

Process technology of luwak coffee through bioreactor utilization

M Hadipernata* and S Nugraha

Indonesian Center for Agricultural Postharvest Research and Development, Ministry of Agriculture

Jl. Tentara Pelajar No 12 Bogor 16114, Jawa Barat Indonesia

*Email: mulya_nata@yahoo.com

Abstract. Indonesia has an advantage in producing exotic coffee that is Luwak coffee. Luwak coffee is produced from the fermentation process in digestion of civet. Luwak coffee production is still limited due to the difficulty level in the use of civet animals as the only medium of Luwak coffee making. The research was conducted by developing technology of luwak coffee production through bioreactor utilization and addition the bacteria isolate from gastric of civet. The process conditions in the bioreactor which include temperature, pH, and bacteria isolate of civet are adjusted to the process that occurs in civet digestion, including peristaltic movement on the stomach and small intestine of the civet will be replaced by the use of propellers that rotate on the bioreactor. The result of research showed that proximat analysis data of artificial/bioreactor luwak coffee did not significant different with original luwak coffee. However, the original luwak coffee has higher content of caffeine compared to bioreactor luwak coffee. Based on the cuping test the bioreactor luwak coffee has a value of 84.375, while the original luwak coffee is 84.875. As the result, bioreactor luwak coffee has excellent taste that similiar with original luwak coffee taste.

Keywords: *Luwak coffee, Bioreactor, Civet*

1. Introduction

Civet (*Paradoxurus Hermaphrodirus*) or Luwak in Indonesian language is wild animal that actively at night and tends to behave cannibals when gathered with a smaller civet. Civet eat fruits such as palm fruit, papaya, and banana. Also, civet animal eat coffee berries because the sweet taste of outer skin of coffee berries. Coffee berries undergoes a fermentation process for 12 hours in a civet digestive system containing enzymes and bacterial isolates. Non-digestible coffee beans come out with feces in the excretion process. Civet feces are collected, cleaned and dried with sunlight.

Luwak coffee has a distinctive taste and unique, so it has a high selling price in the international coffee market [1,2]. The production of luwak coffee is still limited because the civet as the only medium of luwak coffee making. The added value of luwak coffee is very large when compared to ordinary coffee. The price of luwak coffee is more than twenty time of ordinary coffee.

There are several problems from the production of Luwak coffee. The main problem is the possibility of coffee contamination with *E.coli* and *Salmonella* if luwak coffee is not properly cleaned [3]. Furthermore, there is no Halal guarantee products for Moslem people because coffee beans come out with civet feces. In addition, luwak coffee production by civet cause some cases of animal abuse.



The research related to the production of artificial luwak coffee has been done such as research on production of probiotic luwak coffee [4]. The probiotic microbial inoculum is taken from the small intestine of civet animals. During fermentation process of probiotic coffee bean, the probiotic microbial is added into coffee bean in fermentation tank. Fermentation process of probiotic luwak coffee is different compared to fermentation process in civet animal. So, the taste of probiotic luwak coffee does not have the same quality as the original luwak coffee.

The objective research is developing technology of luwak coffee production through bioreactor utilization and addition the bacteria isolate from civet animal. The process conditions in the bioreactor which include temperature, pH, and bacteria isolate of civet are adjusted to the process that occurs in civet digestion, including peristaltic movement on the stomach and small intestine of the civet will be replaced by the use of propellers that rotate on the bioreactor.

2. Materials and Methods

2.1. Materials

Coffee berries was obtained from farmer field in Pangalengan Subdistrict, Bandung, Jawa Barat. Bioreactor used was Biotron Liflus GX type from Intran Technologies Sdn Bhd, Malaysia.

2.2. Methods

Bioreactor luwak coffee was processed by artificial digestive system of civet in bioreactor with 5 lt capacity. The process of fermentation in the bioreactor can be seen in Figure 1. Coffee berries peeled then weighed 1.5 kg and put into the bioreactor. Simulated Gastric Fluid (SGF) with pH 1.2 to 2.5 were added 2.5 liter. Fermentation process in bioreactor according to the condition of civet digest, such as: pH, temperature, fermentation time, propeller stirring rate and bacterial isolates addition. The level of acidity or pH value is 1.5 to 4.5 [5]. Temperature is determined based on the temperature conditions inside the civet digest that is about 25°C to 35°C [6]. Stirring rate is set at 50 rpm.

The first stage of the *in vitro* digesting process is the process of acidifying the coffee beans in the SGF solution. This process lasts for 2 hours. After that pH neutralization using NaOH solution to obtain pH 6.5 to 7.5 for the second stage preparation. The third stage is the addition of lactic acid bacteria. Lactic acid bacteria used is bacterial isolate from civet animal that is 5 isolate from luwak. The amount of BAL added in the bioreactor process is 108 cfu/ml to 109 cfu/ml [6].

After fermentation process for 12 hours in bioreactor, the fermented coffee bean will be washed and then dried to a moisture content of 11-12%. Coffee beans are still wrapped in endocarp skin, then will be peeled using peeler and ready to roasted by using roaster machine, then grinded until ready for consumption. The bioreactor luwak coffee will be analyzed physically, chemically and tastefully and compared with the original luwak coffee. The original luwak coffee produced from civet animals.

The water content analysis was performed by oven method according to AOAC procedure in 1996 [7], fat content according to AOAC procedure in 2006 [8], protein content according to AOAC in 1995 [9], ash content according to AOAC 2006 [8], while carbohydrate analysis based on by difference calculation.

Determination of microbial count was done by using PCA media. A total of 10 g of sample was incorporated into 90 ml 85% NaCl and stirred until homogeneous, then diluted to dilution 10^7 . The amount was then calculated by plate count method with some dilution series after incubation at 37°C. for 48 hours. Then calculated total plate count (TPC) based on standard plate count. Coliform and Salmonella analysis was performed using EMBA media and XLD media. While, yeast mold analysis was done using PDA media.

The cupping test of bioreactor luwak coffee and original luwak coffee were done in cupping test laboratory, Coffee and Cocoa Research Center in Jember. The cupping test was performed by a cupping test expert who has earned an American standard certificate and a European standard certificate.



Figure 1. Fermentation process of coffee in bioreactor

3. Results and Discussion

The fermentation process of coffee in bioreactor describes the fermentation process of coffee that occurs in the stomach, intestines and colon civets. The addition of SGF solution is simulation of the existing solution in the civet stomach. The second stage process in bioreactor is condition in the intestine of civet animals. The third stage is the addition of lactic acid bacteria that resembles the fermentation process in the colon. The amount of BAL added in the bioreactor process is 108 cfu/ml to 109 cfu/ml according to the amount of BAL found in the civet small intestine reaches more than 107-108cfu/ml [6]. Figure 2 shows the product of artificial or bioreactor luwak coffee from coffee berries (a), coffee bean with endocarp skin (b), green bean coffee (c), and roasted coffee. Coffee bean with endocarp skin was obtain after fermentation process in bioreactor, washing treatment and drying with oven or sunlight. Green bean coffee (c) was obtain after peeling process the endocarp skin of coffee. Roasted coffee (d) was obtain after roasting treatment.



Figure 2. Coffee berries (a), coffee bean with endocarp skin (b), green bean coffee (c), and roasted coffee (d)

The research result shows the proximate analysis of coffee for water content, ash content, lipid, protein and carbohydrate in Table 1. There is no significant different for water content, ash content and lipid content of bioreactor/artificial luwak coffee compared with original luwak coffee. On the other hand, protein content of artificial luwak coffee is higher compared with original luwak coffee. Suggestion that bioreactor process appreciably suppresses the degradation of protein that it caused by fermentation process. Protein degradation of protein can be caused by the acid solution in civet digestion, the pH of gastric fluid in civet lower than pH simulated gastric fluid in the bioreactor. Addition of acid solution in this research does not cause damage to coffee beans. Martinez (2007) reported that quality of coffee bean will increase after acid and enzyme treatment [10].

Table 1. Proximate analysis of roasted coffee

Treatment	Water content (%)	Ash content (%)	Lipid (%)	Protein (%)	Carbohydrate (%)
Artificial luwak coffee	2.05 ± 0.11	5.38 ± 0.22	12.21 ± 0.19	13.11 ± 0.23 ^a	67.25 ± 0.33
Original luwak Coffee	2.00 ± 0.15	5.34 ± 0.17	12.13 ± 0.22	11.08 ± 0.31 ^b	69.44 ± 0.28

Data are presented as mean ± SD (n = 3) and values with different superscript letters (a and b) in a line show the significant difference (P < 0.05)

The microbiology analysis of *Salmonella*, *Coliform* and *E.coli* showed negative results which means that the undetected microbes are present in the material. Also, analysis of yeast molds in roasted coffee beans shows a negative result which means that the material does not contain yeast molds. In the calculation of total bacteria on treatment showed that the amount of bacteria in all treatments and controls was negative. Based on Indonesian's national standard for coffee powder, the maximum amount of mold allowed in maximum powdered coffee is 10⁴ colonies/gram, while the maximum total plate count of bacteria is 10⁶ cfu/gram [11].

Table 2. Microbiological analysis of roasted coffee

Treatment	<i>Salmonella</i> (cfu/g)	<i>Coliform</i> (cfu/g)	<i>E. Coli</i> (cfu/g)	Yeast Mold (cf u/g)
Artificial luwak coffee	negatif	negatif	negatif	negatif
Original luwak coffee	negatif	negatif	negatif	negatif

Salmonella, *Coliform* and *E.coli* are indicators of bacteria on the food quality and water sanitation. The existence of these three bacteria on food can cause dangerous diseases for humans. The results of microbiological analysis are still below the maximum limit of food contamination, especially for coffee beverage products in packaging [12]. For coffee beverage products, the maximum limit of *Salmonella* contamination is negative in 100 ml, while the maximum limit of *Coliform* contamination is less than 2 in 100 ml coffee. The maximum limit of *E.coli* contamination on the packaged coffee drink is negative in 100 ml. Based on microbiology analysis, artificial/bioreactor luwak coffee and original luwak coffee meets the requirements of Indonesia's National Standard.

The effect of bioreactor treatment on artificial/bioreactor coffee taste and flavor can be seen in Table 3. The quality scale grouping in cupping test are based on the Specialty Coffee Association of America Cupping Protocols. Cupping test is one of the important testing stages in determining the quality of a product including coffee. The organoleptic test is performed to determine the sensory differences between treatments, to describe the treatment flavor and to determine the consumer's preference for the product. One method of testing coffee flavor is cupping test. The quality parameters tested using this method include flavor, after taste, acidity, body, balance, uniformity, clean cup, sweetness, defects and overall [13].

The treatment that has a total score with a fair quality scale is the treatment with a total score below 6.0. The treatment included in the Good quality scale is treated with a total score of 6.0-6.75. The total score in the range of 7.0 - 7.75 is included in the very good quality scale, while the total score in the range of 8.0 - 8.75 is included in the excellent quality scale. The cupping test results showed that the total value of the flavor of bioreactor Luwak coffee and original luwak coffee has a

value of more than 80 which means included into the class of specialty grade or special coffee group. Total score of artificial coffee and original coffee for coffee characteristic are 84.375 and 84.875. So, Luwak coffee bioreactor and original luwak coffee included into the specialty coffee, and above the premium coffee.

Yusianto *et al.* (2005) and Budryn *et al.* (2009) reported that protein will be degraded into free amino acids which is one of the typical fragrance-forming components in coffee [14,15]. During coffee roasting, maillard reaction is occurs between carbonyl groups especially from reducing sugars with amino acids, peptides and proteins [16]. The initial reaction between aldehyde or ketone groups and free amino groups is called the amino-sugar reaction [17]. The final stage of Maillard's reaction produces brown melanoidin pigments [18]. Maillard's reaction also produces a flavor in coffee [19]. During the roasting process, carbohydrates turn into water soluble polysaccharides, oligosaccharides, monomers, melanoidin, and caramels, as well as volatile components. Carbohydrates contribute to brown changes in roasted coffee, the formation of volatile components and strengthening the body. Based on American's standard coffee, the artificial luwak coffee flavor as well as the original luwak coffee belong to a very good quality scale.

Table 3. Cupping test analysis of artificial/bioreactor luwak coffee and original luwak coffee

Characteristic	Artificial luwak coffee	Original luwak coffee
Fragrance	7.75	8.00
Flavor	7.75	7.75
After Taste	7.75	7.875
Acidity	7.63	7.75
Body	7.75	7.50
Uniformity	10.00	10.00
Balance	7.88	8.00
Clean cup	10.00	10.00
Sweetness	10.00	10.00
Overall	7.88	8.00
Defect	None	None
Total score	84.375	84.875

4. Conclusion

The utilization of bioreactor technology and the addition of bacterial isolates from civet animals have produced artificial luwak coffee that has the same quality and taste as the original luwak coffee. Artificial luwak coffee technology can be developed for higher production scale compared to the production of luwak coffee using civet animal.

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