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# Application of digestive bioprocessing model on coffee fermentation

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**Abstract**
Animal coffee is one of the world's rarest and most costly coffee products. Digestive bioprocessing coffee method can bring about an improvement to the existing coffee marketing. The high cost of animal processed coffee has been due to its processing methods that have not been completely understood. Current research indicates that effects of digestive bioprocessing method on the value and quality of coffee as well as has been focused on the isolation of microbial cultures from the animal excreta and gastrointestinal tract. Therefore, this review will provide a clear understanding of digestive bioprocessing method and its application in coffee fermentation.

**Keywords:** animal coffee, digestive bioprocessing, fermentation, animal excreta, gastrointestinal tract

## Introduction

Coffee is one of the most popular beverages all over the world with widely known for its stimulant effect, health benefits, and economic importance [1, 2]. The overall quality of coffee is impacted by a variety of factors, including post-harvest procedures, which account for around 60% of the quality of green coffee beans especially changes in physicochemical qualities and sensory characteristics [3]. There are three different types of coffee processing methods including dry, wet, and semi-dry processing ones (Figure 1). Dry processing (DP) or natural methods implies to the processing that the whole coffee cherry (pulp, parchment, and bean) is dried together. After DP method, coffee has been described as having a sweet, heavy body, smooth, and complex [3, 4]. Wet processing (WP) is the common methods which requires huge amount of water and some special facilities for washing and fermentation processes, being used by many coffee producers to meet market demands for green coffee beans with higher quality [3, 5]. Coffee beans in semi-processing are fermented but not washed [6]. The variances between these three procedures have an influence on coffee's sensory profile as well as metabolic responses in coffee fruits, resulting in alterations in the chemical components of the beans [7, 8].

Fermentation of coffee is known as one of the critical processing steps during wet processing. During fermentation, the main purpose is to remove mucilage from coffee parchment and reduce water content [9]. Fermentation is carried out naturally by complex microorganisms such as yeast, bacteria, and fungus in typical coffee manufacturing by farmers. Those in coffee fermentation help to produce a variety of metabolites which affect the biochemical composition and sensory profile. However, this natural fermentation of coffee beans results in a wide range of coffee product quality. The use of starter cultures in the fermentation sector has shown favorable outcomes for a variety of fermented beverages, including shorter fermentation times and better coffee quality [10].

Besides the normal coffee beans produced by the conventional approach, there are unique varieties of coffee beans derived from the digestive systems of animals, known as "Animal coffee" [11,12]. Animal coffee (Digestive bioprocessing) is a unique coffee processing method which is passed the digestive tracts of animals, for example, Kopi Luwak (civet coffee) and Black Ivory Coffee (elephant dung coffee) [13]. This type of coffee is the most expensive in the world. Kopi Luwak (Civet coffee) have a unique flavour and aroma as musty, earthy, smooth, syrupy, and chocolaty with a price tag of USD $500 per pound [3, 11]. In comparison to normal coffee, Black Ivory Coffee has been described as having a particularly smooth flavor. It costs $1800 per kilogram, and manufacturing is limited to 200 kilograms each year [3].

Current research indicates that effects of digestive bioprocessing method on the value and quality of coffee as well as has been focused on the isolation of microbial cultures from the animal excreta and gastrointestinal tract. Therefore, this review will provide a clear understanding of digestive bioprocessing method and its application in coffee processing.

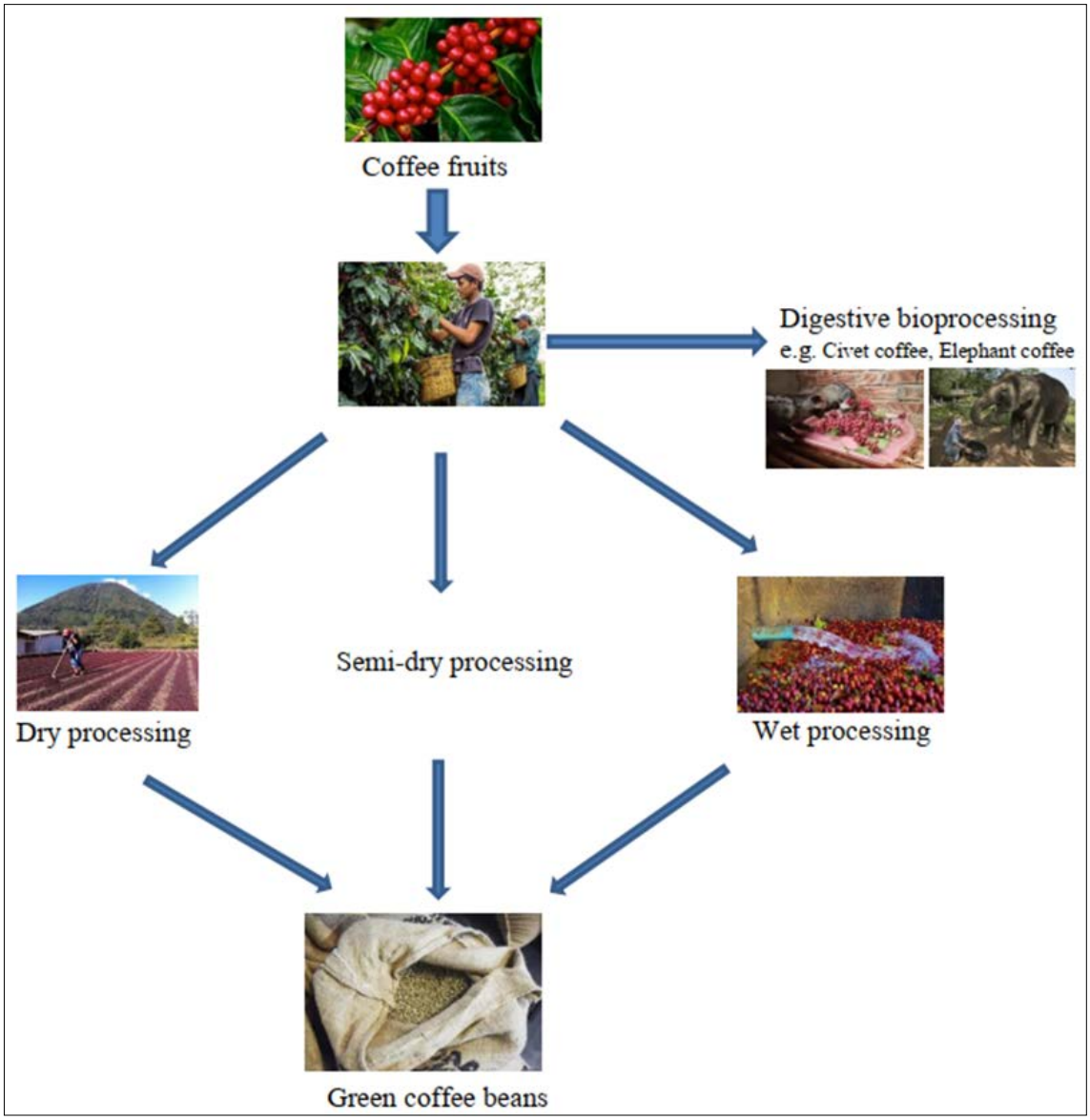


**Fig 1:** Main methods of coffee processing

**Coffee fermentation**

Fermentation is one of the most important post-harvest processing processes in wet processing that results in the highest quality coffee due to chemical reactions that breaks down complex molecules into smaller ones, resulting in liquids and gases (volatile compounds) [14]. In other words, fermentation is a metabolism in which organic substances (mostly sugar) were transformed to other molecules, such as alcohol, acids, gas, or other secondary metabolites by microorganism activities in the absence (anaerobic) or presence (aerobic) of oxygen. Coffee fermentation is a process in which microorganisms, such as yeast, mold and even bacteria break down the mucilage layer from the parchment coffee [15]. It is necessary to remove mucilage from coffee parchment and lower water content during fermentation. Furthermore, the effect of fermentation on the antioxidant activity and sensory profile of green coffee beans has recently been documented [16]. Enzymes found naturally in the coffee berry, as well as microorganisms obtained from the environment, aid in the fermentation process which help to produce a variety of metabolites [4, 14].

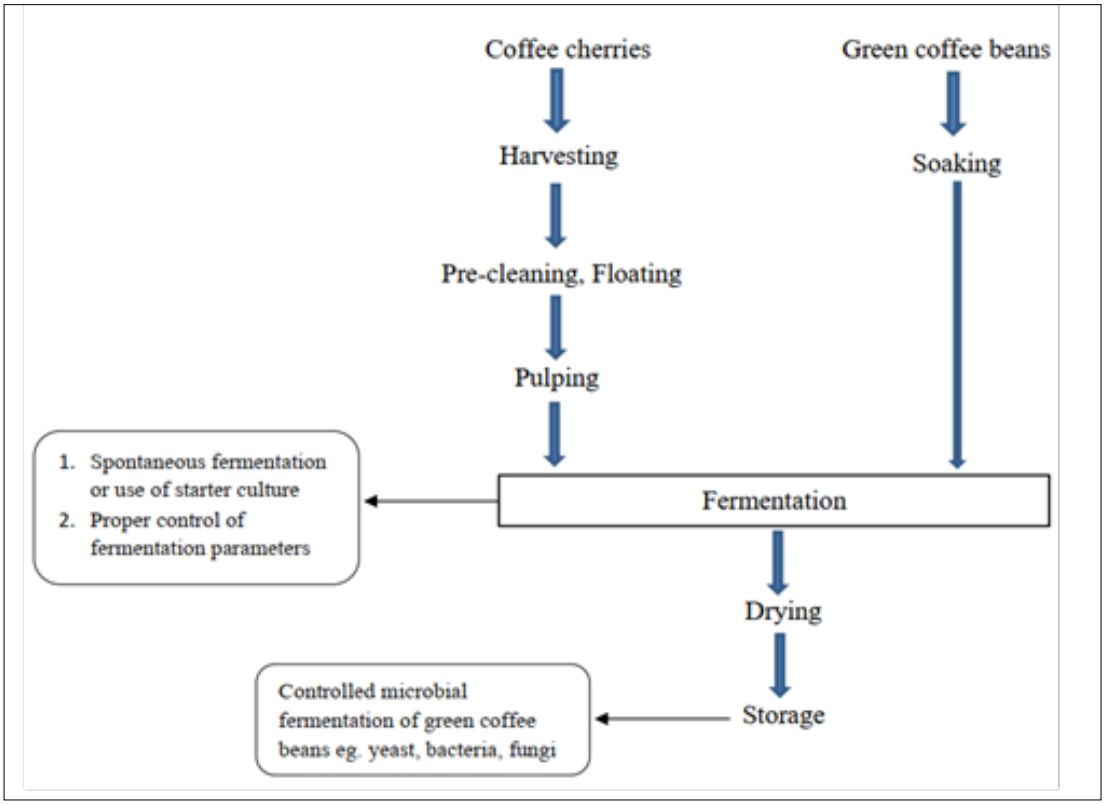


**Fig 2:** Main steps involved in coffee fermentation (modified after Schwan, R. F., *et al*., 2012) [10]

In the research of Melo Pereira, G. V., *et al*. (2014), the strong flavor-producing microorganism *Pichia fermentans* and pectinolytic *Saccharomyces* sp. were selected as starter culture for fermentation. The use of those yeasts on the wet processing of coffee results in a modified-flavor coffee product. It suggests that yeasts can play a significant role in enhancement of coffee quality by the synthesis of volatile compounds [17]. Evangelista, S. R., *et al*. (2014) reported yeasts also contribute to fermentation process in dry process. The coffee inoculated with yeast showed flavorful characteristics compared to control samples which did not have yeast activity. In this study, the treatment of *Candida parapsilosis* UFLA YCN448 and *Saccharomyces cerevisiae* UFLD YCN727 produced coffee with special aroma of caramel, herbs, and fruits [18]. Kwak, H. S., *et al*. (2018) reported similar observations on coffee fermentation with different yeasts (*Saccharomyces* species) at 30ºC for 24 hours improved the functionally of coffee by significantly increasing antioxidant properties as well as influence the aroma and flavor of coffee [16]. Afriliana, A., *et al*. (2018) provided evidence for process of Robusta coffee beans with using kefir, lactic acid bacteria (LAB) starter culture enhanced cup test value [19]. In another study, *Aspergillus tubingensis* was cultured to produce polygalacturonase and feruloyl esterase. Then, those two enzymes were treated on coffee cherries to remove the mucilage within just 3 hours which is significantly efficient compared to traditional method [20]. The food-grade mold *Rhizopus oligosporus* was inoculated to undergo solid-state fermentation of coffee [21]. The fermentation changed significantly (at 95% confidential level) to the composition of aroma compounds in green coffee, such as ethyl palmitate, 3-hydroxybutanoate (fruity odors), pyrazine, thiols, furanones, guaiacols etc. Besides, 2,3-butanediol which gave buttery and unpleasant odors and its presence in commercial green coffee beans might be considered as uncontrolled spontaneous fermentation during post-harvest processing of coffee [22]. However, the main challenge revolving around fermentation in wet processing is the lack of controllability of the process. over-fermentation results in the development of spoilage microorganisms that production of black or ‘‘stinker’’ beans and undesirable chemical compounds as well as creation optimum condition for the growth of filamentous fungi and mycotoxins with poor flavour and aroma characteristics [23, 24].

**Application of Digestive Bioprocessing models on coffee fermentation**

Digestive Bioprocessing method is coffee fermented by digestive systems of certain animals, such as weasel, elephant, or monkey. It is the highest graded coffee in terms of availability and its distinct flavour.

**Civet coffee (Kopi Luwak)**

Civet coffee (or Kopi Luwak in Indonesia, Kape Alamid in The Philippines) is coffee cherries eaten and defecated by the Asian palm civet (*Paradoxurus hermaphroditus*) before further processes have been occurred, such as washing, drying, and roasting [25]. This type of coffee has a unique flavour and aroma as musty, earthy, smooth, syrupy, mint, and chocolaty [3,11]. The civets eat ripe and qualified coffee cherries. This very first step in selection contributes to the quality of the product. They eat coffee berries to consume the sweet pulp. At oral

cavity, the pulp is removed by its teeth, but the bean remains wholesome. In stomach and intestine, the sweet pulp is digested completely by various enzymes, but the bean is just slightly affected. The hard parchment and silver skin which protect the bean from digestion and isolate it from harmful microorganisms in fecal matter. After collecting, the beans are washed, parchment and silver skin are removed before roasting, making the beans are safe to consume [26]. The caffeine content and protein content in Luwak coffee were found to be low due to bioprocessing inside the gastrointestinal tract. Coffee bean proteins are degraded into short peptides and free amino acids which are assisted by the bacteria in the gastrointestinal tract, providing a unique taste to Luwak coffee. Additionally, lipid of civet coffee is higher than its in original coffee [27]. Moreover, citric acid and malic acid were found in Civet coffee, making a special for the quality of this type of coffee [28, 29].

**Elephant dung coffee (Ivory coffee)**

Coffee cherries are gone through the digestive system of elephants. In here, the cherries are fermented together with other food fruits, such as banana or sugarcane. Final coffee has the flavor of those fruits and less bitter than normal one [12]. Nowadays, one kilogram of elephant coffee is even more expensive than civet coffee. It costs nearly 2,000 USD per 1 kg. According to Haile, M., Bae, H. M., & Kang, W. H. (2020), volatile compounds including 2-hydroxymethylpyrrole, 3-methylfuran, 2-methylfuran, 2-ethyl-3-methylpyrazine, and 2-hexanol compounds were only detected in the digesting coffee pulp completely (EP1) which have an odor type of chocolaty, nutty and earthy while propionic acid, 4-ethylguaiacol, 1-furfurylpyrrole, and 2 methylphenol were found only in the EP2 beans (the pulp was not digested) which is pungent, rancid and unpleasant odor type [3]. Moreover, Haile, M., Bae, H. M., & Kang, W. H. (2020) also reported the elephant dung coffee sample has better results of antioxidant activities compared to other coffee at light roasting condition [3].

**Isolation of microbial cultures from the animal excreta and gastrointestinal tract and application in coffee fermentation**

Several publications have applied fermentation by microorganisms that are isolated from the animal excreta and gastrointestinal tract.

Hadipernata, M., & Nugraha, S. (2018) provided a fermentation method based on civet gut bacterial isolates. The conditions in fermentation were kept in the same range as the digestive system of a Luwak. The propellers in the bioreactor replaced the intestine's peristaltic action. As a result, it is stated that the fake Kopi Luwak created is comparable to the genuine Kopi Luwak [30]. Bektiarso *et al*. (2020) investigated the fermentation process in civet using Extreme Low Frequency (ELF) magnetic field radiation to improve *Lactobacillus* growth because *Lactobacillus* plays a role in the fermentation of artificial civet coffee. It can be observed that there was improved growth of Lactobacillus at 300mT which contributed to improving of artificial Civet coffee quality and there was no significant change in the pH during the process of artificial Civet coffee production [31]. Muzaifa, M., *et al*. (2019) studied the coffee fermentation by using *Bacillus subtilis* that was isolated from civet (*Paradoxorus hermaphroditus*) as starter culture, resulting in significantly improve sensory quality (cup test) with the cupping score total of coffee increasing from 82.83 to 84.33 [4]. In another study, *Aspergillus niger*, *Aspergillus unguis, Penicillium citrinum*, and *Wickerhamomyces edaphicus* were isolated and could be applied in the production of civet coffee, which also observed a distinct flavor as natural one [32]. Besides, in another study done by Tien N. T. Hoang, Khanh K. Tran, Phu H. Le. (2015), three highest cellulase producer strains: *Torulaspora delbrueckii*, *Hanseniaspora uvanum*, *Candida boidinii*, and three highest pectinase producers: *Wickerhamomyces anomalus, Enterobacter sacchari, Pantoea vagans* were isolated and identified [33]. However, there is a lacking of research on purification of those enzymes and the isolation and identification of microorganisms that can excrete protease, one of the most important enzymes in the digestive system of civet which can degrade caffeine and contribute to the decaffeination of coffee. Moreover, the application of those microbial enzymes on coffee should also be implemented to investigate the optimal conditions for producing artificial civet coffee which is not only high quality compared to original one, but also at a reasonable price.

The high demand for this product and its rarity making this too expensive. Several commercial processes attempt to mimic the digestive system of those animals and artificially produce this legend coffee. In Vietnam, Trung Nguyen Corporation has manufactured an imitation of civet coffee which is soaked in enzymes claiming that replicates the civet’s digestive process.

**Conclusion**

In conclusion, animal coffee is a premium product which can bring high added value for producers. However, due to the dependence on the digestive system of animals, this kind of coffee cannot be manufactured on a large scale. There are only around 700 kg of civet coffee, 200kg of elephant coffee produced annually worldwide. This reason raises its price to 1,000 or even 3,000 USD per kg. In view of this, there are numerous studies on the application of the fermentation process by using isolated microbes from animal excreta and gastrointestinal tract, especially in Civet. The results were observed that this process has positive impacts on the improvement quality of coffee. Therefore, researches on expanding the scale and lowering the cost of production of civet coffee should be carried out. Mimicking fermentation by microorganisms that are isolated from civet or elephant coffee may be a possible way to do this.

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