coffee fermentation; they usually use a particular microorganism, such as Saccharomyces cerevisiae and lactic acid bacteria [20, 21]. Using a specific microbe, a pure culture or single culture normally would generate a special metabolite. This certainly may not significantly alter and enrich the attributes of the fermented coffee. On the other hand, coffee fermented using mixed culture or various microorganisms involved may potentially enhance the quality of the coffee beans due to the variety of metabolites generated [14, 22, 23]. The mixed culture coffee fermentation naturally occurs in the civet's digestive system. This occurs in civet coffee fermentation involving various types of microbes. In this process, the coffee cherries are consumed by the civet and subsequently fermented by microbial consortia in the digestive system of the civet to form various fermentation end-products, including volatile and non-volatile fatty acids [24]. Hence, the civet coffee may have a superior taste compared to other types of coffee [25]. The study added that when questioned about the advantages of civet coffee compared with other types of coffee, tour guides at 12 of the plantations stated that the main advantage was superior taste. A previous study showed that the addition of inoculum derived from civet digestive microbes enriched with sugar or soluble carbohydrates would significantly reduce sugar content in the coffee beans, in which around 84% of the sugar was converted within 24 hours of incubation [14]. The current study aims to assess and evaluate the use of inoculum from civet fecal suspension for fermenting coffee cherries, including arabica and robusta. The organic acid formation and carbohydrate degradation will be thoroughly evaluated during the fermentation process.

As caffeine content is a significant attribute of coffee beans, the decaffeination process will also be covered in this study. A study mentioned that decaffeination is a process to reduce caffeine content in coffee [26]. The reduction of caffeine content in coffee may minimize the bitter taste of the coffee [27]. Besides, decaffeination is also a method to lower the side effects of caffeine in the body of coffee consumers [28]. Several methods for the decaffeination process include water decaffeination, which involves hot water extraction, supercritical decaffeination using CO_2, solvent decaffeination using solvents such as acetone, chloroform, ethyl acetate, ethanol, and ethyl ether, and biological decaffeination using enzymes. Each method of decaffeination would generate a coffee with special characteristics based on some operating conditions such as temperature, duration, solvent, solvent concentration, and pressure introduced [26]. In this current study, decaffeination will be performed via a fermentation process using a mixed culture of civet digestive microbes, adapted for caffeine utilization through a selective enrichment process. The study hypothesizes that the civet microbiota selectively enriched with caffeine utilization may enhance caffeine degradation of the coffee beans.

2. MATERIALS AND METHODS

2.1 Preparation of coffee fruit

The experiments were carried out using coffee fruits, including arabica coffee (*Coffea arabica*) and robusta coffee (*Coffea canephora*). The fruits were collected from the coffee plantation in the Gayo Highland, Jeget Ayu Village, Jagong Jeget subdistrict, Central Aceh Regency, Takengon, Aceh Province, Indonesia. In this study, the type of coffee used for the experiments is coffee cherry or unpeeled coffee fruits. The collected coffee was the ripe coffee cherries, and they were harvested when their skin color had turned dark red. The dark red coffee cherries were picked after 9 months from flowering. Before the start of the experiments, all collected coffee cherries were sorted and cleaned to remove undesired materials that may hinder the fermentation processes. The original civet coffee beans were also prepared and taken from the coffee plantation. In this research, the civet coffee beans were used to compare data between in vivo and in vitro results [29].

2.2 Culture preparation

The microbial inoculum used for the coffee fermentation was obtained from a selectively enriched civet fecal suspension. The civet fecal suspension used was derived from the civet poop collected from the coffee plantation in the Gayo Highlands, Takengon, Aceh, Indonesia. The civet poop and civet coffee beans used for the current experiments were derived from the wild civet habitat in the Gayo coffee plantation. All collected civet poops were then placed in a sealed bottle. Shortly after collection, the civet poop was kept at the body temperature of the civet, which was around 37±0.5°C [30]. This practice was performed to maintain the activities of the civet's digestive microbes close to their indigenous ecosystem.

Microbial cultivation processes were conducted via selective enrichment by gradually loading culture media and a particular substrate. To enrich microbes' degrading caffeine and to induce the digestive microbes to adapt to caffeine utilization, the only substrate fed into the culture during the cultivation process was caffeine. The microbial culture media were prepared by dissolving 1g of $NaHCO_3$ (Merck, Germany), 1g of Bacto Peptone (Merck, Germany), 1g of NH_4Cl (Merck, Germany), and 1g of caffeine (Sigma Aldrich, USA) in 100ml of distilled water. A hundred grams of civet poop was subsequently mixed with the culture media. The selective enrichment process was carried out by gradually adding 100ml of medium and substrate into a 500ml fed-batch reactor. The reactor was continuously agitated with a stirring rate of 50 rpm. The enrichment process lasted for around 5 days until the volume of the culture reached 500ml, with the headspace about 100cm^3. The culture was continuously stirred at The process of microbial cultivation was performed at mesophilic conditions at around 37±0.5°C. The specific microbial growth during the cultivation period would be shown by an optical density (OD) measurement chart. The physical and chemical properties of the civet fecal suspension used as inoculum for civet coffee in vitro fermentation are depicted in Table 1.

Table 1. Physicochemical characteristics of civet fecal suspension

Parameters	Unit	Value
Total dissolved solids	mg/L	90.5±0.3
pH	-	7.61±0.01
Salinity	mg/L	91±0.2
Optical density	OD600	0.541±0.02
Electrical conductivity	µS/cm	179.8±0.1

2.3 Experimental design and procedures

The experiments were carried out to assess the effects of coffee fermentation using civet fecal suspension enriched with caffeine utilization on the physicochemical properties of various types of fermented coffee beans, including arabica and robusta coffee beans. Characterization of original civet coffee beans was also performed to compare the physicochemical characteristics between coffee fermented in vivo and coffee fermented in vitro using inoculum enriched with caffeine utilization. The process was conducted in a series of batch trials. The working volume of the fermenter used in this test was 80 ml. The starter used for the coffee fermentation was derived from the microbial culture cultivation process, with a concentration of 40% of the total volume. Hence, the ratio of coffee to suspension applied to the anaerobic fermentation process was 2:3. The experiment trials were carried out in duplicate to ensure reproducible and consistent results. To avoid any cross-contamination during coffee fermentation, the process was conducted in closed batch reactors.

The coffee fermentation lasted for 48 hours to allow all coffee fruits to be completely fermented. To have similar environmental characteristics between in-vitro and in-vivo civet coffee fermentation, the operational temperature of this in-vitro civet fermentation was maintained at 37±0.5°C, which was somewhat close to the typical body temperature of civet [30]. Shortly after fermentation was completed, each sample of coffee beans fermented in vitro was assessed and compared with the coffee beans fermented in vivo or the original civet (luwak) coffee beans for their physical-chemical properties.

2.4 Analytical methods

Each collected sample was periodically analyzed to assess the physicochemical characteristics of various types of fermented coffee beans. Before the start of the experiments, each sample of Arabica and Robusta coffee cherries was analyzed for its properties. The pH of the culture derived from the selective enrichment process was monitored regularly using a Lab Benchtop pH meter multifunction with the complete probe, Model MW 102 (Milwaukee, USA). Before the measurement, the pH meter used was calibrated and validated using a buffer solution to ensure accurate and precise data collection [31].

To evaluate the potential microbial growth during the selective enrichment and/or microbial cultivation processes, optical density (OD) was measured using a Lab UV-VIS Spectrophotometer, 325-1000nm, 4nm Ultraviolet (Yuchengtech, China). Before the OD analysis of the test sample, a blank sample of distilled water was introduced into the spectrophotometer. The microbial culture OD measurement was carried out at the 600 nm wavelength [32].

Samples of coffee before and after fermentation were measured for their moisture and solid contents using a laboratory oven (Thermolyne Drying Oven, USA) at a temperature of 105°C for 24 hours. Those samples were subsequently analyzed for ash content using a muffle furnace (Thermolyne Drying Oven, USA) at a temperature of the temperature of 600°C±50°C for around two hours. All procedures to perform the analysis were based on the Standard Methods [33, 34].

The analysis of soluble carbohydrate concentration in the raw, fermented, and original civet coffee beans was performed by using the Glucometer BioSensor AGM-2100 (Gluco Dr.auto, South Korea) with an assay method of electrochemical method (Gold electrode). The measurement process was conducted at room temperature. To acquire accurate and reproducible data, the analyzer was calibrated before use. To prevent an exaggerated reading of the data collected, 10 times the dilution factor was applied to each analyzed sample. All procedures applied for the soluble carbohydrate analysis of the fermented coffee were performed according to the method developed by Darwin [35].

The determination of volatile fatty acids (VFA) was performed to assess the formation of organic acids, especially VFA, during the fermentation process of Arabica dan Robusta coffee. The analysis of VFA was conducted by using the titrimetric method. The measurement of VFA was performed with a Lab Standard pH meter (Milwaukee, USA). Each sample was initially acidified to pH 3.0 with hydrochloric acid to convert bicarbonate to H_2CO_3 ($\rightarrow CO_2$). After the acidification step, the sample was aerated to remove carbon dioxide. Each sample was then back titrated using sodium hydroxide from pH 3.9 to 5.6. To have both precision and accuracy at both low and high VFA levels, a titration volume of 40ml was applied in this analysis. To ensure the accuracy of the obtained results, an assessment of the control sample was conducted periodically. The analytical procedures used were based on the study performed by Lützhøft et al. [36].

As caffeine was an essential substance of the coffee, the current study performed the caffeine analysis on the coffee samples, including coffee cherries and coffee beans fermented in vivo and in vitro. The determination of caffeine concentration of the coffee beans was analyzed by using a laboratory. UV-Visible Spectrophotometer (Yuchengtech, China). The caffeine measurement was performed at the wavelength of 275nm [37]. Before the measurement, 1g of milled fine coffee beans was mixed as well as dissolved into 150ml of hot distilled water. The sample was then filtered using filter paper and placed into a glass beaker. Calcium carbonate (Merck, Germany) 1.5g was added to the mixture, and subsequently, the sample was extracted four times using chloroform (Merck, Germany) 25ml. The extract was then heated using a laboratory oven to remove the remains of the chloroform. Before sample analysis using a Spectrophotometer, the extract of the caffeine was subsequently diluted 10 times dilution of distilled water [38]. The caffeine degradation efficiency was also assessed to evaluate the effectiveness of the decaffeination of the coffee fermented using a starter derived from the selectively enriched microbe caffeine utilization. The caffeine degradation was measured based on the initial and final caffeine content in the coffee beans.

2.5 Statistical analysis

The experimental data collected during in vitro civet coffee fermentation were statistically analyzed using descriptive statistical analysis methods. To ensure reproducible data, the data from fermentation samples collected was replicated in duplicate. The obtained data analysis was to evaluate the effects of caffeine-degrading microbial culture generated from the selective enrichment process on the physical-chemical characteristics of arabica and robusta coffee beans fermented in vitro, compared to civet coffee beans generated in vivo.

3. RESULTS AND DISCUSSION

3.1 Microbial cultivation

The results of the current study showed that the growth of microbes occurred during the cultivation process. The microbial growth, as indicated by the OD number, continuously increased from day 1 to day 4 of incubation and reached a stable value from day 4 to day 5 of the incubation period. During this period, the mixed microbial culture was selectively enriched with the addition of caffeine as the only substrate. This may indicate that the culture has somewhat adapted to the utilization of caffeine. As depicted in Figure 1, the OD of the culture gradually increased from 0.066 to 0.230. Besides, during the incubation period pH of the culture was stable between 7.5 and 7.6, which was quite feasible for the fermentation process [39, 40]. The results suggested that the selectively enriched mixed microbial culture generated was certainly worthwhile to use as an active starter for fermenting coffee fruits to form volatile fatty acids (VFA) and degrade or reduce their caffeine content. This is because caffeine and VFA were essential substances of the coffee beans, determining the quality of the coffee beans and the consumers' preference [41, 42]. Hence, the use of microbial starters adapted to caffeine utilization would be significant for the development of coffee production.

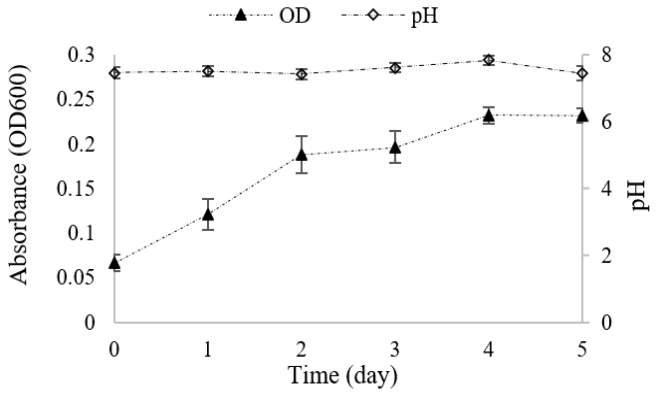

Figure 1. Microbial growth as measured by absorbance (OD600) and pH of the inoculum supplemented with caffeine during the process of selective enrichment

3.2 Characteristics of coffee cherries before fermentation

In this current study, two varieties of coffee cherries utilized in the experiments included arabica and robusta coffee cherries. The initial properties of the coffee cherries before fermentation are depicted in Table 2. Results revealed that robusta coffee cherries had more caffeine (2.11%) than arabica coffee cherries (1.83%). This agreed with some studies describing that robusta coffee had relatively higher levels of caffeine content in comparison to that of Arabica [43, 44]. They reported that Robusta coffee beans not only have more caffeine content than Arabica coffee beans, but they also carry a stronger and more naturally bitter taste [45]. Hence, the use of a starter enriched with caffeine utilization generated in this study would potentially be feasible to reduce the caffeine content of the coffee and thereby generate decaffeinated coffee beans. This is significant because some people prefer decaffeinated coffee over regular coffee. This is because a study mentioned that the consumption of decaffeinated coffee may have health benefits, such as improving insulin sensitivity [46].

Results revealed that the alkalinity of robusta coffee cherries was slightly higher (1.74 mmol/L) than arabica coffee cherries (1.18 mmol/L). Besides, both coffee cherries were somewhat too acidic, and the pH of robusta coffee cherries was slightly higher (pH 5.60) than arabica coffee cherries (pH 5.48). Hence, the use of inoculum that had a higher pH level (pH 7.5) than that of the coffee cherries would be significant. This is because the combination may generate synergetic effects during the fermentation process [47, 48], and thereby could optimize the conversion of organic materials of the coffee cherries to form useful metabolites that may improve the quality of coffee beans.

As presented in Table 2, both arabica and robusta coffee cherries had substantial nutrients and minerals required for fermentation, including solid content (60-61%) and ash content (5.0-6.5%). Further, the coffee cherries used in these experiments contained soluble carbohydrates, which were between 19 and 21mmol/L glucose. The presence of carbohydrates in the fermentation process is very crucial. This is because the carbohydrates may be utilized by fermentative microbes as their sources of carbon and energy, and converted into fermentation end-products [49]. The metabolites, or fermentation end-products, generated during the coffee fermentation included volatile and non-volatile fatty acids. These components normally diffuse into the coffee beans, alter their properties, and eventually enhance their flavor and aroma [50].

Table 2. Physico-chemical characteristics of *arabica and robusta* coffee cherries before fermentation

Parameters	Unit	Arabica	Robusta
Caffeine	g/100g	1.83±0.02	2.11±0.03
Alkalinity	mmol/L	1.18±0.03	1.74±0.01
pH	-	5.48±0.02	5.6±0.1
Solid content	%	59.96±0.01	60.5±0.15
Ash content	%	4.97±0.02	6.52±0.02
Moisture content	%	40±0.2	39.4±0.1
Soluble carbohydrate	mM glucose	21.09±0.03	19.43±0.01

3.3 Fermentation process of coffee cherries by the civet digestive mixed microbial culture

3.3.1 pH of the coffee during in vitro civet coffee fermentation

The results of the current study revealed that within 2 hours of the fermentation period, the pH of both arabica and robusta coffee cherries inoculated with a mixed culture of civet digestive microbes dropped from around 6 to 5.50 (Figure 2). After 2 hours of fermentation period, the pH of the fermented arabica coffee cherries continuously decreased from 5.20 to 4.31, while the pH of the fermented robusta coffee cherries did not undergo a significant drop as arabica coffee did. Results showed that from 2 hours to 48 hours of incubation, the pH of the fermented robusta coffee cherries dropped from 5.71 to 5.65. This occurred since robusta coffee had higher alkalinity (1.74 mmol/L) than arabica coffee (1.18 mmol/L). The high alkalinity of the robusta coffee cherries suggests they have high buffering capacity that may have the ability to neutralize the acids formed during the fermentation process [51].

3.3.2 VFA formation during in vitro civet coffee fermentation

As a volatile organic compound, volatile fatty acids (VFA) formation during coffee fermentation may also affect the

quality of coffee beans by improving as well as enriching their aroma and flavors [52]. In this present study, VFA formation occurred within 2 hours of incubation in at which time both arabica and robusta coffee generated around 1.4 g/L VFA (Figure 3). Some factors affecting the formation of VFA during the fermentation process typically include the types of microorganisms present in the culture, the types of substrates supplemented and their composition, conversion rate, alkalinity, and pH of the fermentation culture [53, 54].

The production of VFA in the fermented arabica coffee reached a peak at 4 hours of incubation, in which the concentration of VFA produced was about 3.45 g/L VFA. At this period, the pH of the fermented arabica coffee was around 5.10, which may be feasible for VFA production [55]. From 20 hours to 24 hours of incubation, the concentration of VFA in the fermented arabica coffee dropped from 3.40 to 1.40 g/L (Figure 3). The decrease in VFA during this period may be attributed to an extremely low pH level (4.20), which may inhibit the formation of VFA. On the other hand, the formation of VFA in the fermented robusta coffee reached a peak at 20 hours of incubation, at approximately 3.50 g/L. At this stage, the pH of the fermented robusta coffee was around 5.0. After 20 hours of incubation, a drop of VFA content in the fermented robusta coffee occurred, in which at this phase the concentration decreased from 3.50 to 1.04 g/L. This phenomenon would also be related to a drop in pH in the fermented robusta coffee from 5.0 to 4.60, which may restrict the VFA production. This is because some VFA producers are suppressed at an extremely low pH (<5.0) [56]. Studies report that the formation of VFA as a fermentation end-product may be altered when the pH is too acidic. When the pH of the culture drops from 5.0 to 4.0, the formation of metabolites in the fermentation process may be taken over by lactic acid producers. The formation of lactic acid may occur at an extremely low pH (<4.50). The lactic acid could be generated under extremely acidic conditions, as the pKa of lactic acid is about 3.80 [57, 58].

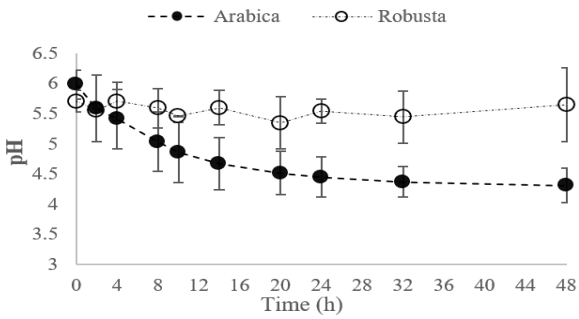

Figure 2. pH of different types of coffee cherries during the fermentation period

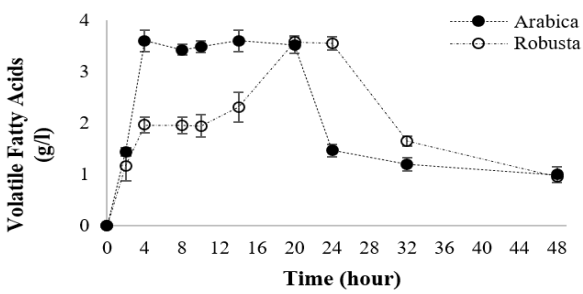

Figure 3. VFA formation of different types of coffee cherries during the fermentation period

3.3.3 Carbohydrate concentration during coffee fermentation

Results of the present study revealed that within 4 hours of incubation, soluble carbohydrates in the fermented arabica and robusta coffee dropped drastically from 20 to 5.0 mmol/l glucose. At this stage, the rate of the carbohydrates converted during the fermentation process was around 2.50 mmol/l per hour. A high conversion rate would be attributed to an active starter introduced to the culture as the starter was generated from a selective enrichment process (Figure 1). After a 24-hour fermentation period, the soluble carbohydrates detected were merely 1.3 mmol/l glucose (Figure 4). During the fermentation of coffee cherries, fermentative microbes degrade and convert their pulps and mucilage to form metabolites, including volatile fatty acids, lactic acid, and alcohols. The fermentation end-products would alter the physicochemical properties of the coffee beans and subsequently modulate the coffee flavors and aroma [59].

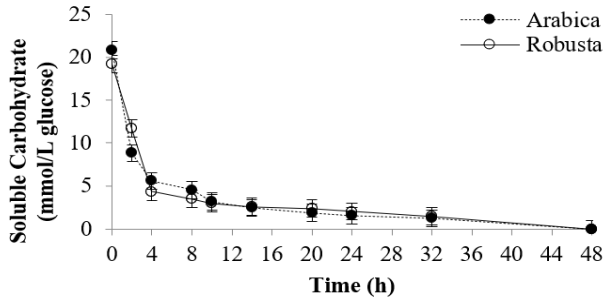

Figure 4. Soluble carbohydrate concentration of different types of coffee cherries fermented with the civet digestive mixed microbial culture

3.3.4 Caffeine degradation during in vitro civet coffee fermentation

The process of decaffeination via fermentation is a significant method since it can reduce and degrade the caffeine content of the coffee beans without changing the flavors and aroma of the coffee [26, 60]. Compared with other decaffeination methods (such as supercritical CO_2, solvent decaffeination, water decaffeination), the advantages of bio-fermentation decaffeination include retaining flavor precursors, lower energy, and investment cost [61]. Besides, by affecting multiple compounds rather than just a single component, fermentation significantly impacts aroma and taste.

The results of the present study revealed that during the coffee fermentation, the process of decaffeination of Arabica coffee was faster than that of Robusta coffee. As depicted in Figure 5, within 24 hours of incubation, caffeine content of arabica coffee decreased from 3.90 to 2.0% while within this period, caffeine content of robusta coffee reduced from 4.00 to 3.00%. At this stage, the rate of decaffeination of the fermented arabica coffee (0.08% per hour) was two times higher than that of the fermented robusta coffee (0.04% per hour). After 48 hours of incubation, the caffeine content of the fermented robusta coffee (1.7%) was slightly higher than that of the arabica coffee (1.4%). Overall, both fermented arabica and robusta coffee merely reached around 63% and 60% of caffeine degradation, respectively. The low efficiency of caffeine degradation may be attributed to the extremely low pH in the fermentation culture. Besides, as the coffee fermentation culture is too acidic, it would restrict the microbial activity and thereby may inhibit the fermentative microbes from degrading the caffeine of the coffee.

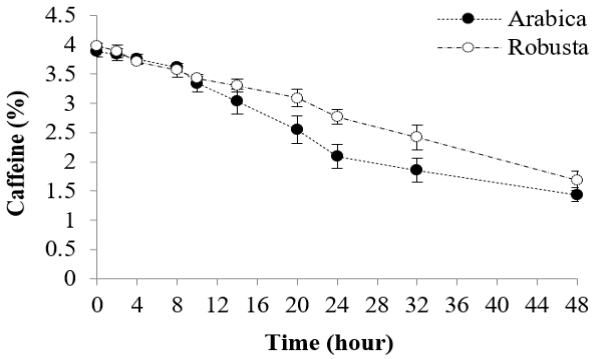

Figure 5. Caffeine of different types of coffee cherries fermented with the civet digestive mixed microbial culture

3.4 Physicochemical characteristics of civet coffee fermented in vitro and in vivo

The results of the present study revealed that the caffeine content of both arabica (1.46%) and robusta coffee (1.65%) beans was somewhat higher than that of the original civet (luwak) coffee (1.32%) (Table 3). This suggested that the decaffeination that occurred in the in-vivo fermentation of civet was much better than in-vitro fermentation. This would be related to the active complex microbial consortia and buffering system present in the digestive system of the civet [30]. The low decaffeination rate of coffee fermented in vitro would also be attributed to the pH level. The results showed that the pH of coffee fermented in vivo by civet (pH 5.33) was higher than that of coffee fermented in vitro, including arabica (pH 3.81) and robusta (pH 4.97) coffee beans. The low pH in the in vitro fermentation may restrict the degradation of caffeine in the coffee beans. Further, an extremely low pH in the fermentation culture would potentially inhibit the formation of volatile fatty acids. As presented in Table 3, the VFA generated in the civet coffee or in-vivo process (2.3 g/L) was double compared of the in-vitro fermentation of arabica (1.10 g/L) and robusta (1.04 g/L) coffee.

Table 3. Physicochemical characteristics of various types of fermented coffee beans

Parameters	Unit	Arabica	Robusta	Civet/Luwak Coffee
Caffeine	g/100g	1.46 ± 0.015	1.65 ± 0.01	1.32 ± 0.02
Carbohydrate	mM	ND	0 ± 0.02	0 ± 0.01
pH	-	3.81 ± 0.03	4.97 ± 0.02	5.33 ± 0.01
VFA	g/L	1.10±0.03	1.04±0.01	2.30±0.15
Moisture content	%	53.02±0.02	51.57±0.02	32.15±0.01
Ash content	%	1.45±0.015	1.41±0.01	1.50±0.02
Solid content	%	46.90±0.03	48.4±0.01	67.85±0.01
Moisture content after drying	%	11.45 ± 0.01511.62 ±0.02		11.95 ± 0.01

ND: Not Detected

The results suggested that the high quality of the civet coffee beans may not only be attributed to their low caffeine content but also could be related to the high VFA generated. Since VFA is a volatile compound, its formation during the fermentation process may significantly improve the aroma and enrich the flavors of the fermented coffee beans [5, 62]. Besides, to have the fermented coffee beans have similar characteristics as the civet coffee beans pH of the fermentation culture should be maintained not lower than 5.0. This is significant because an extremely low pH not only inhibits the formation of VFA [63] as a volatile compound but also restricts the decaffeination process [64]. As shown in Table 3, the pH of civet coffee performed in vivo is higher than coffee fermented in vitro; this could be attributed to the buffering system in the in vivo process that can maintain the pH level. On the other hand, the in vitro fermentation performed in this study did not use any buffers or add alkaline substances that could prevent the pH from a significant drop when the organic acid is produced during the fermentation process.

4. CONCLUSION

The decaffeination process of coffee via microbial fermentation was quite effective in reducing the caffeine content of the coffee beans. The rate of decaffeination of the fermented arabica coffee was double (0.08% caffeine/h) that of the fermented robusta coffee (0.04% caffeine/h). The caffeine content of the coffee beans fermented in vitro (arabica and robusta coffee beans) was higher compared to that of the coffee fermented in vivo or civet coffee beans. The caffeine degradation efficiency of both fermented arabica and robusta coffee was around 63% and 60%, respectively. VFA formed in the civet coffee beans via the in-vivo process (2.3 g/L) was double compared to the in-vitro fermentation of arabica (1.10 g/L) and robusta (1.04 g/L) coffee. An extremely low pH in the coffee fermentation culture of this study may not only restrict the degradation of caffeine but also inhibit the formation of VFA. Hence, for future study, an increased pH level (such as maintaining pH 5.5-6.5) in the coffee fermentation culture may potentially improve caffeine degradation and enhance VFA formation.

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REFERENCES

[1] Haile, M., Kang, W.H. (2019). The role of microbes in coffee fermentation and their impact on coffee quality. Journal of Food Quality, 2019(1): 4836709. https://doi.org/10.1155/2019/4836709

[2] Samoggia, A., Riedel, B. (2018). Coffee consumption and purchasing behavior review: Insights for further research. Appetite, 129: 70-81. https://doi.org/10.1016/j.appet.2018.07.002

[3] Torok, A., Mizik, T., Jambor, A. (2018). The competitiveness of global coffee trade. International Journal of Economics and Financial Issues, 8(5): 1.

[4] Liang, N., Kitts, D.D. (2014). Antioxidant property of coffee components: Assessment of methods that define mechanisms of action. Molecules, 19(11): 19180-19208. https://doi.org/10.3390/molecules191119180

[5] de Melo Pereira, G.V., de Carvalho Neto, D.P., Júnior,

A.I.M., do Prado, F.G., Pagnoncelli, M.G.B., Karp, S.G., Soccol, C.R. (2020). Chemical composition and health properties of coffee and coffee by-products. Advances in Food and Nutrition Research, 91: 65-96. https://doi.org/10.1016/bs.afnr.2019.10.002

[6] Mahmud, M.C., Shellie, R.A., Keast, R. (2020). Unravelling the relationship between aroma compounds and consumer acceptance: Coffee as an example. Comprehensive Reviews in Food Science and Food Safety, 19(5): 2380-2420. https://doi.org/10.1111/1541-4337.12595

[7] da Silva, M.M.P., Tarone, A.G., Giomo, G.S., Ferrarezzo, E.M., Guerreiro Filho, O., Teramoto, J.R.S. (2024). Predicting best planting location and coffee cup quality from chemical parameters: An evaluation of raw Arabica coffee beans from São Paulo over two harvests. Food Research International, 195: 114911. https://doi.org/10.1016/j.foodres.2024.114911

[8] Gebreselassie, H., Tesfaye, B., Gedebo, A., Tolessa, K. (2024). Evaluation of physical and chemical characteristics of bean and cup quality of Arabica coffee genotypes grown in Southern Ethiopia. Heliyon, 10(14). https://doi.org/10.1016/j.heliyon.2024.e34378

[9] Brando, C.H., Brando, M.F. (2014). Methods of coffee fermentation and drying. Cocoa and Coffee Fermentations, 367-396.

[10] Girma, B., Sualeh, A. (2022). A review of coffee processing methods and their influence on aroma. International Journal of Food Engineering and Technology, 6(7): 7-16. https://doi.org/10.11648/j.ijfet.20220601.12

[11] Febrianto, N.A., Zhu, F. (2023). Coffee bean processing: Emerging methods and their effects on chemical, biological and sensory properties. Food Chemistry, 412: 135489. https://doi.org/10.1016/j.foodchem.2023.135489

[12] Silva, C.F. (2014). Microbial activity during coffee fermentation. Cocoa and Coffee Fermentations, 368-423.

[13] de Carvalho Neto, D.P., de Melo Pereira, G.V., Finco, A.M., Letti, L.A., da Silva, B.J., Vandenberghe, L.P., Soccol, C.R. (2018). Efficient coffee beans mucilage layer removal using lactic acid fermentation in a stirred-tank bioreactor: Kinetic, metabolic and sensorial studies. Food Bioscience, 26: 80-87. https://doi.org/10.1016/j.fbio.2018.10.005

[14] Muliawati, A., Bulan, R. (2022). Performance of mixed-microbial culture from civet fecal suspensions on physicochemical composition of wet fermented Arabica coffee. Current Research in Nutrition and Food Science, 10(3): 918-927. http://dx.doi.org/10.12944/CRNFSJ.10.3.9

[15] Osorio, V., Medina, R., Acuña, J.R., Pabón, J., Álvarez, C.I., Matallana, L.G., Fernández-Alduenda, M.R. (2023). Transformation of organic acids and sugars in the mucilage and coffee beans during prolonged fermentation. Journal of Food Composition and Analysis, 123: 105551. https://doi.org/10.1016/j.jfca.2023.105551

[16] Elhalis, H., Cox, J., Zhao, J. (2023). Coffee fermentation: Expedition from traditional to controlled process and perspectives for industrialization. Applied Food Research, 3(1): 100253. https://doi.org/10.1016/j.afres.2022.100253

[17] Júnior, A.I.M., de Carvalho Neto, D.P., de Melo Pereira, G.V., da Silva Vale, A., Medina, J.D.C., de Carvalho, J.C., Soccol, C.R. (2021). A critical techno-economic analysis of coffee processing utilizing a modern fermentation system: Implications for specialty coffee production. Food and Bioproducts Processing, 125: 14-21. https://doi.org/10.1016/j.fbp.2020.10.010

[18] Bastian, F., Hutabarat, O.S., Dirpan, A., Nainu, F., Harapan, H., Emran, T.B., Simal-Gandara, J. (2021). From plantation to cup: Changes in bioactive compounds during coffee processing. Foods, 10(11): 2827. https://doi.org/10.3390/foods10112827

[19] Ferreira, L.J.C., de Souza Gomes, M., de Oliveira, L.M., Santos, L.D. (2023). Coffee fermentation process: A review. Food Research International, 169: 112793. https://doi.org/10.1016/j.foodres.2023.112793

[20] Vinícius de Melo Pereira, G., Soccol, V.T., Brar, S.K., Neto, E., Soccol, C.R. (2017). Microbial ecology and starter culture technology in coffee processing. Critical Reviews in Food Science and Nutrition, 57(13): 2775-2788. https://doi.org/10.1080/10408398.2015.1067759

[21] da Mota, M.C.B., Batista, N.N., Rabelo, M.H.S., Ribeiro, D.E., Borém, F.M., Schwan, R.F. (2020). Influence of fermentation conditions on the sensorial quality of coffee inoculated with yeast. Food Research International, 136: 109482. https://doi.org/10.1016/j.foodres.2020.109482

[22] Mahyudi, R., Sundari, D., Muliawati, A. (2021). Influence of solid state fermentation on Robusta coffee (Coffea cannephora) beans inoculated with microbial culture of civet fecal suspensions. Research on Crops, 22(4): 968-976. hhttps://doi.org/10.31830/2348-7542.2021.158

[23] Cruz-O'Byrne, R., Piraneque-Gambasica, N., Aguirre-Forero, S. (2021). Microbial diversity associated with spontaneous coffee bean fermentation process and specialty coffee production in northern Colombia. International Journal of Food Microbiology, 354: 109282. https://doi.org/10.1016/j.ijfoodmicro.2021.109282

[24] Cardoso, W.S., Agnoletti, B.Z., de Freitas, R., de Abreu Pinheiro, F., Pereira, L.L. (2021). Biochemical aspects of coffee fermentation. In Quality Determinants in Coffee Production, pp. 149-208. https://doi.org/10.1007/978-3-030-54437-9_4

[25] Carder, G., Proctor, H., Schmidt-Burbach, J., D'cruze, N. (2016). The animal welfare implications of civet coffee tourism in Bali. Animal Welfare, 25(2): 199-205. https://doi.org/10.7120/09627286.25.2.199

[26] Chindapan, N., Puangngoen, C., Devahastin, S. (2025). Caffeine removal and compositions losses from whole Robusta coffee beans during conventional and ultrasound-assisted aqueous decaffeination. Journal of Food Engineering, 387: 112349. https://doi.org/10.1016/j.jfoodeng.2024.112349

[27] Torrez, V., Benavides-Frias, C., Jacobi, J., Speranza, C.I. (2023). Ecological quality as a coffee quality enhancer. A review. Agronomy for Sustainable Development, 43(1): 19. https://doi.org/10.1007/s13593-023-00874-z

[28] Poole, R.L., Tordoff, M.G. (2017). The taste of caffeine. Journal of Caffeine Research, 7(2): 39-52. https://doi.org/10.1089/jcr.2016.0030

[29] Darwin, Bulan, R., Hapirayani, Muliawati, A., Faradilla, Irnanda, A.I., Kaban, M.B. (2023). Carbohydrate enrichment in in-vitro civet coffee fermentation: Impact on arabica coffee beans. International Journal of Design & Nature and Ecodynamics, 18(6): 1485-1492.

https://doi.org/10.18280/ijdne.180623
[30] Watanabe, H., Ng, C.H., Limviphuvadh, V., Suzuki, S., Yamada, T. (2020). Gluconobacter dominates the gut microbiome of the Asian palm civet Paradoxurus hermaphroditus that produces kopi luwak. PeerJ, 8: e9579. https://doi.org/10.7717/peerj.9579
[31] Darwin, Pratama, A., Mardhotillah. (2021). Biodiesel Production from Waste Frying Oils by Potassium Methoxide Supported on Activated Carbon Catalysts from Lignocellulosic Biomass, Acta Technologica Agriculturae, 24(4): 153-158. https://doi.org/10.2478/ata-2021-0026
[32] McBirney, S.E., Trinh, K., Wong-Beringer, A., Armani, A.M. (2016). Wavelength-normalized spectroscopic analysis of Staphylococcus aureus and Pseudomonas aeruginosa growth rates. Biomedical Optics Express, 7(10): 4034-4042. https://doi.org/10.1364/BOE.7.004034
[33] Darwin, D.D., Purwanto, S., Rinaldi, R. (2019). Removal of organic pollutants from tofu-processing wastewater through anaerobic treatment process with short hydraulic retention time. Environmental Research, Engineering and Management, 75(1): 34-42. https://doi.org/10.5755/j01.erem.75.1.21532
[34] Darwin, Diana, N., Mardhotillah, Pratama, A. (2021). Anaerobic co-digestion of cow manure and palm oil mill effluent (POME): Assessment of methane production and biodegradation efficiency. International Journal of Design and Nature and Ecodynamics, 16(6): 671-676. https://doi.org/10.18280/ijdne.160608
[35] Darwin. (2019). Determination of glucose concentration in anaerobic acidification cultures by portable glucose monitoring system. Asian Journal of Chemistry, 31: 763-766.
[36] Lützhøft, H.C.H., Boe, K., Fang, C., Angelidaki, I. (2014). Comparison of VFA titration procedures used for monitoring the biogas process. Water Research, 54: 262-272. https://doi.org/10.1016/j.watres.2014.02.001
[37] Robertson, A.H., Fisher, H.J., Horwitz, W. (2012). Official Method of Analysis of the Association of Official Agricultural Chemysts. Literary Licensing, LLC, USA.
[38] Danhelova, H., Hradecky, J., Prinosilova, S., Cajka, T., Riddellova, K., Vaclavik, L., Hajslova, J. (2012). Rapid analysis of caffeine in various coffee samples employing direct analysis in real-time ionization-high-resolution mass spectrometry. Analytical and Bioanalytical Chemistry, 403: 2883-2889. https://doi.org/10.1007/s00216-012-5820-2
[39] Lin, C.Y., Hung, C.H., Chen, C.H., Chung, W.T., Cheng, L.H. (2006). Effects of initial cultivation pH on fermentative hydrogen production from xylose using natural mixed cultures. Process Biochemistry, 41(6): 1383-1390. https://doi.org/10.1016/j.procbio.2006.01.021
[40] Darwin, Ilham, M., Fazil, A. (2018). Performance and kinetic study of the anaerobic co-digestion of cocoa husk and digested cow manure with high organic loading rate. INMATEH-Agricultural Engineering, 55(2): 131-140.
[41] Angeloni, S., Mustafa, A.M., Abouelenein, D., Alessandroni, L., Acquaticci, L., Nzekoue, F.K., Petrelli, R., Sagratini, G., Vittori, S., Torregiani, E., Caprioli, G. (2021). Characterization of the aroma profile and main key odorants of espresso coffee. Molecules, 26(13): 3856. https://doi.org/10.3390/molecules26133856
[42] Rusinek, R., Dobrzański Jr, B., Oniszczuk, A., Gawrysiak-Witulska, M., Siger, A., Karami, H., Ptaszyńska, A.A., Żytek, A., Kapela, K., Gancarz, M. (2022). How to identify roast defects in coffee beans based on the volatile compound profile. Molecules, 27(23): 8530. https://doi.org/10.3390/molecules27238530
[43] Shokouh, P., Jeppesen, P.B., Christiansen, C.B., Mellbye, F.B., Hermansen, K., Gregersen, S. (2019). Efficacy of arabica versus robusta coffee in improving weight, insulin resistance, and liver steatosis in a rat model of type-2 diabetes. Nutrients, 11(9): 2074. https://doi.org/10.3390/nu11092074
[44] Paramartha, D.N.A., Fatinah, A., Nofrida, R., Rahayu, N., Anggraini, I.M.D., Utama, Q.D. (2023). The chemical characteristics of arabica and robusta green coffee beans from Geopark Rinjani, Indonesia. Biotropia, 30(3): 318-328.
[45] Deribe, H. (2019). Review on factors which affect coffee (Coffea Arabica L.) quality in South Western, Ethiopia. International Journal of Forestry and Horticulture, 5(1): 12-19. http://dx.doi.org/10.20431/2454-9487.0501003
[46] Reis, C.E., Paiva, C.L.D.S., Amato, A.A., Lofrano-Porto, A., Wassell, S., Bluck, L.J., Dórea, J.G., da Costa, T.H. (2018). Decaffeinated coffee improves insulin sensitivity in healthy men. British Journal of Nutrition, 119(9): 1029-1038. https://doi.org/10.1017/S000711451800034X
[47] Darwin, Cheng, D.J.J., Liu, Z., Gontuphil, J. (2016). Anaerobic co-digestion of cocoa husk with digested swine manure: Evaluation of biodegradation efficiency in methane productivity. Agricultural Engineering International: CIGR Journal, 18(4): 147-156.
[48] Darwin, Triovanta, U., Rinaldi, R. (2019). Two-stage anaerobic co-digestion of landfill leachate and starch wastes using anaerobic biofilm reactor for methane production. Progress in Agricultural Engineering Sciences, 15(1): 53-70. https://doi.org/10.1556/446.15.2019.1.4
[49] Liu, C., Ren, L., Yan, B., Luo, L., Zhang, J., Awasthi, M.K. (2021). Electron transfer and mechanism of energy production among syntrophic bacteria during acidogenic fermentation: A review. Bioresource Technology, 323: 124637. https://doi.org/10.1016/j.biortech.2020.124637
[50] Elhalis, H., Cox, J., Frank, D., Zhao, J. (2020). The crucial role of yeasts in the wet fermentation of coffee beans and quality. International Journal of Food Microbiology, 333: 108796. https://doi.org/10.1016/j.ijfoodmicro.2020.108796
[51] Ramos, S.C., Kim, S.H., Jeong, C.D., Mamuad, L.L., Son, A.R., Kang, S.H., Cho, Y.I., Kim, T.G., Lee, J.S., Cho, K.K., Lee, S.S., Lee, S.S. (2022). Increasing buffering capacity enhances rumen fermentation characteristics and alters rumen microbiota composition of high-concentrate fed Hanwoo steers. Scientific Reports, 12(1): 20739. https://doi.org/10.1038/s41598-022-24777-3
[52] Zhao, L., Wang, Y., Wang, D., He, Z., Gong, J., Tan, C. (2023). Effects of different probiotics on the volatile components of fermented coffee were analyzed based on headspace-gas chromatography-ion mobility spectrometry. Foods, 12(10): 2015. https://doi.org/10.3390/foods12102015

[53] Darwin, Pratama, A., Mardhotillah (2021). Organic waste conversion via continuous anaerobic co-digestion of oil palm empty fruit bunches and cow manure: Evaluation of feeding regime on methane production. Acta Technologica Agriculturae, 24(1): 8-13.
[54] Darwin, Charles, W., Cord-Ruwisch, R. (2019). Anaerobic acidification of sugar-containing wastewater for biotechnological production of organic acids and ethanol. Environmental Technology, 40(25): 3276-3286. https://doi.org/10.1080/09593330.2018.1468489
[55] Darwin, Cord-Ruwisch, R. (2019). Thermodynamics of anaerobic digestion: Mechanism of suppression on biogas production during acidogenesis. INMATEH-Agricultural Engineering, 57(1): 287-301.
[56] Lv, N., Cai, G., Pan, X., Li, Y., Wang, R., Li, J., Li, C., Zhu, G. (2022). pH and hydraulic retention time regulation for anaerobic fermentation: Focus on volatile fatty acids production/distribution, microbial community succession and interactive correlation. Bioresource Technology, 347: 126310. https://doi.org/10.1016/j.biortech.2021.126310
[57] Darwin, Bulan, R., Humaira, T., Muliawati, A., Agustina, F., Baszanova, G.S.T. (2023). Impact of concentrate supplementation on biodegradability and acidosis in in-vitro rumen fermentation of forage. International Journal of Design & Nature and Ecodynamics, 18(4): 877-883.
[58] Darwin, Triovanta, U., Rinaldi, R., Pratama, A. (2019). Anaerobic acidification of coconut water waste by Lactobacillus acidophilus culture for biotechnological production of lactic acid. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 67(6): 1433-14440. https://doi.org/10.11118/actaun201967061433
[59] Todhanakasem, T., Van Tai, N., Pornpukdeewattana, S., Charoenrat, T., Young, B.M., Wattanachaisaereekul, S. (2024). The relationship between microbial communities in coffee fermentation and aroma with metabolite attributes of finished products. Foods, 13(15): 2332. https://doi.org/10.3390/foods13152332
[60] Lukman, K., Muhammad, A., Shehu, D., Babandi, A., Yakasai, H.M., Ibrahim, S. (2023). Potential of microbial-decaffeination process: A review. Journal of Applied Sciences and Environmental Management, 27(9): 1915-1924. https://doi.org/10.4314/jasem.v27i9.4
[61] Shofinita, D., Lestari, D., Purwadi, R., Sumampouw, G. A., Gunawan, K.C., Ambarwati, S.A., Achmadi, A.B., Tjahjadi, J.T. (2024). Effects of different decaffeination methods on caffeine contents, physicochemical, and sensory properties of coffee. International Journal of Food Engineering, 20(8): 561-581. https://doi.org/10.1515/ijfe-2024-0013
[62] Hashimoto, E.H., de Cassia Campos Pena, A., da Cunha, M.A.A., de Freitas Branco, R., de Lima, K.P., Couto, G. H., Binder Pagnoncelli, M.G. (2024). Fermentation-mediated sustainable development and improvement of quality of plant-based foods: From waste to a new food. Systems Microbiology and Biomanufacturing, 1-32. https://doi.org/10.1007/s43393-024-00292-6
[63] Jankowska, E., Chwiałkowska, J., Stodolny, M., Oleskowicz-Popiel, P. (2015). Effect of pH and retention time on volatile fatty acids production during mixed culture fermentation. Bioresource Technology, 190: 274-280. https://doi.org/10.1016/j.biortech.2015.04.096
[64] Gummadi, S.N., Santhosh, D. (2006). How induced cells of Pseudomonas sp. increase the degradation of caffeine. Central European Journal of Biology, 1: 561-571. https://doi.org/10.2478/s11535-006-0032-4