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# Advancements in civet coffee production and analytical techniques: From aroma profiling to market dynamics and ethical considerations

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ABSTRACT

*Background:* Civet coffee is produced from coffee beans that have been consumed and excreted by civets. However, its production raises several ethical concerns. The increasing cases of adulteration create significant challenges in verifying the authenticity of civet coffee. Currently, there is no comprehensive review addressing alternative and sustainable production methods for civet coffee. This manuscript aims to fill this knowledge gap and serve as a reference for improving civet coffee fermentation.

*Scope and approach:* This paper explores innovative techniques for producing civet coffee while considering ethical and sustainability issues. It examines market trends and authentication methods, focusing on technologies such as electronic nose (E-Nose) technology, proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS), UV–visible spectroscopy, infrared spectroscopy (NIR and FTIR), and nuclear magnetic resonance (NMR). Additionally, it discusses the health-promoting effects of civet coffee.

*Key findings and conclusions:* Alternative methods to the *in vivo* fermentation of civet coffee, using isolated bacteria (i.e., *Bacillus subtilis*), yeast, and fungi from the civet's gastrointestinal tract or excreta in glass jars or bioreactors, have significantly enhanced the fermentation process. These methods resulted in improved and consistent coffee quality, flavor, and nutritional value. Technologies like e-nose PTR-ToF-MS effectively distinguish civet coffee from non-civet variants through aroma and marker profiling. Additionally, UV–Vis, NIR, FTIR, NMR, GC/MS, and GC/FID, combined with chemometrics, have demonstrated significant efficacy in distinguishing farmed and wild types of civets. Despite its high price, civet coffee continues to see a growing global demand as consumers increasingly prioritize quality, health benefits and authenticity. Innovative analytical techniques are essential for maintaining the integrity of civet coffee. However, addressing animal welfare concerns in civet coffee production is crucial to meet the expectations of ethically and sustainability-conscious consumers.

## 1. Introduction

Coffee fruits are products derived from woody, perennial evergreen dicotyledonous coffee plants belonging to the *Rubiaceae* family. These fruits are cultivated worldwide, primarily in subtropical and equatorial regions. Various coffee plant varieties exist, with the most prominent being the arabica coffee tree (*Coffea arabica*) and the robusta coffee tree (*Coffea canephora*), which together account for approximately 75–80% and 20% of global production, respectively (Coffee Research Institute, 2024). Coffee cherries are harvested from the trees and undergo a multi-step processing method that involves removing the outer layers, including the skin, pulp, mucilage, and parchment surrounding the coffee seeds. This is followed by drying and roasting to produce the coffee beans. Coffee is a popular beverage consumed worldwide,

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primarily made from the fermentation of roasted coffee beans. Arabica coffee contains lower levels of caffeine and chlorogenic acid compared to robusta beans (Freitas et al., 2023; Sunarharum et al., 2014). Coffee ranks among the most consumed beverages globally, alongside tea and beer, due to its distinctive flavor, aroma, and the stimulating effects of caffeine and chlorogenic acid (Denoeud et al., 2014; Taveira et al., 2014; Freitas et al., 2023; Freitas et al., 2024).

Recently, civet or *Kopi Luwak* coffee has gained immense popularity due to its believed health benefits and unique taste, attributed to various bioactive compounds (Febrina et al., 2021; Ifmalinda et al., 2019; Fitri et al., 2019). Civet coffee was first discovered by local farmers in Indonesia in the 19th century (Fitri, Laga, et al., 2021; Ifmalinda et al., 2019). It is often considered more flavorful and better tasting than other coffee types, a distinction attributed to its unique production process (Hadipernata & Nugraha, 2017; Muzaifa et al., 2018; Muzaifa et al., 2019). In addition to its exceptional taste, civet coffee is rich in nutrients such as proteins (13.66 ± 0.12%), lipids (14.79 ± 0.08%), and other bioactive compounds, including caffeine (1.10 ± 0.02), chlorogenic acids (0.88 ± 0.44%), polyphenols (7.56 ± 0.03%), and caffeic acid (0.056 ± 0.001%) (Fitri, Laga, et al., 2021; Muzaifa et al., 2020; Yoshikazu et al., 2017). Civet coffee is uniquely produced using the Asian palm civets (*Paradoxurus hermaphroditus* (APCs)). In this process, the civet is fed either *C. arabica* or *C. canephora* cherries, which undergo partial fermentation in its digestive tract before being excreted. After excretion, the partially fermented beans are carefully sorted, cleaned, dried, and roasted. This distinctive *in vivo* production method imparts the civet coffee with a unique aroma and flavor, often described as having fermented undertones. As a result, civet coffee is regarded as one of the most prestigious and expensive coffee varieties worldwide (Jumhawan et al., 2015a, 2015b; Suhandy & Yulia, 2017; Pesce, 2013; Fitri et al., 2019; Eleven Coffees, 2024).

As civet coffee rapidly emerged as one of the most profitable business ventures, many farmers have turned to its production to meet the growing demand. However, this unconventional method of caging and force-feeding civets with coffee cherries has drawn criticism from animal rights activists worldwide, who highlight the poor living conditions and potential mistreatment of the civets. Additionally, foragers of wild civet coffee may face significant dangers, including the risk of bites from venomous snakes and scorpions which can threaten their lives. Furthermore, civet coffee production can lead to adverse environmental impacts, including deforestation. There are also health and safety concerns regarding the contamination of civet coffee with harmful bacteria or chemicals due to inadequate hygiene practices during processing. Many view civet coffee as a luxury product produced at the expense of animal welfare and environmental sustainability, raising ethical questions about its consumption (Sabin, 2018). Thus, there is a pressing need for alternative and sustainable production methods that can satisfy the increasing consumer demand for civet coffee. To our knowledge, no comprehensive review exists on the alternative and sustainable production of civet coffee. This manuscript aims to fill that knowledge gap, providing a reference for ethically improving the fermentation process of civet coffee. The primary goal of this review is to investigate and suggest alternative methods for producing civet coffee without endangering the lives of civets. Additionally, this review explores the chemical composition (including proximate composition and abundance of volatile organic compounds), global market trends, and authentication techniques relevant to civet coffee.

## 2. Global market value and patronage of civet coffee

Civet coffee is a high-value product primarily due to its unique fermentation process occurring within the adult civet, along with the various nutrients it may contain. This has led to a significant demand for civet coffee (Muzaifa et al., 2020). A study suggest that civet coffee possesses antibacterial properties that may help prevent oral infections. Additionally, it is considered gentler on the stomach, making it suitable for ulcer patients, as it contains lower acidity levels (Achmadi, 2019). Consequently, many coffee enthusiasts prioritize the health benefits over the cost. Though, initially sourced entirely from wild civets, production was limited to just 250–500 kg annually. This scarcity drove prices to soar, fueling a surge of greed in the market (Sabin, 2018). Additionally, the production processes for civet coffee are labor-intensive, causing farmers to further inflate prices.

Recent data suggests that the global market value of civet coffee is between $6.5 to $8.23 billion, with an annual growth rate of 4.9%. It is projected to reach $10 billion to $11.43 billion by 2030 (Straits Research, 2022; Maximize Market Research, 2024). As of 2023, the civet coffee market is primarily dominated by North America, Asia-Pacific, and Europe, while South America, the Middle East, and Africa account for a small share of the market (Maximize Market Research, 2024). Prices for civet coffee vary based on the type of civet involved in its production, with wild civets generally fetching higher prices than caged civets. Wild civet coffee is priced higher due to its smoother texture and greater abundance of volatile compounds compared to coffee produced by caged civets (Marcone, 2004). Furthermore, the higher prices reflect the considerable effort involved in producing wild civet coffee.

Despite its high cost, the demand for civet coffee has steadily increased since 2011 (Straits Research, 2022). The price of civet coffee can range from $100 to $1300 per kg, depending on whether it is farmed or wild. For instance, a low-grade farmed civet coffee from Bali can cost around $100 per kg, which is particularly steep given the prevailing socio-economic inequalities (Eleven Coffees, 2024). Additionally, reports from 2021 indicate that the price of civet coffee in Vietnam was approximately $500 per kg (Thout, 2012). Historical data shows that the price of brewed civet coffee per cup was $60 in 2006 (Australia), $108 in 2008 (UK), and ranged from $25 to $30 in New Zealand. In the UK, the price per cup reached £70 in 2012 (The Standard, 2012).

In the UK, coffee imports primarily come from Brazil ($52.9M) and Vietnam ($46.3M), with Indonesian ($24.9M) civet coffee making up only 5% of the market. Although the import volume of civet coffee is low, it maintains a strong market presence compared to other coffee products (OEC World, 2024).

## 3. Nomenclature, geographical distribution, feeding habits, and diseases of civets

APCs belongs to the order *Carnivora* and the family *Viverridae*, which encompasses several subfamilies, including *Viverrinae*, *Paradoxurinae*, *Hemigalinae*, *Fossinae*, *Galidinae*, *Herpestinae* and *Cryptoproctinae*, along with 36 genera and 70 species. These small, musk-like mammals are nocturnal frugivores and opportunistic carnivores (Chaudhary, 2021; Chua et al., 2012; Nakabayashi et al., 2012; Rode-Margono et al., 2014). They typically have a lifespan ranging from 15 to 28 years (Jennings & Veron, 2011; Animalia, 2021). APCs are characterized by their robust build, covered in coarse, shaggy fur that varies in color from black to grey and brown. They possess a bushy tail and a tapered snout, with distinctive black markings on their paws, ears, and muzzle. Their body size, tail length, and weight vary significantly, measuring between 17 and 28 inches, 16–26 inches, and 2–5 kg, respectively (Duckworth et al., 2008). These civets inhabit a wide range of regions across Asia (i.e., Indonesia, the Philippines, Singapore, Sri Lanka, Bangladesh, Bhutan, India, Nepal, China, Thailand, Vietnam, Cambodia, Brunei, and Laos) as well as parts of the East Indies, sub-Saharan Africa (i.e., Ghana, Nigeria, Zambia, South Africa, and Madagascar), and southwestern Europe (Chua et al., 2012; Jennings, Zubaid, & Veron, 2010; Jennings & Veron, 2011; Rode-Margono et al., 2014).

APCs have a diverse diet that includes a variety of fruits (i.e., coffee cherries, berries, mangoes, and bananas), seeds, rodents, birds, insects, reptiles, and palm flower sap, among other foods (Animalia, 2021; Duckworth et al., 2008; Khan et al., 2019; Krishnakumar & Balakrishnan, 2003). Like all animals, civets are susceptible to a range of diseases, including viral infections such as severe acute respiratory

syndrome coronavirus, H5N1 influenza virus, rabies virus, and canine parvovirus, as well as bacterial, protozoan, and nematode infections (Demeter et al., 2009; Matsumoto et al., 2011; Moinet et al., 2010; Wicker et al., 2017). Symptoms of these diseases can vary widely, including loss of appetite, diarrhea, fever, and potentially death. Additionally, studies have documented specific diseases, symptoms, and affected species within the *Viverridae* family of APCs (Wicker et al., 2017).

### 3.1. Microbiome of the civet (microbial composition of gut/stomach)

The gut microbiome refers to a complex community of microorganisms, including bacteria, fungi, protozoa, archaea, viruses, and yeasts, living in the gastrointestinal tract (GIT) of animals and humans, along with their genetic material and metabolic byproducts (Turner, 2018). The composition of microorganisms in a civet's GIT can be influenced by various factors, such as diet, age, infections, antibiotic use, and environmental contaminants (Wen & Duffy, 2017). It has been reported that the physiochemical properties and aroma of civet beans can also affect the composition of a civet's gut microbiome (Watanabe et al., 2020). Suhandono et al. isolated and characterized a diverse range of bacteria, specifically lactic acid bacteria (LAB) and non-lactic acid bacteria (NLAB), from the GIT of an adult *P. hermaphroditus javanica* (Suhandono et al., 2016). Among the GIT organs examined, the colon exhibited the highest concentration of NLAB at 9.58 log CFU/mL, closely followed by the small intestine with 9.10 log CFU/mL; the stomach had the lowest concentration at 6.10 log CFU/mL. In contrast, LAB were most abundant in the small intestine (9.36 log CFU/mL), followed by the colon (9.20 log CFU/mL), while the stomach had the least at 7.06 log CFU/mL (Suhandono et al., 2016). The 16S rDNA analysis of the isolated strains identified several species, including *Bacillus methylotrophicus* strain PY5 (L2LAB1), *Enterobacter cloacae* QJ.993364.1 (L3L8), *Enterobacter* sp. (L3L6), *Enterobacter cloacae (L9L1)*, *Enterobacter cloacae* QQ.993364.1 (L6L7), *Cedecea davisae* JQ396389.1 (L8L4), *Enterobacter* sp. 1045 (L7L9), and *Enterobacter agglomerans* strain (L7L8). These strains were identified as the predominant culturable *Enterobacteriaceae* in the GIT of an adult *P. hermaphroditus javanica* (Suhandono et al., 2016). The observed variations in bacterial load can be attributed to fluctuations in the composition of gastric fluids, including changes in pH, as food moves through different sections of the GIT (Suhandono et al., 2016). Additionally, other bacterial species such as *Lactobacillus plantarum*, *Lactobacillus brevis*, *Leuconostoc paramesenteroides*, *Leuconostoc mesenteroides*, *Leuconostoc dextranicum*, and *Enterococcus faecium* (previously classified as *Streptococcus*) were also identified in the stool of adult APCs (Fauzi, 2008; Fitri, Tawali et al., 2021).

A further comprehensive analysis through gene sequencing (16S rRNA) revealed the presence of several *Gluconobacter* species including *G. frateurii*, *G. japonicus*, *G. nephelii*, and *G. wancherniae* which were found to be dominant in fresh fecal samples from wild civets (Watanabe et al., 2020). Notably, farmed civets exhibited a significantly higher concentration of lactic acid bacteria, with levels reaching $7.9 \times 10^8$ CFU/mL, compared to $5.8 \times 10^8$ CFU/mL in their wild counterparts (Muzaifa et al., 2018). Additionally, Muzaifa et al. conducted a study isolating and characterizing three yeast species (designated as YC1, YC2, and YC3) from farmed civets, focusing on their sugar utilization, proteolytic activity, and temperature tolerance (Muzaifa, 2019). The findings indicated that the optimal temperature for these yeast isolates ranged from 15 °C to 35 °C. All isolates demonstrated the ability to utilize lactose and mannitol, but not sucrose. Specifically, YC1 was capable of utilizing glucose, while YC2 could utilize maltose. Although YC1 and YC2 showed promising proteolytic potential, YC3 did not exhibit similar capabilities (Muzaifa, 2019).

## 4. Production of civet coffee

### 4.1. Classic in vivo fermentation methods

It is important to note that there is limited literature regarding *in vivo* civet coffee production, with most research concentrated in Indonesia. The production process involves capturing healthy APCs from the wild, confining them in cages, and force-feeding them coffee cherries. The cherries remain in the civet's stomach for natural fermentation, which takes approximately 8–12 h. This fermentation process is facilitated by gastric acid (specifically hydrochloric acid), proteolytic enzymes, the activity of specific microbes (i.e., LAB), and the favorable internal conditions of the civet's GIT, which break down the fruit and expose the coffee beans to further enzymatic activity (Fitri, Tawali et al., 2021; Marcone, 2004; Murthy & Naidu, 2011; Salengke et al., 2019) (Fig. 1). Healthy APCs are selected because sick APCs can affect their digestion and metabolism potentially leading to lower quality beans with less distinctive flavors. Research indicates that the proteolytic enzymes in the civet's GIT degrade proteins into peptides and amino acids (Muzaifa et al., 2018). Additional studies have documented the protease, lipase, and cellulase activities exhibited by LAB isolated from the civet's GIT (Fitri, Tawali et al., 2021). It was observed that*Leuconostoc pseudomesenteroides* Ni1324 demonstrates higher protease (0.051 U/mL vs. 0.041 U/mL) and lipase (0.639 U/mL vs. 0.306 U/mL) activities compared to *Weissella cibaria* MG5327. Conversely, *W. cibaria* MG5327 exhibits significantly higher cellulase activity (0.039 U/mL) than *L. pseudomesenteroides* Ni1324 (0.029 U/mL). These differences in enzymatic activity influence the fermentation rate, the development of aromatic compounds, and the overall quality of civet coffee beans (Fitri, Tawali et al., 2021). Furthermore, biochemical reactions generate heat within the civet's intestine and contribute to the formation of aromatic compounds. Dominant compounds, including pyrrole, pyrazine, thiols, and furanones, are produced during roasting, further enhancing the flavor profile (Lee et al., 2015; Montavon et al., 2003). LAB, such as *Lactobacillus trevis*, can convert available carbohydrates in coffee beans into lactic acid under anaerobic conditions (Fauzi, 2008). During the fermentation process, metabolites like acetic acid and acetaldehyde are produced, which serve as flavor enhancers for coffee beans (Van Kranenburg et al., 2002). After approximately 8–12 h of fermentation, the beans are expelled in chain-like clusters, collected from the wild or cages, then washed and dried. The dried beans, referred to as green beans, are subsequently polished, sorted, and packaged for further processing (Fig. 1).

Coffee beans sourced from Indonesian and African civets exhibit notable differences in their nutritional composition, total microbial counts, and physical attributes such as color, size, and shape. These variances can be attributed to the distinct metabolic activities that occur during the fermentation process in these civets (Hadipernata and Nugraha, 2017; Marcone, 2004). However, the experimental conditions (temperature, pH, quality of cherries, etc), number of biological replicates among other factors (i.e., methods, equipment, reagents) may influence the overall qualities of these parameters.

Following fermentation, the coffee beans undergo roasting, a process that involves exposing them to high temperatures ranging from 200 to 250 °C. This step reduces moisture content while contributing to the development of the desired aroma and flavor profile (Rao et al., 2011). Roasting typically occurs through two methods: continuous and non-continuous, lasting about 5–10 min and 20 min, respectively. The degree of roasting significantly influences the intensity of the coffee's aroma and flavor (Rao et al., 2011). During roasting, various physical (color, size, and shape) and chemical changes (including the Maillard reaction, caramelization, and lipid oxidation) take place, shaping the final characteristics of the coffee (Somoza, 2005; Summa et al., 2007). The Maillard reaction is a complex series of interactions between amino acids and reducing sugars that occurs during the roasting of coffee. Initially, reducing sugars react with amino acids to form glycosylamines,

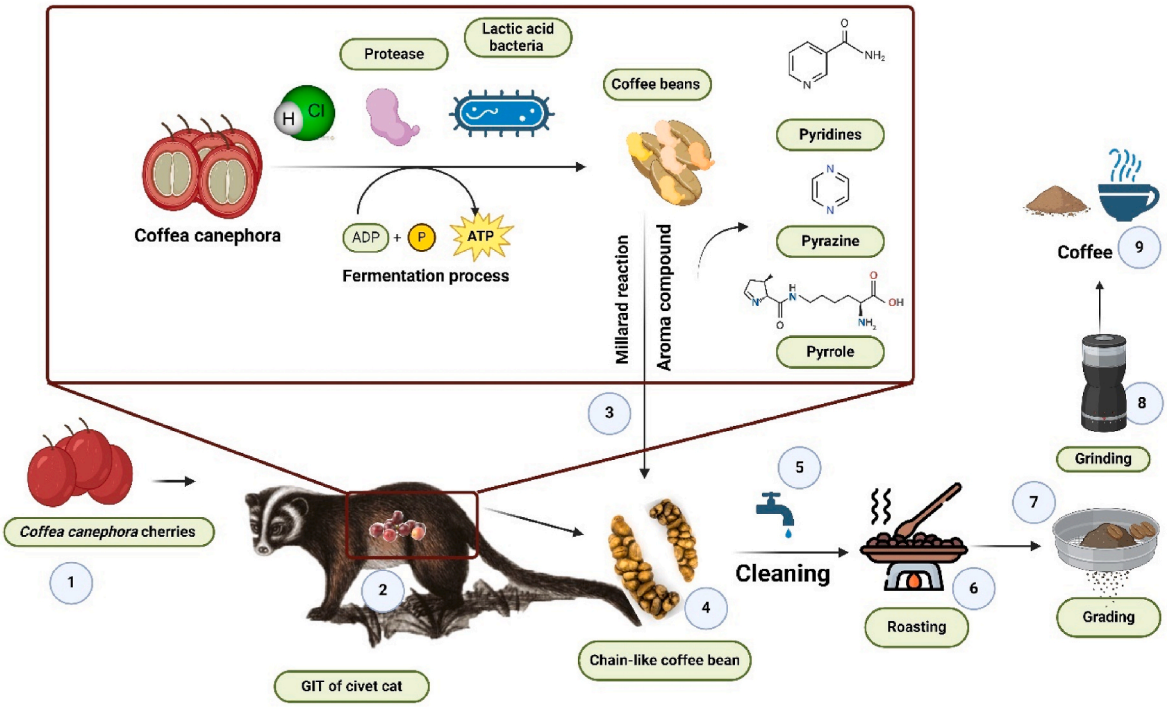


**Fig. 1.** GIT of civet cat and processes involved in production of Civet Coffee (*in vivo* fermentation). (1) Civet select and consume the best quality ripe cherries; (2) beans inside pass through their digestive system; (3) microbiota in the GIT along with enzymes break down the cherry's pulp and ferment it, influencing the flavor profile of the beans; (4) undigested beans are excreted by the civets; (5) collected thoroughly cleaned to remove any impurities and then dried, (6) roasted, (7) graded and (8) ground and (9) brewed to create a unique coffee drink with distinct flavors (i.e., smooth and less acidic).

which then rearrange into Amadori and Heyns compounds. As roasting continues, these compounds undergo further transformations, ultimately leading to the formation of melanoidins, which impart the characteristic brown color and rich flavors to the coffee. In contrast, caramelization is the thermal decomposition of sugars that occurs during roasting. At higher temperatures, sugars, particularly monosaccharides undergo dehydration, fragmentation, and polymerization, resulting in a variety of volatile compounds, including furans and caramels. These compounds enhance the sweetness and complexity of roasted coffee. The caramelization process also contributes to the distinctive color of the final product, with darker roasts displaying deeper hues due to the increased formation of caramelized sugars. Lipid oxidation refers to the breakdown of lipids (fats) during the roasting and drying processes, significantly impacting coffee's flavor and aroma. Unsaturated fatty acids in coffee beans can react with oxygen, leading to the formation of peroxides and free radicals. While this oxidative process can produce undesirable flavors often described as rancid or stale, some oxidation products can positively influence aroma, adding complexity to the coffee profile especially in lightly roasted beans (Nagaraju et al., 1997; Summa et al., 2007; Córdoba et al., 2021; Toci et al., 2020; Tarigan et al., 2022; da Costa et al., 2023).

Reports indicate that the proteolytic enzymes, such as pepsin and trypsin, present in the civet's GIT penetrate the beans and hydrolyze proteins into amino acids and free amino acids. This process exposes the beans to chemical reactions, resulting in more unique flavors compared to control beans (Marcone, 2004). Furthermore, notable differences in proteolysis between Indonesian and African civet types have been observed, leading to variations in Maillard browning products and, consequently, distinct flavor and aroma profiles (Marcone, 2004). The beans transition in color from grey to light brown, dark brown, or black, accompanied by a decrease in pH. This pH reduction is attributed to the formation of various organic acids during the roasting process (Nagaraju et al., 1997).

### 4.2. Microbial culture/inoculation method and enzymatic treatment

An alternative approach to the *in vivo* fermentation process of civet coffee involves using isolated *B. subtilis* from the civet's GIT or excreta, as outlined by Fauzi and Hidayati (2016) (Fig. 2). This species is identified through 16S rRNA sequencing and cultured on De Man–Rogosa–Sharpe (MRS) agar before being transferred to MRS broth for 48 h at 37 °C. A sterile medium is prepared using coffee fruit skin extracts which serve as cheap nutrients, into which the cultured *B. subtilis* is inoculated and allowed to ferment for 48 h at 37 °C. This culture then serves as a starter for *in vitro* coffee fermentation. In a glass jar, approximately 500 g of de-pulped coffee beans are inoculated with 10% (v/w) of the starter culture and fermented for 48 h at 37 °C. A control sample is typically set up without *B. subtilis* (Muzaifa, 2019). The fermentation process results in coffee beans with lower pH, higher titratable acidity, and improved sensory attributes compared to the control sample (Fauzi & Hidayati, 2016; Muzaifa, 2019; Fitri, Laga, et al., 2021). Additionally, *Gluconobacter* species, such as *G. frateurii*, *G. japonicus*, *G. nephelii*, and *G. wancherniae*, can be isolated from civet feces, cultured, and used as a starter for producing civet coffee (Watanabe et al., 2020). *Gluconobacter* encodes genes responsible for cell motility and the metabolism of hydrogen sulfide and sulfur-containing amino acids, which may enhance the fermentation of civet coffee. The authors also suggest *Citrobacter* and *Clostridium* as potential candidates for civet coffee production due to their ability to express proteolytic enzymes that penetrate and expose amino acids to Maillard reaction, contributing to the development of unique flavors (Watanabe et al., 2020).

A recent study has indicated that supplementing media with glucose in a mixed culture significantly enhances the fermentation of civet coffee over a 12-h period, compared to a starch-fed culture which requires 24 h (Darwin et al., 2023). Chen and his colleagues attempted to simulate civet coffee production using exogenous enzymes, specifically pepsin and pancreatin, in a glass jar. They observed that the treated coffee

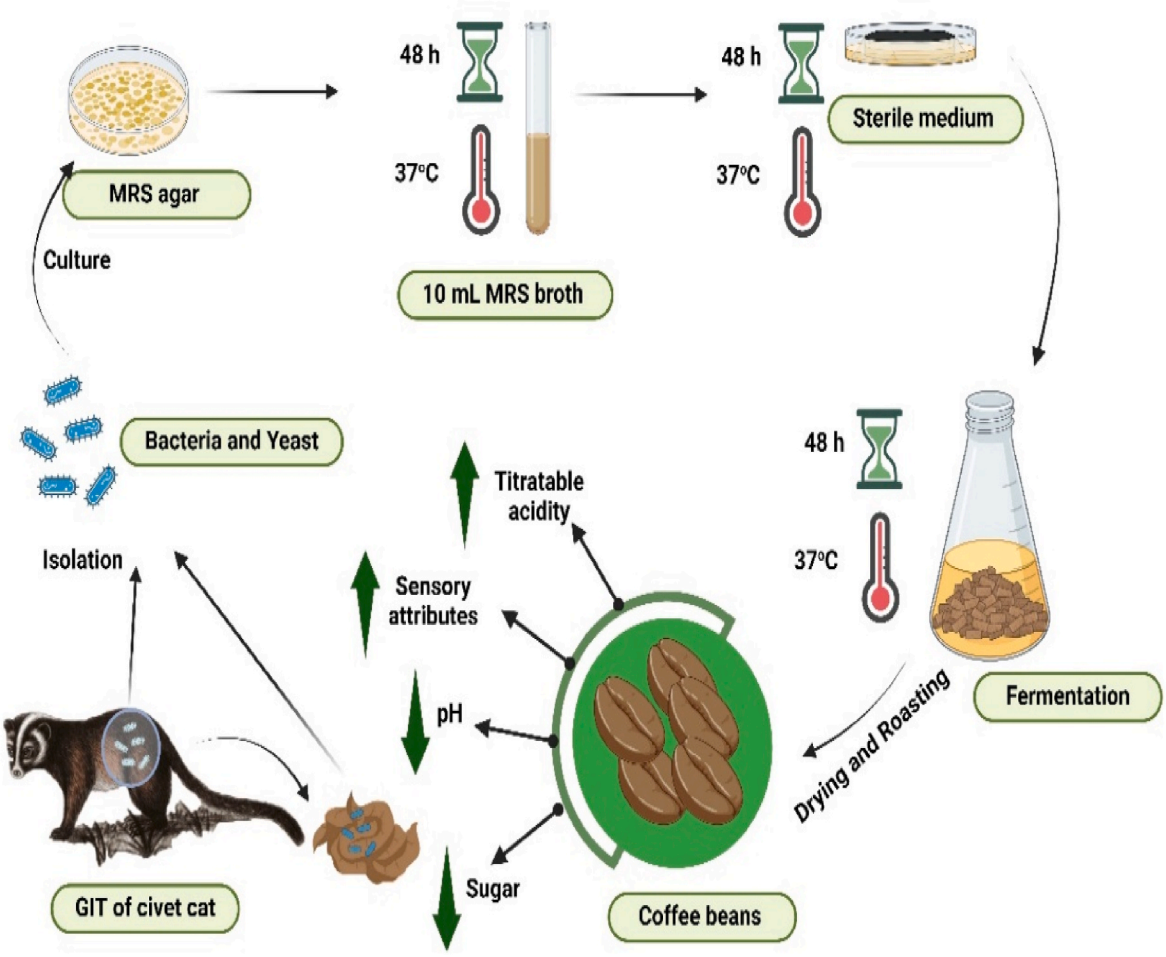


**Fig. 2.** Alternative method of producing civet coffee (Microbial culture/inoculation method).

exhibited rough surfaces with numerous micro-pits, while the control coffee had smooth surfaces. Additionally, the compound responsible for coffee's bitterness was significantly reduced in the treated coffee compared to the control, a finding further confirmed by sensory analysis. Sensory panelists rated the treated coffee as 38%–24% less bitter than the control (Chen et al., 2024). The use of starter cultures and exogenous enzymes can enhance the fermentation process and improve coffee quality, resulting in enhanced flavor and increased phenolic compounds. However, further research is needed to better understand the mechanisms underlying flavor development by fine-tuning parameters such as the source of coffee cherries, enzyme dosage, fermentation duration, and concentrations of bacterial and yeast species, among other important factors.

### 4.3. *Ex-situ approach*

A literature search revealed only one paper (Hadipernata and Nugraha, 2017) addressing the *ex situ* production of civet coffee. Therefore, caution is warranted when interpreting the findings.

Using glass tubes or jars to mimic *in vivo* fermentation of civet coffee has several limitations. For instance, the glass containers often fail to replicate the complex environmental conditions of a civet's GIT, such as temperature fluctuations, pressure variations, and the presence of diverse microbial communities. The microbial population in a glass jar is typically less diverse than that found in the natural gut environment, which may adversely affect fermentation dynamics and flavor development. *In vivo* fermentation occurs within a dynamic system where contents are continuously mixed and moved. In contrast, static glass containers restrict movement, hindering interaction between microbes and substrates. Additionally, the accumulation of metabolites and changes in pH can differ significantly in a glass jar compared to the gut, potentially influencing fermentation outcomes. The interactions among microbes, enzymes, and substrates in a natural setting are complex and may not be fully captured in glass containers, oversimplifying the fermentation process. Furthermore, results from small-scale glass jars may not easily translate to larger-scale production, where different dynamics and factors come into play. Controlling all relevant variables, such as oxygen levels and gas production, can also prove challenging in a glass jar, impacting fermentation efficiency and the unique civet coffee flavor development.

Given these limitations, some researchers suggest alternative methods for producing civet coffee on an industrial scale using bioreactors (Fig. 3). To implement this, it is essential to isolate bacteria and yeast from the civet feces and conduct DNA and RNA sequencing, along with other molecular techniques, to accurately identify their species. In addition, the isolates should undergo further testing, including colony morphology screening, cell morphology analysis, and biochemical assessments (e.g., sugar utilization potential, vitality tests using intracellular pH, vital dye staining, and $CO_2$ production). These tests may provide valuable insights into the metabolic state of the isolates (Kwolek-Mirek & Zadrag-Tecza, 2014). After completing these screenings, viable isolates can be tested in a laboratory setting that simulates the GIT conditions of a civet cat. This process may involve using response surface methodology (RSM) to optimize parameters such as temperature, isolate concentration, pH levels, source of cherries, volume of simulated gastric fluid (SGF), stirring rates, and dosing rates. These

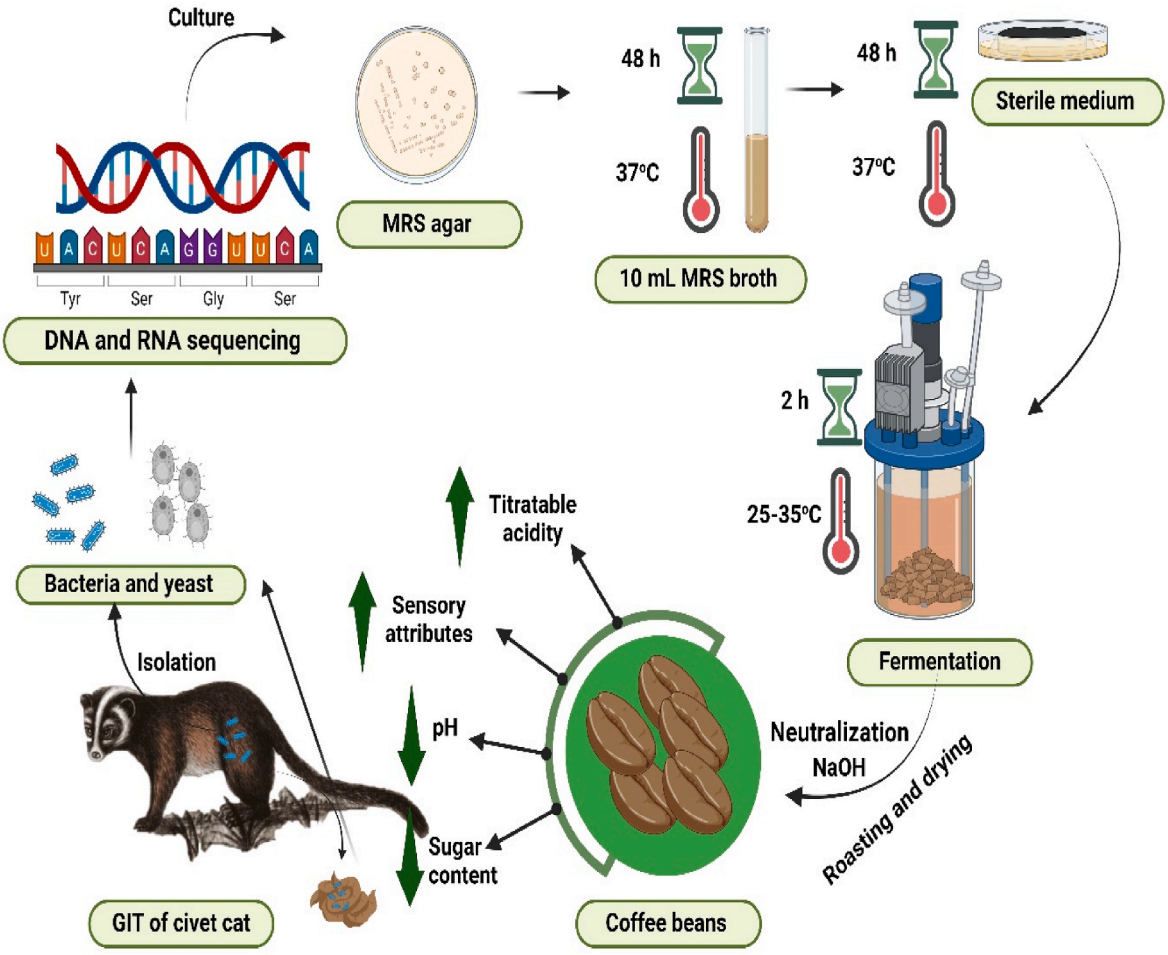


**Fig. 3.** Ex-situ bioreactor method for producing civet coffee.

factors are crucial in determining the overall quality of the civet coffee produced.

Other researchers have explored the use of mini fermenters (bioreactors) to produce civet coffee by mimicking the gastric and intestinal conditions of civet. Following the method described by Hadipernata and Nugraha, 2017, approximately 1.5 kg of peeled coffee cherries were transferred into a bioreactor containing SGF (2.5 L) with a pH ranging from 1.2 to 2.5. The pH was adjusted to between 1.5 and 4.5, and the beans were left to rest for 2 h at 25 °C–35 °C. The beans were then neutralized using sodium hydroxide (NaOH) to achieve a pH of 6.5–7.5, after which they were inoculated with $10^8$–$10^9$ CFU/mL of isolated LAB from the civet. Fermentation was allowed to occur for 2 h at 25 °C–35 °C. It is speculated that there was no difference in taste and overall product quality between coffee produced through the bioreactor and *in vivo* fermentation (Hadipernata and Nugraha, 2017). However, the authors did not adequately characterize the composition of the SGF. Additionally, the specific species of LAB used was not thoroughly identified through colony morphology, cell morphology, and biochemical testing. Furthermore, the optimal dosages of buffers and exogenous enzymes suitable for the fermentation process were not provided. Therefore, future studies should address these aspects to enhance the understanding and effectiveness of the bioreactor fermentation method.

### 4.4. The potential mechanism of flavor generation in civet coffee

Despite the long history of civet coffee, research focused on its flavor production remains scarce. Therefore, it is essential to propose potential mechanisms involved in flavor generation, which include microbiota, metabolic pathways, and associated genes.

Flavor development in coffee begins in the coffee plant, where key flavor precursors are synthesized and accumulate in the cherries. This flavor evolution continues throughout the coffee processing stages, spanning cherry harvesting, processing (such as fermentation, drying and monsooning), roasting, grinding, and brewing (including decoction and infusion). Factors such as geographical location, climate, ripeness, and harvesting methods significantly influence the flavor characteristics of coffee (Freitas et al., 2024; Sunarharum et al., 2014).

What sets civet coffee apart from other coffee types is its reliance on the selection of high-quality ripe cherries and fermentation that occurs in the civet's GIT (Fauzi & Hidayati, 2016; Muzaifa et al., 2019; Watanabe et al., 2020). Specifically, LAB, yeasts, and *Bacillus* spp. play vital roles in fermentation within the civet's gut. These microbes contribute to flavor development through various metabolic pathways (Ashika & Pushpa, 2021; Fauzi & Hidayati, 2016; Hadipernata and Nugraha, 2017; Muzaifa et al., 2019; Watanabe et al., 2020). For example, the metabolic pathways involving LAB and *Bacillus* spp. begin with glycolysis, where sugars from the cherries (Darwin et al., 2022) are converted into pyruvate, catalyzed by key enzymes such as hexokinase, phosphofructokinase, and pyruvate kinase. This is followed by the conversion of pyruvate into acids (e.g., lactic and acetic acids), carbon dioxide, and alcohols (e.g., furfuryl alcohol, maltol), facilitated by enzymes like lactate dehydrogenase (*LDH*, including *ldhL* and *ldhD*) and alcohol dehydrogenase. Lipase metabolizes lipids into glycerol and free fatty acids, which can subsequently be converted into esters (such as ethylene diacetate, allyl isobutyl oxalate, etc) by esterases, thereby contributing to the flavor of civet coffee (Wang et al., 2021; Eiteman & Ramalingam,

2015; Yılmaz et al., 2024; Kleerebezem et al., 2020; Goelzer et al., 2008; Farag et al., 2023). Additionally, diacetyl and acetoin (including butyryl lactone) can be synthesized with the assistance of acetolactate synthase and acetoin reductase (Farag et al., 2023). *Bacillus* spp. also plays a significant role by metabolizing amino acids (such as lysine and leucine) into volatile esters that enhance the aroma of civet coffee (Goelzer et al., 2008; Yılmaz et al., 2024). Furthermore, through proteolysis, *Bacillus* spp. break down proteins, releasing free amino acids that can participate in the Maillard reaction during roasting (i.e., pyrroles, pyridines), contributing to the overall flavor profile (Muzaifa et al., 2019). Yeasts present in the civet's GIT convert cherry sugars into pyruvate, generating adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH) in the process, catalyzed by AGP1 and Actin-Like Protein 1 (ALP1) (Eder et al., 2018; Procopio et al., 2011; Takagi, 2019). Pyruvate is then further metabolized to produce additional ATP, NADH, and $FADH_2$ via the tricarboxylic acid (TCA) cycle, aided by hexose transporter (HXT3) and MAE1. The pentose phosphate pathway also plays a crucial role, catalyzing the production of NADPH and ribose-5-phosphate, which are essential for biosynthetic reactions, facilitated by fatty acid synthetase. Additionally, branched-chain amino acid permease encodes a transporter for branched-chain amino acids such as valine, leucine, and isoleucine, further contributing to the unique flavor of civet coffee (Eder et al., 2018; Procopio et al., 2011; Takagi, 2019). Previous studies have shown that yeast metabolizes lipids in cherries, producing fatty acids and esters that enhance the flavor profile of civet coffee (Suhandono et al., 2016). Additionally, the types of cherry consumed by civets can significantly influence the flavor composition. Research on the sensory profile of civet coffee revealed notes of guava and papaya in the processed coffee beans, directly linked to the fruits the civets were fed (Muzaifa et al., 2018). Another study found that coffee samples from wild civets exhibited dominant flavors of nuts, chocolate, fish, herbs, and earthiness, reflecting their natural diet. Despite these intriguing aromatic traits from wild civets, coffee sourced from caged civets tends to possess superior flavor attributes. This improvement is attributed to their diet, which includes high-quality cherries that contribute to flavor development (Muzaifa et al., 2018).

These metabolic pathways and associated genes collaborate to produce the unique flavors and aromas characteristic of civet coffee. The interaction between these microorganisms and the civet's GIT environment, or conditions that mimic it, further influences these metabolic processes, leading to the coffee's distinctive flavor qualities. Therefore, comprehensive research into the microbiota, metabolic pathways, and genes involved in the generation of flavor and aroma in civet coffee is essential to better understand the underlying mechanisms.

## 5. Chemical composition of civet coffee

Measuring the chemical composition of civet coffee is essential for ensuring product quality, enhancing flavor profiles, and providing vital nutritional information (Table 1). This analysis enables producers to maintain consistency, optimize processing techniques, and cater to consumer preferences. Additionally, understanding the chemical components supports sustainable coffee production and bolsters the marketing of civet coffee as a premium product. Ultimately, this measurement not only safeguards consumer health but also enhances the overall appreciation of civet coffee in the marketplace.

Green civet coffee primarily consists of key chemical components, including carbohydrates, lipids (12.30%), nitrogen-containing compounds (i.e., proteins (13.36%) and caffeine (1.20%)), organic acids (3.73%), moisture (12%), and ash (5.2%) (Darwin et al., 2023; Muzaifa et al., 2020). These elements serve as the major precursors to flavor and taste (Baggenstoss et al., 2008). Carbohydrates account for approximately 40–65% of green civet coffee, comprising both water-soluble and water-insoluble sugars (Luigi et al., 2017). Studies have shown that farmed and wild civet coffee beans contain 67.25 ± 0.33% and 69.44 ± 0.28% carbohydrates, respectively (Hadipernata & Nugraha, 2017), indicating no significant treatment effects on carbohydrate content due to similar processing methods. In addition to carbohydrates, green coffee beans also contain various acids, including volatile acids (i.e., acetic and formic acids (traces to 0.06%)), non-volatile acids (including chlorogenic, citric, malic, and quinic acids (1.3–2.9%)), and phenolic acids (6.2–14.1%) which are crucial for the perceived acidity and brightness of civet coffee (Muzaifa et al., 2020; Viani & Petracco, 2012). Civet coffee contains higher concentrations of hexadecenoic (44.3%) and octadecenoic acids (7.12%) compared to the control arabica coffee (40.3% vr. 2.46 %, respectively) (Ifmalinda et al., 2019) which may be attributed to the acidic environment of the civets GIT and the action of the proteolytic enzymes on the beans. A study by Jumhawan et al. demonstrated that malic and citric acids serve as key discrimination markers for civet coffee, providing reliable fingerprints for authenticating genuine civet coffee and distinguishing it from counterfeit products (Jumhawan et al., 2013). Analysis using proton nuclear magnetic resonance ($^1$H NMR) fingerprinting of civet coffee extracts revealed a range of key metabolites, including caffeine, trigonelline,

**Table 1**
Nutritional composition of civet coffee.

| Nutritional Content | Green coffee (%)[a] | Roasted coffee (%)[a] | Infused civet coffee (%)[b] | Farmed civet coffee (%)[α] | Wild civet coffee (%)[c] | Wild civet coffee (%)[d] | Ethiopian Nekemte civet (%)[d] | Wild civet green bean (%)[e] | Wild civet coffee (%)[e] |
|---|---|---|---|---|---|---|---|---|---|
| Lipids | 12.30 ± 0.33 | 14.79 ± 0.08 | NR | 12.21 ± 0.19 | 12.13 ± 0.22 | 13 | 12.5 | 9.3 | 12.2 |
| Proteins | 13.36 ± 0.47 | 13.66 ± 0.12 | 1.1 ± 0.01 | 13.11 ± 0.23 | 11.08 ± 0.31 | 13.5 | 12.1 | 8.8 | 10.12 |
| Caffeine | 1.20 ± 0.02 | 1.10 ± 0.02 | 0.361 ± 0.03 | NR | NR | NR | NR | 0.52 | 0.47 |
| Chlorogenic acid | 3.73 ± 0.61 | 0.88 ± 0.44 | 0.293 ± 0.001 | NR | NR | NR | NR | NR | NR |
| Ash | NR | NR | NR | 5.38 ± 0.22 | 5.34 ± 0.17 | 3.6 | 3.2 | NR | NR |
| Carbohydrate | NR | NR | NR | 67.25 ± 0.33 | 69.44 ± 0.28 | 60.7 | 61.3 | NR | NR |
| References | Muzaifa et al. (2019) | Muzaifa et al. (2019) | Nishiguchi et al. (2017) | Hadipernata and Nugraha (2017) | Hadipernata and Nugraha (2017) | Marcone (2004) | Marcone (2004) | Ifmalinda et al. (2019) | Ifmalinda et al. (2019) |

NR not reported.
[a] Data presented are mean ± standard deviation of triplicate treatments.
[b] Data presented are mean ± standard error of the mean of five replicate treatments.
[c] Data presented are mean ± standard deviation of triplicate treatments.
[d] Data presented are mean of duplicate treatments.
[e] Number of replicates note reported.

N-methylpyridinium, kahweol, sucrose, caffeoyl shikimic acid, quinic acid, malic acid, lactic acid, acetic acid, sterols, fatty acids, GABA, and various caffeoylquinic acids (5-CQA, 4-CQA, and 3-CQA) as primary components (Farag et al., 2023; Febrina et al., 2021). The concentrations of trigonelline (10.11 ± 0.06 mM), sucrose (35.10 ± 0.17 mM), quinic acid (11.81 ± 0.04 mM), and alanine (2.58 ± 0.04 mM) were significantly higher in farmed civet coffee compared to the wild variety (Febrina et al., 2021) which may be attributed to the difference in treatments such as quality of cherries fed, stress due to confinement and dietary patterns (i.e., foraging for insects, meat and vegetable (Marcone, 2004). Furthermore, ultra-performance liquid chromatography coupled with high-resolution mass spectrometry (UPLC-ESI–HRMS) of methanolic extracts from civet coffee identified twenty-four metabolites across various classes, including three organic acids (i.e., malic acid, citric acid, quinic acid) two sugars (i.e., o-malonyl-hexopyranoside, di-o-hexoside), eight phenolic acids, five diterpenes, four fatty acids, and an amino acid (i.e., caffeoyl-n-tryptophan) (Farag et al., 2023). The abundance of amino and fatty acids in civet coffee increases during processing, serving as precursors for flavor compounds in the final product (Fitri, Laga, et al., 2021). The higher levels of valine (1.998 mg/g) and proline (0.758 mg/g) in civet coffee have been linked to the production of pyrroles and pyridines through reactions with Maillard intermediates (Muzaifa et al., 2019). Regarding fatty acid content, research indicates that civet coffee contains 9.3% fat in its green form and 12.2% in its roasted form, compared to 8.5% and 11.7% for regular arabica coffee (Ifmalinda et al., 2019). This enhanced concentration of amino and fatty acids in civet coffee, compared to raw and green coffee beans, is believed to result from enzymatic activity during gut fermentation (Fitri, Laga, et al., 2021; Ifmalinda et al., 2019; Marcone, 2004). Fermentation also reduces tannin-like constituents in the beans, which play a crucial role in flavor and taste development. Additionally, previous studies indicate that thoroughly roasted civet coffee beans contain more lipids (14.79 ± 0.08%), protein (13.66 ± 0.12%) nutrients than their green counterparts (12.30 ± 0.33% vrs 13.36 ± 0.47%, respectively) (Muzaifa et al., 2019). This can be attributed to a reduction in antinutrient factors, such as chlorogenic acid and other compounds, as well as enhanced solubility and concentration of beneficial compounds (Table 2). The roasting process also promotes Maillard and caramelization reactions due to the application of heat. Overall, the roasting process transforms the chemical composition of civet coffee, improving the bioavailability of certain nutrients and contributing to the unique flavor and aroma profile of the roasted beans.

The color of civet coffee beans is notably darker with a reddish hue, distinguishing them from both ordinary arabica and robusta coffee beans (Muzaifa et al., 2018). Scanning Electron Microscope (SEM) surface analysis revealed that civet coffee beans have a smoother texture compared to conventional coffee. This unique characteristic is attributed to the distinctive fermentation process occurring in the civet's GIT, which causes the outer layer of the beans to remove, aided by peristalsis (Ashika & Pushpa, 2021; Marcone, 2004). Further examination identified micro-pitting on the coffee beans, a result of enzymatic action and the acidic environment within the civet's GIT (Marcone, 2004; Stephanie & Emmanuel, 2011). Caffeine, a central nervous system stimulant, is one of the most recognized compounds found in coffee. This alkaloid contributes to the characteristic bitterness and stimulating properties of civet coffee (Muzaifa et al., 2020; Wale et al., 2024; Wondimkun et al., 2022). The caffeine content in unroasted civet coffee beans is significantly lower (41.77 mg/kg) relative to the control (44.94 mg/kg), a phenomenon attributed to the utilization of caffeine in the synthesis of purines and other nitrogen-containing compounds (Ashika & Pushpa, 2021; Stephanie & Emmanuel, 2011). It is important to note that purines and other nitrogen-containing compounds are converted into aromatic and flavor compounds during roasting. This transformation further explains why civet coffee has a more complex flavor profile compared to conventional coffee beans.

Another important alkaloid in coffee is trigonelline, which plays a crucial role in the Maillard reaction during roasting, enhancing the coffee's aroma and flavor. In civet coffee, trigonelline undergoes transformation during digestion, potentially increasing sweetness and reducing bitterness (Freitas et al., 2023, 2024; Ripper et al., 2022; Wale et al., 2024). Analysis of civet coffee from various regions in Indonesia revealed trigonelline concentrations of 1.01–1.20 g/100 g dry weight (Ripper et al., 2022), significantly higher than the 0.14 μg/mg reported by Farag et al. (2023). Chlorogenic acids (CGAs), phenolic compounds, also play a vital role in determining coffee's acidity and astringency (Freitas et al., 2023; Ripper et al., 2022; Wale et al., 2024). It has been reported that roasting significantly reduced CGA concentrations (0.88 ± 0.44%) compared to green beans (3.73 ± 0.61%), due to their instability under heat (Muzaifa et al., 2020).

The protein content in civet coffee is reported to be low due to the action of proteolytic enzymes in the civet's GIT. Ifmalinda and colleagues (2019) reported protein contents of 9.48% for regular green coffee and 11.3% for regular roasted coffee, compared to 8.80% and 10.12% for green and roasted civet coffee, respectively. These proteolytic enzymes break down proteins into short peptides and free amino acids, contributing to the unique flavor profile of civet coffee (Hanifah & Kurniawati, 2013; Ifmalinda et al., 2019). The fermentation process enhances the taste and flavor of civet coffee, facilitated by the optimal growth temperature of 37 °C for the microorganisms involved (Ifmalinda et al., 2019; Panggabean, 2011). Additionally, a study has reported increased lipid content in coffee beans (19.76%) subjected to fermentation in the civet (Mahendradatta, Zainal, & Tawali, 2011). Finally, mineral analysis revealed that the levels of most minerals in civet coffee were significantly lower than in the control coffee, with potassium at 0.75% versus 5.76%, calcium at 0.18% versus 0.85%, and copper at 1.39% versus 0.09%. However, bromine was an exception, showing higher levels in civet coffee at 3.37% compared to 1.735% in the control. This suggests that most minerals are absorbed through the civet's GIT (Stephanie & Emmanuel, 2011).

**Table 2**
The concentrations of bioactive compounds in roasted and green civet coffee.

| Name of compound | Civet coffee type | Quantity (%) | Reference |
|---|---|---|---|
| Caffeine | Roasted coffee | 1.10 | Muzaifa et al. (2020) |
| | Green coffee | 1.20 | |
| Caffeine | Green coffee | 1.34 | Darwin et al. (2022) |
| Caffeine | Roasted coffee | 0.46 | Ripper et al. (2022) |
| | Green coffee | 0.56 | |
| Caffeine | Roasted coffee | 0.52 | Ifmalinda et al. (2019) |
| Caffeine | Roasted coffee | 47.59 (mg/kg) | Stephanie and Emmanuel (2011) |
| | Green coffee | 41.77 (mg/kg) | |
| Caffeine | Roasted coffee | 1.18 | Patria, Abubakar, & Muzaifa, 2018 |
| Caffeine | Green coffee | 6.63 | Febrina et al. (2021) |
| Chlorogenic acid | Roasted coffee | 0.88 | Muzaifa et al. (2020) |
| | Green coffee | 3.73 | |
| Chlorogenic acid | Roasted coffee | 6.64 | Ripper et al. (2022) |
| | Green coffee | 6.57 | |
| Malic acid | Green coffee | 9.06 | Febrina et al. (2021) |
| Quinic acid | Green coffee | 11.81 | |
| Trigonelline | Green coffee | 10.11 | |
| Trigonelline | Roasted coffee | 1.05 | Ripper et al. (2022) |
| | Green coffee | 1.18 | |
| α-tocopherol | Roasted coffee | 0.35 (mg/kg) | Stephanie and Emmanuel (2011) |
| | Green coffee | 0.33 (mg/kg) | |

## 6. Volatile composition of civet coffee

The volatile and aromatic compounds in civet coffee are crucial for defining its complex flavor and rich aroma, enhancing the overall sensory experience. These compounds not only serve as indicators of quality but also influence consumer preferences and may offer health benefits. Additionally, they contribute to the coffee's cultural significance and culinary versatility.

Several gas chromatography–mass spectrometry (GC-MS) methods can be employed to profile the volatile composition of coffee, including headspace solid phase microextraction (HS/SPME), stir bar sorptive extraction (SBSE), headspace sorptive extraction (HSSE), solvent assisted flavor evaporation (SAFE). Each method has its own advantages and disadvantages, and the choice of technique depends on factors such as the desired sensitivity and selectivity for specific volatiles (Adadi, 2022; Adadi et al., 2019; Richter et al., 2017). It is also important to consider that the source of civet coffee, treatment methods (e.g., derivatization), extraction solvents, the chosen GC-MS method, fibers, the mode of injection (robotic or manual), sample size, and replicates can significantly influence the abundance of detected volatiles.

According to available literature (Cheong et al., 2013; Almerido et al., 2015; Ifmalinda et al., 2018; Ongo et al., 2020; Farage et al., 2023), HS/SPME-GC-MS is widely used for volatile profiling of civet coffee due to its reproducibility, ability to be fully automated, and capacity to extract multiple samples simultaneously (Adadi, 2022; Adadi et al., 2019; Richter et al., 2017).

Coffee contains a variety of compounds, each contributing to its quality and biological activity. Among these, volatile compounds are particularly important, as they are key constituents responsible for the aroma and flavor in coffee and coffee-derived products (Arya & Rao, 2007; Dong et al., 2019). Civet coffee, in particular, features a diverse range of volatile compounds, including pyridines, pyrazoles, aldehydes, and furans, all of which significantly enhance its unique aroma (Ashika & Pushpa, 2021). Research has identified over 800 volatile compounds in coffee, such as aldehydes, ketones, furans, pyrroles, sulfur compounds, and pyrazines, that contribute to its complex aroma profile (Toledo et al., 2016). Notably, sulfur-containing volatiles have emerged as significant contributors to the aroma development of coffee. The bitterness of civet coffee is attributed to the presence of carboxylic acids, including acetic, propionic, and butanoic acids (Buffo & Cardelli-Freire, 2004; Cheong et al., 2013), which could serve as discriminating markers to differentiate it from other coffee products.

A metabolomic analysis of arabica and robusta civet coffee sourced from the Philippines using HS/SPME-GC-MS identified a total of 47 volatile metabolites, with furfural being the most dominant ($1.4 \times 10^{-8}$ % peak area). Other notable volatile markers included acetic acid, 5-methylfurfural, 2-formylpyrrole, maltol, phenol, and 4-ethylguaiacol, which could be utilized for future fingerprinting in conjunction with chemometric tools (Ongo et al., 2020). Importantly, furfural is produced through the hydrolysis of pentose sugars, particularly xylose, during the roasting process (Bradbury, 1990). Additionally, Almerido et al. reported a total of 96 volatile compounds in robusta and excelsa civet coffee from the Philippines, also utilizing HS/SPME-GC-MS (Almerido et al., 2015). The tentatively identified volatiles were categorized into several chemical groups, including hydrocarbons, alcohols, aldehydes, ketones, acid anhydrides, furans, pyrans, esters, phenols, pyrroles, thiazoles, pyridines, pyrazines, amines, and other nitrogen heterocyclic compounds. The authors noted a lower abundance of furans (20.31%), and pyrans (20.31%) in robusta civet beans, although their levels increased (44.04%) with higher roasting temperatures (Almerido et al., 2015). The caramel-like flavor of civet coffee has been linked to the presence of furans and pyrans, while the woody and smoky aromas are attributed to pyrazines and phenols, respectively. Roasting significantly impacts the volatile composition of civet coffee; for instance, the abundance of 2-furan-methanol increases after roasting, whilst levels of 2-methyl-butanal decrease (Almerido et al., 2015; Silwar & Lullmann, 1993). Further analysis of civet coffee from Indonesia using SPME/GC-MS identified 75 volatile compounds across thirteen chemical groups, including six alcohols, two aldehydes, four aliphatic hydrocarbons, two aromatic hydrocarbons, seven esters, five ethers/oxides, fourteen furans/pyrans, three ketones, six monoterpene hydrocarbons, three phenolics, twelve pyrazines, and ten sesquiterpene hydrocarbons (Farag et al., 2023). Civet coffee exhibited a higher abundance of furan/pyrrole, pyrazines, and phenolic volatiles compared to roasted coffee with cardamom and roasted *c. arabica*. The authors attributed the elevated levels of pyrazines (i.e., methylpyrazine (6.05 ± 1.102%), 2, 6-dimethylpyrazine (1.62 ± 1.205%), etc) and phenolics (i.e., 4-ethylguaiacol (9.59 ± 4.57%), 4-vinylguaiacol (1.78 ± 1.197%), O-guaiacol (1.52 ± 0.078%)) to the effects of gut fermentation on the biotransformation of amino acids, sugars, and phenolic precursors (Farag et al., 2023). In contrast, roasted coffee infused with cardamom and roasted *C. arabica* exhibited a greater abundance of monoterpenes (such as α-Terpineol (5.69 ± 5.262%), camphor (0.23 ± 0.127%), isoterpinolene (0.6 ± 0.103%), and terpin-4-ol (0.95 ± 0.754%) as well as sesquiterpene hydrocarbons (including α-Farnesene (1.02 ± 1.11%), β-Curcumene (3.68 ± 0.702%), β-Eudesmene (1.19 ± 0.518%), calamenene (0.17 ± 0.151%), β-Caryophyllene (2.09 ± 1.751%), curcumene (7.28 ± 0.257%), germacrene (6.25 ± 5.915%), α-Humulene (0.45 ± 0.31%), α-Bergamotene (2.94 ± 3.195%), and β-Farnesene (0.63 ± 0.386%)). Additionally, di-furfuryl ether (2.56 ± 0.609%) was identified as a key discriminant marker for distinguishing civet coffee from control coffee (Farag et al., 2023). Furthermore, researchers have compared potential discriminant markers with authentic standards (such as malic acid, citric acid, glycolic acid, pyroglutamic acid, caffeine, and inositol) using advanced chemometric techniques, successfully differentiating civet coffee from commercial control coffee and counterfeit varieties (Jumhawan et al., 2013).

### 6.1. Determination of aroma of civet coffee using proton transfer reaction -time of flight-mass spectrometry (PTR-ToF-MS)

Proton Transfer Reaction-Time of Flight Mass Spectrometry (PTR-ToF-MS) is an innovative technique that enables direct, rapid, and sensitive online monitoring of volatile compounds in food and environmental samples (Biasioli et al., 2011). While this emerging method has been widely utilized to analyze volatiles in beer, coffee, and various other food products (Biasioli et al., 2011; Richter et al., 2017; Wan et al., 2024), there is limited research exploring its application in profiling the volatile composition of civet coffee (Ozdestan et al., 2013). This manuscript aims to highlight the importance of adopting this advanced technique for researchers studying civet coffee and its volatile compounds.

PTR-ToF-MS operates by utilizing gaseous-phase hydronium ions generated from an ion source. This method facilitates the monitoring of volatile organic compounds through the reaction between $H3O^+$ ions and the target analytes, allowing for effective proton transfer (Lindinger et al., 1998; Richter et al., 2017). PTR-ToF-MS can directly measure gas-phase of samples, enabling real-time monitoring of dynamic changes while maintaining an improved detection limit. Additionally, the use of hydronium ions for chemical ionization reduces the formation of artifacts, thereby enhancing the accuracy of tentative volatile identification (Biasioli et al., 2011; Richter et al., 2017). The technique has demonstrated its capability to identify and differentiate coffees from various geographical origins, including Brazil, Colombia, Costa Rica, Ethiopia, Guatemala, and India, based on their volatile compounds (Yener et al., 2015). A study characterized approximately 110 coffee samples from Asia, Africa, Central, and South America using PTR-ToF-MS in conjunction with chemometric tools such as partial least squares discriminant analysis (PLS-DA) (Ozdestan et al., 2013). The results revealed a clear distinction among Espresso coffees, civet coffee, and organic coffees based on their volatile profiles. The chemometric analysis demonstrated a significant ability to discriminate civet coffee from

other samples, a distinction likely attributed to the unique fermentation processes occurring in the civet's GIT. Notable volatile compounds associated with civet coffee include pyrrole (*m/z* 68.0500), furan (*m/z* 69.0336), terpenes (*m/z* 67.0542), vanillin (*m/z* 153.0520), and pyridine (109.0749) (Ozdestan et al., 2013). Moreover, when combined with chemometric techniques such as orthogonal projection to latent structures discriminant analysis (OPLS-DA), PLS-DA, and principal component analysis (PCA), PTR-ToF-MS has proven to be an excellent method for differentiating civet coffee based on its volatile profile (Monteiro et al., 2018). Overall, the application of PTR-ToF-MS in civet coffee research holds significant potential for enhancing the understanding of its unique aroma characteristics and could pave the way for more precise quality control and authentication methods in the coffee industry.

## 7. Analytical techniques for the authentication of civet coffee

Quality control is a fundamental aspect of food safety within the food industry, underpinned by rigorous laws, regulations, policies, and guidelines aimed at ensuring food protection (Aung & Chang, 2014). Coffee ranks among the most widely consumed beverages globally, and within the diverse array of coffee products, civet coffee stands out as the most expensive and highly valued, owing to its unique composition and fermentation process. The high market value and consumer demand for civet coffee have led some producers to resort to fraudulent practices, misrepresenting other coffee products as civet coffee. Consequently, effective authentication methods for civet coffee are crucial to safeguarding consumer interests and ensuring the integrity of the product (Taveira et al., 2014; Adier et al., 2020). Recently, various techniques have been employed to distinguish and verify civet coffee, including e-nose (Table 3), UV–Visible spectroscopy, infrared spectroscopy, and $^{1}$H NMR (Table 4).

### *7.1. Determination of the odorants and tastants of civet coffee using electronic nose (e-nose) technology*

All coffees and coffee products possess unique sensory properties, making it challenging to distinguish them based solely on their distinct aromas and tastes using conventional sensory evaluation methods. This difficulty is particularly pronounced when differentiating between civet coffee, regular coffee, and counterfeit varieties (Kulapichitr et al., 2019). Effectively distinguishing civet coffee from non-civet coffee based on aroma profiles is crucial for detecting adulterated civet coffee in the market (Dong et al., 2019; Wakhid et al., 2020). Traditionally, GC-olfactometry (GC-O) and sensory analysis have been employed to analyze coffee aromas; however, these techniques are often time-consuming, invasive, and costly. Recently, new technologies such as the e-nose have been utilized to differentiate civet coffee from non-civet varieties based on aroma. This innovative approach is highly sensitive, rapid, cost-effective, straightforward, and non-invasive (Korel & Balaban, 2009, pp. 365–374). However, factors such as sensitivity, selectivity, response time, stability, environmental conditions (temperature, humidity), sample size, and data processing can influence the results of the analysis. Notably, there are relatively few studies reported on the use of e-nose technology to distinguish between civet and non-civet coffee based on aroma. Below is a summary of these studies, including details such as sample size and other relevant factors to better understand the application of e-nose in civet coffee analysis (Table 3). The e-nose utilizes a gas sensor array to capture aroma signals in a data format, allowing for the extraction of statistical values for classification and interpretation (Wijaya et al., 2017). The process of classifying civet coffee aromas using the e-nose method involves several steps, including ground truth data acquisition, statistical feature extraction, classification, and performance evaluation (Ashika & Pushpa, 2021; Wakhid et al., 2020). Marcone (2004) successfully differentiated Indonesian and Ethiopian civet coffee and their respective controls, achieving a discrimination index of 95% with the Alpha MOS Fox 3000 e-nose, which is equipped with 12 metal oxide sensors. It was observed that the aroma profiles of civet coffee were modified due to the gastric juices and proteolytic enzyme activities in the civet's GIT. Ongo et al. (2012) reported a remarkable 100% accuracy in distinguishing between arabica and robusta civet coffee and non-civet coffee using the e-nose. Furthermore, chemometric analyses, such as PCA and hierarchical cluster analysis (HCA), revealed clear groupings among civet coffee, non-civet coffee, and their controls, highlighting unique aroma characteristics as quality indicators (Ongo et al., 2012). Other studies have integrated the Fox 3000 e-nose with six metal oxide semiconductor sensors and the Astree II potentiometric e-tongue, which features seven chemical sensors, alongside chemometric techniques (e.g., PCA and HCA) to differentiate coffee odorants based on various roasting conditions (Dong et al., 2019).

Harsono et al. reported that combining the e-nose with MQ sensors and machine learning techniques was effective in distinguishing arabica civet coffee from robusta coffee based on aroma, with the K-Nearest Neighbor (KNN) model yielding the highest accuracy of 97.7% (Harsono et al., 2020, pp. 333–339). Others have successfully integrated artificial intelligence with e-noses to create digital fingerprints that can accurately differentiate between civet and non-civet coffee (Hendrawan et al., 2022; Magfira et al., 2023). Building on these findings, future investigations could explore advanced machine learning algorithms and larger datasets to refine the discrimination process, potentially leading to more precise identification methods. Additionally, incorporating sensor fusion techniques and real-time data analysis could improve the robustness and applicability of e-nose technology in diverse production environments.

**Table 3**
Details of e-nose analysis of civet coffee.

| References | Coffee type | Sample size | Accuracy (%) | Multivariate analyses | E-nose and sensor type | Main findings |
|---|---|---|---|---|---|---|
| Marcone (2004) | Robusta civet coffee and African civet coffee | 6 | 95 | PCA | Alpha MOS Fox 3000 E-nose equipped with 12 metal oxide sensors. | There is a marked differentiation between Indonesian and African civet coffee |
| Ongo et al., 2012 | Arabica and robusta civet coffee | 8 | 100 | PCA, CA | Electronic Olfactory System, EOS835 with six metal oxide (MOX) sensors | E-nose effectively distinguished the aroma quality of Philippine civet coffee from control beans. |
| Harsono et al. (2020) | Arabica civet coffee and robusta coffee | 9 | 97.7% | LR, LDA, KNN | E-nose with the five (MQ) sensors | The aroma of civet coffee was successfully differentiated from both blended coffee and regular coffee varieties |
| Ongo et al., 2015 | Arabica and robusta civet coffee and their corresponding controls | 8 | 100 | PCA, CA | Electronic Olfactory System, EOS835 with six metal oxide (MOX) sensors | E-nose technology identified distinctions between civet and non-civet coffee from various geographical locations |

LR: Logistic Regression; LDA: Linear Discriminant Analysis; KNN: K-Nearest Neighbors; PCA: Principal Component Analysis; CA: Cluster Analysis.

**Table 4**
Authentication of civet coffee using various techniques.

| References | Coffee type | Markers used | Sample size | Sample preparation | Accuracy (%) | Multivariate analyses | Methods | Main Findings |
|---|---|---|---|---|---|---|---|---|
| Suhandy et al. (2019) | Peaberry, civet coffee | NR | 80 | Destructive | NR | PCA, PLS-DA | VS | The two coffee species were differentiated based on particle size |
| Suhandy and Yulia (2017) | Robusta civet coffee, Arabica coffee | Caffeine and caffeic acid | 98 | Destructive | 97 | PCA, PLS | VS | The determination of civet coffee content in Arabica-civet blends was significant. Absorbance measurements at 276 nm and 320 nm, linked to caffeine and caffeic acid, were crucial for identification |
| Adier et al. (2020) | Robusta civet coffee, robusta coffee | NR | 160 | Destructive | 100 | QDA, LR | VS | Civet coffee exhibited lower absorbance values in the visible spectrum compared to non-civet coffee, facilitating its discrimination |
| Yulia and Suhandy (2017) | Robusta civet coffee, Robusta coffee | NR | 100 | Destructive | 100 | PLS-DA, SIMCA | VS | Civet coffee was distinguished from non-civet variants with 100% accuracy, sensitivity, and specificity |
| Arboleda (2018) | Robusta civet coffee, Robusta coffee | NR | 218 | Nondestructive | 95–100 | DA, SVM, KNN | NIR | NIR spectroscopy revealed differences between civet and non-civet coffee at wavelengths of 907, 1088, 1540, and 1658 nm. Additionally, data training using a feedforward backpropagation artificial neural network achieved classification scores ranging from 95 to 100%. |
| Suhandy et al. (2018) | civet coffee | NR | 126 | Nondestructive | | PCA, PLS-DA | NIR | PLS regression model based on full-spectrum NIR spectroscopy quantified the degree of adulteration in civet coffee, achieving a high coefficient of determination during calibration and validation |
| Suhandy & Yulia 2018 | Civet, Peaberry, Pagar Alam coffee | NR | 90 | Destructive | 100 | PCA | FS | Discriminatory analysis between civet and non-civet coffees was successful at wavelengths of 370 nm and 453 nm, leading to the development of a highly effective discrimination model with 100% sensitivity and specificity for Peaberry, Civet, and Pagar Alam coffee |
| Prajna et al. (2023) | Wild and farmed civet coffee | NR | NR | Destructive/ nondestructive | 100 | HCA, PCA, SVM, RF | NIR | Successful discrimination between wild and farmed civet coffee was achieved based on treatment methods (unground, ground, unroasted, unroasted–unground, and roasted–unground). Moreover, sample treatments such as roasting and grinding significantly enhanced the accuracy of the discrimination models employed |
| Farag et al., 2023 | Civet coffee, Robusta coffee, Arabica coffee | kahweol, chlorogenic acid lactones, and elaidic acid | NR | Destructive | NR | PCA, HCA, OPLS-DA | $^{1}$H NMR, LC-MS, SPME/GC-MS | Civet coffee was differentiated from non-civet coffee based on the unique metabolites present in the beans and their size |
| Jumhawan et al. (2013) | Civet coffee, Robusta and Arabica coffee | malic acid, citric acid, glycolic acid, pyroglutamic acid, caffeine, and inositol | 21 | Destructive | NA | PCA, OPLS-DA | GC-MS | Civet coffee was successfully distinguished from other blends and counterfeit coffee using key markers such as citric acid, malic acid, and inositol |
| Jumhawan et al., 2015a | Civet, non-civet coffee | malic acid, citric acid | NR | Destructive | NA | PCA | GC-MS | A metabolomics approach combined with orthogonal projections to latent structures (OPLS) was employed to predict and distinguish the mixing composition of both known and unknown civet coffee. |
| Prajna et al., 2024 | Wild, farmed civet, | NR | 14 | Destructive | NA | PCA, SVM, RF | HS/GCMS | RF algorithm outperformed SVM techniques in discriminating wild civet coffee from farmed varieties based on their chemical profiles. The integration of Boruta feature selection with RF further enhanced discrimination accuracy, achieving a remarkable 100% accuracy rate |

PCA: Principal component analysis; PLS-DA: Partial least squares discriminant analysis; PLS: Partial least squares regression; LR: Logistic regression; QDA: Quadratic discriminant analysis; SIMCA: Soft independent modeling of class analogy; DA: Discriminant analysis; SVM: Support vector machine; RF: Random forest; HCA: Hierarchical cluster analysis; OPLS-DA: Orthogonal partial least squares-discriminant analysis; NA: Not applicable; VS: Visible spectroscopy; NIR: Near Infrared

Spectroscopy; NMR: Nuclear magnetic resonance: GC-MS: Gas chromatography–mass spectrometry; SPME/GC-MS: Solid Phase Microextraction/Gas chromatography–mass spectrometry, HS/GC-MS: Headspace/Gas chromatography–mass spectrometry; LC-MS: Liquid chromatography–mass spectrometry; FS: Fluorescence spectroscopy.

### 7.2. UV–visible spectroscopy techniques

The authentication of food products using UV–Visible spectroscopy presents a promising approach with several advantages (Nawrocka and Lamorska, 2013). This technique is relatively straightforward, delivers rapid results, and is more cost-effective compared to other spectroscopic methods. Additionally, it effectively detects and quantifies compounds with conjugated double bonds, which can be instrumental in identifying specific marker compounds associated with civet coffee. However, it has limitations, such as providing less detailed structural information and necessitating careful sample preparation to minimize interference from overlapping absorbance peaks, which can complicate result interpretation.

Recent studies have demonstrated the effectiveness of UV–Visible spectroscopy (239–405 nm) coupled with chemometric techniques such as SPA-LDA and PLS-DA to identify adulteration in a set of 106 ground roasted coffee samples (45 non-adulterated and 57 adulterated with husks and sticks) sourced from 14 different regions in Brazil, achieving 100% accuracy (Souto et al., 2015). Additionally, research on the effects of particle size in ground roasted peaberry and civet coffee (n = 80) utilized UV–Visible spectroscopy (190–1100 nm) alongside PLS-DA. The findings revealed distinct absorbance spectra corresponding to different particle sizes, with samples sized at 297 μm (mesh 50) exhibiting higher absorbance compared to those at 1680 μm (mesh 12). Consequently, the PLS-DA model proved more effective for discriminating between civet and peaberry ground coffee based on particle size compared to PCA (Suhandy et al., 2019). Furthermore, other studies have utilized UV–Visible spectroscopy (450 nm and 650 nm) in conjunction with various models, including linear discriminant analysis, quadratic discriminant analysis, and logistic regression, to authenticate and differentiate 120 samples of civet coffee from non-civet coffee (Adier et al., 2020). Notably, both the quadratic discriminant analysis and logistic regression models achieved 100% accuracy in distinguishing between the two types of coffee. The authors emphasized logistic regression as an optimal model due to its high precision and the fastest training time (14.050 s) among the various models evaluated, making it a promising choice for future modeling of civet coffee (Adier et al., 2020). In another study, multivariate analyses, including soft independent modelling of class analogies (SIMCA), PCA, and PLS-DA, were employed alongside UV–Visible spectroscopy (200–400 nm) to differentiate between civet and non-civet coffee (Yulia & Suhandy, 2017). This research identified distinct peaks at wavelengths of 276 nm and 320 nm, which indicated the presence of caffeine and caffeic acids, respectively. Moreover, the supervised discrimination models, specifically SIMCA and PLS-DA, demonstrated remarkable effectiveness in accurately grouping the coffee variants, achieving 100% accuracy, sensitivity, and specificity (Yulia & Suhandy, 2017). Additionally, the analysis demonstrated impressive results in assessing the authenticity of civet coffee, achieving accuracy rates of 95%, 91%, and 100% for accuracy, sensitivity, and specificity, respectively (Suhandy et al., 2016). These metrics indicate a high level of reliability in detecting adulteration in civet coffee samples. Such robust performance underscores the effectiveness of the analytical method employed, providing confidence in their ability to differentiate genuine civet coffee from adulterated or counterfeit products. Collectively, these studies lay the groundwork for developing a rapid and dependable method for the authentication of civet coffee, utilizing UV–Visible spectroscopy and chemometric techniques while taking into account sample and particle size in future applications.

### 7.3. Infrared (IR) spectroscopy techniques

Infrared spectroscopy is a widely utilized analytical technique for examining both organic and inorganic species within the infrared region of the electromagnetic spectrum. This method is divided into three primary zones: far-infrared, mid-infrared, and near-infrared. Among these, the mid-infrared region (4000–400 $cm^{-1}$) and the near-infrared region (14,000 to 4000 $cm^{-1}$) are the most commonly employed for various analytical applications (Ibañez & Cifuentes, 2001). The technique is based on the principle of absorption, which manifests as combination and overtone bands corresponding to molecular vibrations of functional groups such as -CH, -OH, and -NH. These groups are typically found in oils, carbohydrates, and proteins (Karoui, Downey, & Blecker, 2010; Wadood, Boli, Xiaowen, Hussain, & Yimin, 2020). Coffee contains a variety of compounds, including caffeine, chlorogenic acids, and caffeic acids, which exhibit bonds that produce absorbance bands at different wavelengths. These bands differ among coffee species, enabling effective discrimination between genuine coffee varieties and counterfeit products (Suhandy & Yulia, 2017). The authentication of civet coffee utilizing near-infrared (NIR) and mid-infrared (MIR) spectroscopy is discussed in detail below.

#### 7.3.1. Near-infrared (NIR) spectroscopy techniques

NIR spectroscopy is a non-destructive analytical technique that enables the assessment of samples without altering their properties. It is rapid and effective for evaluating bulk characteristics, such as moisture content and fat levels, which play a significant role in determining coffee quality. However, this method has some limitations, including the need for extensive calibration with known standards, reduced effectiveness in identifying specific compounds due to broad absorption bands, and the requirement for advanced chemometric techniques for data analysis.

NIR spectroscopy operates within the infrared region of the electromagnetic spectrum, specifically in the wavelength range of 14,000 to 4000 $cm^{-1}$ (or 700–2500 nm). It provides concise structural information based on vibrational transitions associated with various bonds in functional groups, such as C-H, N-H, and O-H (Yulia & Suhandy, 2017). Over the years, NIR has been successfully employed in the authentication of various food products, including coffee, honey, and milk powder (Maraboli et al., 2002; Prajna et al., 2023; Yulia & Suhandy, 2017). It has been particularly useful in determining the chemical composition of coffee, including moisture and caffeine content (Shen et al., 2018). These studies have revealed distinct peak shapes and intensities in the spectra, which can be utilized to differentiate between coffee species (Huck et al., 2005). In a notable study, Arboleda (2018) coupled NIR (907–1650 nm) with artificial neural networks (ANN) to analyze 218 samples of civet and non-civet coffee. The dataset was divided into 130 samples for training, 40 for testing, and 48 for validation. The results demonstrated clear discrimination between civet and non-civet coffee based on differences in chemical composition, reflected in varying absorption bands. The analysis using ANN achieved an impressive accuracy level of 95% in correctly grouping civet coffee from non-civet varieties (Arboleda, 2018). Others have successfully distinguished 126 adulterated civet coffee samples from non-adulterated ones using NIR spectroscopy (1300–2500 nm) in conjunction with chemometric techniques such as PCA and PLS (Suhandy et al., 2018). The calibration and validation coefficients ($R^2$) were 0.96 and 0.92, respectively, indicating a strong goodness of fit for the model and demonstrating its reliability. Moreover, hydroxyl (–OH) functional groups, particularly chlorogenic acids, were detected in the 1400–1500 nm range, suggesting their potential as markers for differentiating civet coffee from non-civet varieties. Additionally, the intensity of absorbance was found to decrease with increasing levels of adulteration (Suhandy et al., 2018).

Furthermore, the combination of NIR (400–2500 nm) and chemometric methods achieved 100% accuracy in discriminating civet coffee samples based on variations in production processes, sampling locations, and types (wild versus farmed civets) (Prajna et al., 2023). The authors noted that the processes of roasting and grinding significantly enhanced the accuracy of distinguishing between wild and farmed civet coffee, likely due to the liberation of chemical compounds, which led to differences in absorbance (Prajna et al., 2023). Additionally, research by Yulia and Suhandy (2018) examined the effect of particle size on the authentication of civet coffee using NIR spectroscopy. Their study revealed that reducing the particle size of civet coffee to approximately 212 μm resulted in a decrease in absorbance peaks within the wavelength range of 1300 nm–2500 nm. This observation is indicative of lower moisture content, reduced aroma, and the presence of certain volatile compounds (Yulia & Suhandy, 2018).

#### 7.3.2. Mid infrared or Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infrared (FT-IR) spectroscopy offers comprehensive insights into functional groups and molecular structures, enabling the analysis of a diverse range of sample types, including both liquids and solids, with rapid analysis times. However, there are some drawbacks to consider. FT-IR instruments can be relatively expensive, and certain sample types may require specific preparation to minimize interference. Additionally, the interpretation of spectra can be challenging due to overlapping peaks. Notably, despite its advantages over other spectroscopic techniques, a literature search reveals a lack of research on the application of FT-IR in the quality control of civet coffee.

The mid-infrared region, ranging from 4000 to 400 $cm^{-1}$, provides critical information for structural identification based on the rotational and vibrational properties of molecular bonds. In the food industry, FT-IR is predominantly used within this wavelength range because of its sensitivity and non-destructive nature (Munyendo et al., 2022). In coffee analysis, important compounds such as caffeine, lipids, and carbohydrates exhibit characteristic absorption bands at approximately 2840–2940 $cm^{-1}$, 1747 $cm^{-1}$, and 900–1400 $cm^{-1}$, respectively. These specific wavelengths, along with others, are instrumental in distinguishing between different coffee species (Craig et al., 2015).

### 7.4. Nuclear magnetic resonance (NMR) spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical technique that capitalizes on the interaction of atomic nuclei in a magnetic field. This method is predominantly employed in quality control and research to elucidate the molecular composition of substances. In the food industry, NMR provides detailed insights into the molecular structures present in food products (Hoult & Bhakar, 1997). One of the key advantages of NMR is its ability to perform quantitative analysis of specific compounds within complex mixtures without altering the samples during examination. It also offers comprehensive information regarding molecular structure, dynamics, and interactions. However, NMR equipment can be quite costly, requiring significant investment. Additionally, the technique is relatively slow and necessitates specialized knowledge and experience for effective spectrum interpretation.

In coffee analysis, various compounds such as caffeine, furfuryl alcohol, and 5-hydroxymethylfurfural have been quantified using NMR, making it a promising tool for the authentication of civet coffee (Lachenmeier et al., 2019). Civet coffee, like other coffee varieties, contains distinctive markers such as trigonelline, citric acid, lactic acid, and malic acid, which differentiate it from counterfeit products (Jumhawan et al., 2013). A recent study investigating the metabolite profiles of arabica civet coffees using NMR identified a range of unique compounds, including alanine, quinic acid, citric acid, malic acid, caffeine, trigonelline, sucrose, lactic acid, and formic acid. These metabolites can serve as markers for distinguishing between different coffee products (Febrina et al., 2021). Furthermore, the study found that civet coffee exhibited a higher abundance of metabolites compared to control samples, which is attributed to the fermentation processes occurring in the GIT of civets. Specifically, the concentrations of lactic acid, citric acid, malic acid, and trigonelline were significantly higher, while levels of caffeine, sucrose, and chlorogenic acids were lower (Febrina et al., 2021). $^{1}$H NMR spectroscopic method was utilized to investigate the effects of various factors such as the device used, extraction time, and sample sources on the concentrations of key markers, including 16-O-methylcafestol, kahweol, furfuryl alcohol, and 5-hydroxymethylfurfural (HMF) in coffee (Okaru et al., 2020). The validation results demonstrated satisfactory levels of specificity, selectivity, and linearity. Furthermore, the recovery rates for all analytes were acceptable, except for 16-O-methylcafestol and kahweol, indicating their potential for distinguishing civet coffee from non-civet varieties (Okaru et al., 2020). $^{1}$H NMR technique proves to be effective in differentiating civet coffee from other brands. It is important to recognize that each spectroscopic method possesses unique advantages and limitations in the authentication of civet coffee. Therefore, a combination of these techniques, paired with chemometric analysis, may offer an optimal strategy to leverage their strengths and mitigate their weaknesses, resulting in more reliable and comprehensive authentication outcomes.

### 7.5. Gas chromatography (GC) coupled to and mass spectrometry (GC/MS) or Flame Ionization Detector (GC/FID)-based metabolomics approaches

Gas Chromatography-Mass Spectrometry (GC/MS) and Gas Chromatography-Flame Ionization Detection (GC/FID) are both powerful analytical instruments widely employed to quantify volatile compounds and metabolites in products such as beer and coffee. These techniques operate based on the mass-to-charge ratio of ions and their ability to ionize, producing detectable signals. In the food and beverage industry, GC/MS and GC/FID are commonly used to analyze the chemical composition of samples, helping to identify potential adulteration or contamination. The advantages of these instruments include their rapid analysis times, reliability, cost-effectiveness, good selectivity and sensitivity, and compatibility with universal detectors. However, high operational and maintenance costs are notable drawbacks (Adadi, 2022; Adadi et al., 2021; Adadi et al., 2019; Green et al., 2024).

Researchers have employed GC/MS and GC/FID, often in conjunction with chemometric techniques, to distinguish civet coffee from other varieties (Green et al., 2024; Jumhawan, Putri, Bamba, & Fukusaki, 2015a, 2015b). For example, analysis of civet coffee and ordinary coffee powder using GC/FID and GC/MS revealed 678 and 182 peaks, respectively. Furthermore, similar metabolite patterns were observed across various classes, including glycolic acid, malic acid, pyroglutamic acid, citric acid, quinic acid, inositol, sucrose, and chlorogenic acid (Jumhawan et al., 2015a, 2015b). OPLS-DA modeling of the GC/FID ($R^2X = 0.88$) and GC/MS ($R^2X = 0.84$) data demonstrated clear separation between sample groups, exhibiting excellent goodness of fit. Additional validation tests confirmed a distinct separation between civet coffee (both farmed and commercial) and regular coffee, with $R^2 = 0.982$ and $Q^2 = 0.741$ (Jumhawan et al., 2015a, 2015b). Researchers have utilized GC/MS to effectively quantify and identify discriminant markers essential for the authentication of civet coffee. In one study, 21 coffee bean samples, including both *Coffea arabica* and *Coffea canephora*, were analyzed from three distinct cultivation areas. The analysis was complemented by multivariate statistical techniques such as PCA and OPLS-DA. The authors identified citric acid, malic acid, and inositol-pyroglutamic acid as key markers that successfully differentiated between authentic and counterfeit civet coffee, as well as between regular and blended coffee samples (Jumhawan et al., 2013). This identification of specific markers is crucial for establishing the authenticity of civet coffee, which often faces challenges related to adulteration and mislabeling. Thus, the combination of GC/MS, GC/FID,

chemometrics and markers presents a robust analytical framework for the authentication of civet coffee. By analyzing the chemical composition of coffee and comparing it to established marker profiles of verified authentic civet coffee, these techniques can significantly enhance quality assurance. This not only aids consumers in making informed choices but also helps producers maintain the integrity of their products in a competitive market. Furthermore, the application of these analytical techniques can contribute to regulatory compliance and bolster consumer confidence in the authenticity of civet coffee products. By ensuring that only genuine civet coffee reaches the market, producers can uphold their reputation and protect the unique qualities that distinguish this premium coffee variety.

## 8. Biological activities of civet coffee

The biological activities of civet coffee are still in the early stages of research, as indicated by the limited number of *in vitro* studies available (Febrina et al., 2021, 2023). These studies have primarily concentrated on antioxidant assays, including 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$), and ferric-reducing antioxidant power (FRAP), alongside alpha-glucosidase inhibition assays.

As consumers become increasingly aware of the health benefits associated with nutritious food products, producers are responding by developing products that exhibit biological activities or contain beneficial compounds (Table 2). This trend is particularly relevant to the civet coffee market, which is gaining traction in the food industry. Understanding the biological properties of civet coffee enhances its appeal as a premium, health-promoting beverage. Several studies have highlighted its antioxidant and antidiabetic properties, suggesting potential health benefits for consumers (Febrina et al., 2021, 2023). Research has shown that civet coffee contains higher levels of phenolic compounds and organic acids, such as acetic acid, lipid, trigonelline (10.11 ± 0.06 mM), quinic acid (11.81 ± 0.04 mM), citric acid, and malic acid (9.06 ± 0.03 mM), compared to arabica and robusta coffees (Tables 2–4) (Febrina et al., 2021, 2023). The presence of these compounds contributes to the antioxidant activity of civet coffee, which showed a significantly higher percentage of inhibition compared to arabica coffee (Febrina et al., 2023). Particularly, these compounds have been studied to mitigate free radicals either by transferring a hydrogen atom, or an electron followed by a proton, and/or the via the sequential proton loss electron transfer (Alisi et al., 2020). Furthermore, civet coffee exhibits greater inhibitory activity against $DPPH^{\bullet}$ ($IC_{50}$ = 30.90 ± 0.08 mg/L), $ABTS^{\bullet+}$ ($IC_{50}$ = 37.43 ± 0.15 mg/L), and FRAP ($EC_{50}$ = 30.67 ± 0.13 mg/L) than arabica coffee, with this activity attributed to its organic acid content (Febrina et al., 2021). Partial least squares (PLS) analysis demonstrated a strong correlation between the levels of malic acid in civet coffee and its percentage inhibition. Malic acid is known to reduce free radicals by donating electrons and enhancing the activity of antioxidant enzymes (Febrina et al., 2023; Mousavi et al., 2022). These metabolites have been extensively studied for their antioxidant and antimicrobial properties, suggesting that their concentration in civet coffee may offer health benefits to consumers (Ripper et al., 2022). Furthermore, the presence of malic acid and trigonelline is associated with the antidiabetic activity of civet coffee, as they exhibit inhibitory effects against α-glucosidase (Febrina et al., 2021). Notably, both wild ($IC_{50}$ = 3.94 ± 0.04 mg/mL) and caged ($IC_{50}$ = 4.77 ± 0.13 mg/mL) civet coffee demonstrated stronger α-glucosidase inhibitory activity compared to arabica coffee, potentially reducing sugar formation during digestion and lowering the risk of diabetes (Febrina et al., 2021).

Additionally, civet coffee contains diterpenes, sterols, and tocopherols. The presence of chlorogenic acids may further enhance glucose uptake and insulin sensitivity by acting as competitive inhibitors that occupy the active sites of enzymes like α-glucosidase. PLS-DA results also indicated a strong positive correlation between malic acid and α-glucosidase inhibitory activity ($r = -0.976$, $p < 0.05$) (Febrina et al., 2021). Moreover, the caffeine content in civet coffee has been linked to various activities, including antifibrotic and anti-cellulite effects (Chaiyasut et al., 2018; Kusumah & Gonzalez de Mejia, 2022). Recent reports indicate that caffeine may inhibit the expression of α-smooth muscle actin (SMA) and the conversion of myofibroblasts (MYO-SF) in response to transforming growth factor β (TGF-β1) stimulation. Specifically, caffeine intake has been shown to block the expression of the profibrotic cytokine TGF-β1, leading to reduced collagen deposition and diminished collagen mRNA levels (Talpan et al., 2023). Regarding its anti-cellulite properties, caffeine has been found to decrease the excessive accumulation of subcutaneous fat in blood vessels and to lower levels of proinflammatory cytokines, such as Intercellular Adhesion Molecule 1 (ICAM-1) and Vascular Cell Adhesion Protein 1 (VCAM-1) (Kassem et al., 2023). Furthermore, brewed arabica and robusta coffees have been reported to contain both high molecular weight compounds (such as polysaccharides and melanoidins) and low molecular weight compounds (including chlorogenic acids and caffeine), which are associated with immune modulation and anti-inflammatory effects, respectively, in macrophages (Passos et al., 2021). Chlorogenic acids exhibit strong antioxidant and anti-inflammatory properties, inhibiting the production of pro-inflammatory cytokines and enzymes, such as cyclooxygenase (COX) and lipoxygenase (LOX), thereby reducing inflammation. Similarly, caffeine exerts anti-inflammatory effects by antagonizing adenosine receptors, which decreases the release of pro-inflammatory mediators. Specifically, caffeine has been reported to reduce the expression of interleukins, including IL-1β and IL-6, while increasing levels of IL-10 and IL-13. It also appears to decrease components of the inflammasome, such as caspase-1 (casp1) (Eichwald et al., 2023). Melanoidins can also modulate immune responses by influencing cytokine production, thereby helping to regulate inflammation and immune activity (Cevik & Sarioglu, 2020; Chen & Schwarzschild, 2000; Farah & Donangelo, 2006).

Although research on the biological activities of civet coffee is limited, the higher levels of phenolics, polysaccharides, and alkaloids present in civet coffee suggest significant biological properties, similar to those reported for various coffee species (Febrianto & Zhu, 2023). It is important to recognize that the results from these *in vitro* studies (Febrina et al., 2021, 2023) may not directly translate to laboratory mice (*in vivo*) or cell cultures (*ex vivo*), necessitating caution when interpreting their findings. Nevertheless, these preliminary studies lay a valuable groundwork for future *in vivo* and *ex vivo* investigations into the biological activities of various types of civet coffee (farmed, wild, and ex situ) and their potential health benefits for consumers. However, it is crucial to acknowledge that effects can vary depending on factors such as coffee type, preparation methods, and individual health conditions. Further *in vitro*, *in vivo*, and clinical research is essential to fully elucidate these mechanisms and their implications for health.

## 9. Future directions

Future research in the field of civet coffee production should focus on several key areas to address existing challenges and improve sustainability and ethical practices. Firstly, there is a need for further investigation into alternative fermentation methods, such as the use of isolated microbial cultures and bioreactor systems. This research should aim to optimize fermentation conditions, assess the impact on coffee quality and flavor, and evaluate the feasibility of large-scale implementation. Additionally, future studies should explore the microbial ecology of civet's GIT to better understand the role of the gut microbiota in coffee fermentation. This research could involve metagenomic analysis to identify microbial communities and their functional properties, as well as studies on the interaction between microbiota and coffee beans during fermentation. Additionally, research into gut microbiota, their metabolism under simulated conditions, and the identification of species responsible for the unique flavor of civet coffee is warranted. This research could provide valuable insights for scaling up the production of

*ex vivo* civet coffee.

Furthermore, there is a need for research on the sensory characteristics and consumer preferences of civet coffee produced using different fermentation methods. Comparative sensory evaluations can provide valuable insights into the flavor profiles and quality attributes of civet coffee, helping to inform production practices and market strategies.

Despite the comprehensive discrimination between wild and farmed civet coffee using authentication techniques, volatile profiles combined with chemometrics, there has been no study on the discrimination of *ex vivo* and *in vivo* civet coffee. However, based on the available findings, there may be a clear grouping of *ex vivo* and *in vivo* civet coffee based on aroma and structural characteristics. Therefore, future studies should investigate the effects of authentication techniques on the discrimination between *ex vivo* and *in vivo* civet coffee.

Future research should address the ethical considerations surrounding civet coffee production, including animal welfare concerns and the impact on civet populations and their habitats. This could involve studies on alternative approaches to civet coffee production that minimize harm to animals, as well as initiatives to promote sustainable sourcing practices and conservation efforts. Overall, future research in these areas will contribute to the development of more sustainable, ethical, and high-quality civet coffee production practices, ensuring the long-term viability of this unique and culturally significant beverage.

Lastly, artificial intelligence should be employed to enhance civet coffee production by optimizing various aspects of fermentation and authentication. AI-driven technologies can refine the fermentation process by analyzing microbial interactions and optimizing fermentation parameters (Cheng et al., 2023; Hosseinzadeh et al., 2022), thereby preserving the unique flavor profiles of civet coffee. Additionally, predictive analytics can be integrated with advanced techniques such as SPME/GC-MS, e-nose, PTR-ToF-MS, NIR, FTIR, NMR, GC/MS, and GC/FID to ensure thorough discrimination between genuine civet coffee and counterfeit products. This approach should consider key factors such as marker compounds, sample size, and sources of samples to enhance accuracy. By leveraging these capabilities, the civet coffee industry can achieve improved sustainability, quality, and ethical practices.

## 10. Conclusions

In conclusion, the production of civet coffee has advanced significantly with the exploration of alternative fermentation methods, such as *in vitro* fermentation using isolated *B. subtilis* and bioreactor-based approaches. These techniques present promising solutions to address animal welfare concerns associated with traditional *in vivo* fermentation, all while preserving the unique aroma and flavor profiles characteristic of civet coffee. However, several challenges persist, including the need for more thorough characterization of fermentation substrates and microbial species, as well as the optimization of fermentation conditions to ensure consistent product quality.

Technologies such as SPME/GC-MS, e-nose, and PTR-ToF-MS have proven effective in differentiating civet from non-civet variants based on aroma and marker profiles. Additionally, methods like UV–Visible spectroscopy, NIR, FTIR, NMR, GC/MS, and GC/FID, combined with chemometric techniques, have demonstrated significant efficacy in distinguishing civet coffee, even between farmed and wild types, from counterfeit products. Integrating artificial intelligence with these analytical technologies and chemometric analyses represents an optimal strategy to capitalize on their strengths and address their limitations, ultimately leading to more reliable and comprehensive authentication outcomes. Furthermore, when developing models to distinguish civet coffee from non-civet variants, it is essential to implement well-designed experiments, maintain balanced sample sizes, and incorporate key marker compounds to achieve robust authentication. Future research should prioritize these areas to enhance the sustainability and ethical integrity of civet coffee production. By adopting innovative approaches and prioritizing animal welfare, the civet coffee industry can continue to thrive while fostering responsible and environmentally conscious practices.

## CRediT authorship contribution statement

Parise Adadi: Conceptualization, Writing – original draft, Writing – review & editing. Emmanuel Ofosu Mensah: Writing – original draft, Writing – review & editing. Beatrice Blay: Writing – original draft. Mirja K. Ahmmed: Writing – original draft, Writing – review & editing, Desigin and editing images. Kazi Sumaiya: Data curation and designing images. Dominic Agyei: Writing – review & editing. Biniam Kebede: Writing – review & editing.

## Conflicts of interest

The authors declare no conflict of interest.

## Data availability

No data was used for the research described in the article.

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