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Luwak coffee in vitro fermentation : literature review

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Abstract. Luwak coffee is one of the Indonesia traditional coffee that known globally because of its unique aroma and flavor. Luwak coffee was produced by a fermentation process in luwak digestion by involving microbe that can be found in luwak digestion. Luwak coffee fermentation process using luwak animal has many weakness such as inefficient production processes, and final product quality that always makes consumers hesitate in terms of its hygiene and halal. Therefore some research to produce coffee with the quality of luwak coffee without using luwak began to be developed. Several methods of fermentation to produce coffee with quality luwak coffee are discussed in this paper, such as fermentation by *in vitro* technique

19 Introduction

Coffee is one of the agricultural commodities that become a potential source of foreign exchange for the country. That plant has a high economic value because it is an international drink favored by people in various parts of the world. World coffee consumption continues to increase every year. The International Coffee Organization (ICO) reports that world coffee consumption in 2016-2017 reached 157.8 million / 60 kg of coffee bags with an annual growth rate of 2% [1].

Indonesia has an important role in the international coffee trade. Indonesia's geographical position with a tropical climate causes coffee plants to grow well and causes Indonesia to become the world's fourth-largest coffee exporter after Brazil, Vietnam and Colombia [1]. Such climate conditions also cause Indonesia to produce coffee with a variety of unique varieties. One of the most famous Indonesian coffee globally is luwak coffee.

Luwak coffee is coffee produced from the digestive results of mongoose species (*Paradoxurus hermaphroditus*). That animal has the ability to choose coffee fruit that has been ripped optimally for later consumption. During the digestion process, only coffee skin is digested while coffee beans come out together with dirt. The coffee beans produced have a unique aroma and flavour which is often described as earthy, musty, syrupy, smooth, and chocolate undertones [2].

The uniqueness of the origin and flavour of luwak coffee makes luwak coffee become one of the most expensive coffee in the world. World consumer interest in luwak coffee causes the increase of luwak coffee demand. The increasing demand for luwak coffee causes the luwak animal cultivation business to also increase. This can lead to endangering the sustainability of wild mongoose in nature. In addition, the general public still often doubts about halal and hygienic coffee produced from luwak



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animal feces. Therefore, various methods for producing coffee with unique flavors such as luwak coffee, but not using luwak animals began to be developed. One alternative coffee production that can be done is to do fermentation using microbes from the digestion of luwak by *in vitro* technique. This paper describes several methods of coffee fermentation *in vitro* that have been carried out by several researchers.

2. Luwak Coffee

Luwak coffee is coffee produced by mongoose animals (*Paradoxurus hermaphroditus*) or commonly known as luwak. These animals belong to the type of wild mammals classified into the Viverridae family and the *Paradoxurus* genus. In general, there are around 65 subspecies of civet animals throughout the world. However, the luwak animal that is widely raised and bred in Indonesia is the Bulan and Pandan type. Luwak is a type of nocturnal animal that is active at night. Physically, the luwak puppies have a length from the tip of the head to the tail around 43 cm, while the adult mongoose is 92 cm with a body weight reaching 4.5 kg. Luwak that can be used to produce coffee optimally is an adult mongoose with the age of 1 year old [3]



Figure 1. Luwak [4].

Taxonomically, luwak animals are a type of carnivorous animal, but the fact is that these animals also often consume fruits that taste sweet and one of the fruits that are often sought after by civet animals is coffee. The fruit of coffee consumed by mongoose is optimal cooked coffee (red color). Coffee consumed by mongoose is not fully digested in the digestion of mongoose. Luwak coffee beans have a hard texture so they cannot be digested and released by the civet in full form together with dirt. Coffee beans run into a natural fermentation process that takes place in the digestion of mongoose. Various enzymes and civet digestion microbes cause changes in the chemical composition of coffee beans. The fermentation process in mongoose digestion lasts 8-12 hours. Generally, coffee is given to luwak in the afternoon and the feces that contains coffee beans can be harvested in the morning [3].



Figure 2. Luwak coffee [4].

Fermentation process involving microbes and enzymes contained in the digestion of civet animals has been proven to improve the quality of coffee beans. The fermentation process causes luwak coffee

beans to have a distinctive aroma and taste that other coffee beans don't have. Coffee beans undergo a wet treatment process because the acidification process in the luwak's stomach and then undergoes fermentation by microbes contained in the intestine. The wet treatment process is useful for removing coffee mucilage which can damage the taste of coffee during the drying and storage process. In addition, the forming compounds of flavor and aroma of coffee are formed by the activity of proteolytic enzymes [2], as well as proteolytic microbes that are able to break down proteins into simpler components such as peptides and amino acids [5]. Reducing sugars and amino acids from hydrolysis are the main precursor compounds in the formation of aroma in coffee [6]. The reaction between reducing sugars and amino acids or commonly called the Maillard reaction occurs at the roasting stage. In Maillard reactions, aromatic compounds are formed such as pyrazines, pyrroles, thiols, furanones, pyridines, and thiophenes [7].

In addition to proteolytic bacteria, lactic acid bacteria are also found as bacteria that play a role in improving the taste of luwak coffee. Lactic acid bacteria will convert carbohydrates into lactic acid under anaerobic conditions [8]. In addition to lactic acid, some flavor-forming products are also formed during metabolic process. These compounds include diacetyl, acetone, acetaldehyde, and acetic acid [9]. Lactic acid bacteria are known a group of bacteria that are able to produce various types of enzymes such as malolactic enzymes, proteases and peptidases, glycosidases, saccharide-breaking enzymes, esterases, ureases, phenoloxidases, and lipases so that this type of bacteria is widely used in the fermentation of food and beverage products to improve the product quality [10].

Besides having a distinctive aroma and flavor, luwak coffee has many health benefits. Luwak coffee does not interfere with the heart and stomach, which is the biggest problem coffee lovers. Luwak coffee also contains a number of phytochemical compounds that can function as antioxidants to reduce the risk of cancer and prevent cell damage. In addition, the content that is owned by luwak coffee is also able to reduce the risk of diabetes [11].

3. Luwak coffee *in vitro* fermentation

The fermentation process of coffee using luwak animals has several weaknesses. The increasing demand for world luwak coffee resulted in the increased hunting of luwak animals. This can threaten wild mongoose populations in nature. Another weakness is people's perception of the hygiene of the final coffee product. The release of coffee along with dirt from luwak animals caused some people to be reluctant to consume luwak coffee despite having many health benefits. Therefore, some efforts to produce luwak coffee with luwak coffee without using luwak animals began to be developed, one of the efforts is to do the fermentation using microbes from luwak digestion [12-14]. Such a fermentation process is known as *in vitro* fermentation technique

In vitro fermentation of coffee begins by isolating and identifying microbes contained in the digestion of mongoose animals. Microbes found in the digestion of luwak can be found as long as the digestion of mongoose includes the stomach, small intestine and large intestine. The number of microbial colonies found in the small intestine and large intestine is more than those found in the stomach. Very low pH conditions in the stomach cause only a small number of microbes that can live. The largest number of colonies is found in the large intestine of the mongoose. *Enterobacter cloacae* and *Lactobacillus brevis* are types of microbes found in all digestive tracts of luwak [15].

In general, microbes found in the digestion of mammals are microbes derived from the type of Lactic Acid Bacteria (LAB). The group of Lactic Acid Bacteria is a microbe that is widely used in the fermentation process because of its ability to produce various types of enzymes. The LAB also plays a role in carrying out coffee fermentation in the digestion of mongoose.

To perform luwak coffee *in vitro* fermentation, the bacteria that play a role in breaking down the chemical composition of coffee, in this case means LAB, isolated from the digestion of the mongoose. The type of LAB which can be isolated from the digestion of luwak animals is *Lactobacillus* and *Bifidobacterium*. *Lactobacillus* was isolated from the small intestine (*intestinum*) while *Bifidobacterium* was isolated from the appendix (*caecum*) of the mongoose animal [16]. Besides isolating directly from digestion, LAB can also be isolated from mongoose manure. LAB can be found

from luwak feces, namely *Lactobacillus plantarum*, *Lactobacillus brevis*, *Leuconostoc paramesenteroides*, *Leuconostoc mesenteroides*, *Leuconostoc dextranicum* and *Streptococcus faecium* [8].

The microbes from luwak which have been isolated and identified are then used to produce civet coffee in a bioreactor. Fermentation conditions in the bioreactor need to be regulated to or duration of fermentation. The fermentation conditions in the bioreactor need to be adjusted to the conditions in the digestion of mongoose to produce maximum results [12].

Most of the LAB isolated from luwak such as *Lactobacillus plantarum*, *Lactobacillus brevis*, *Leuconostoc paramesenteroides*, *Leuconostoc mesenteroides*, *Leuconostoc dextranicum* and *Streptococcus faecium* are able to grow well and produce acid at 37°C with pH ranging from 3.37-4.56 [8]. The optimum temperature and pH of LAB growth can be used as a condition for fermentation of civet coffee in a bioreactor to produce optimum quality civet coffee. To produce coffee with the best taste, fermentation in the bioreactor by utilizing microbes from the digestion of civet can be done for 16 hours.

Luwak coffee fermented by *in vitro* can be analyzed through a cupping test to determine the quality of the sensory. There are several parameters used in cupping test analysis including flavor, after taste, acidity, body, balance, uniformity, clean cup, sweetness, defects and overall. Some research results show that civet coffee fermentation *in vitro* by utilizing microbes from the digestive luwak can produce coffee with almost the same quality than original luwak coffee [12] even better than the original civet coffee [13]. Producing luwak coffee with *in vitro* fermentation is considered more effective than using a civet animal because it can produce civet coffee with an amount that can be adjusted to the needs and more economical operational costs [14]

4. Conclusion

Increasing demand for luwak coffee causes the need for alternative production of luwak coffee to maintain the sustainability of luwak in nature by *in vitro* technique. There are several studies that have been published that focus on coffee fermentation on bioreactor using microbes from luwak. Nevertheless, some researches related to the optimization of *in vitro* luwak coffee fermentation still need to be developed to produce high-quality coffee with the more efficient process

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