INVESTIGATE THE ROLE OF MICROBES IN TEA AND COFFEE FERMENTATION AND THEIR IMPACT ON QUALITY OF TEA AND COFFEE

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Daria Hayesalea

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ABSTRACT

This study aims to develop a yeast culture technique for use in the fermentation of Robusta and Arabica coffee while identifying processing methods and oxygen conditions that enhance coffee quality, using *Saccharomyces cerevisiae* SafAle s-33 in fermentation. Notably, SIAF fermentation resulted in higher concentrations of volatile compounds such as pyrazine ethyl, pyrazine 2,6-dimethyl, and furfuryl, which enriched the coffee's aroma with nutty notes (almond, hazelnut, peanut, and walnut) and chocolate and cocoa characteristics. Additionally, furfuryl compounds imparted sweet, caramel, and toasty, bread-like aromas Sensory evaluation by Q-graders and PCA analysis revealed distinct profiles of organic acids and volatile compounds in roasted coffee beans processed with SIAF and anaerobic fermentation. These findings suggest that yeast-based fermentation, particularly SIAF, can enhance Robusta coffee's sensory profile, offering a promising approach for improving its market potential.

Additionally, the study investigated the fermentation of green tea using fungal strain BT01, identified as *Aspergillus cristatus*, and its effects on tea quality. Tea bricks (BT01) were successfully inoculated with *A. cristatus*, leading to a 1.5-fold increase in caffeine content after 16 days of fermentation. GC-MS analysis identified 88 volatile compounds, with linalool and methyl salicylates as components distinguishing fermented tea from controls. Additionally, post-fermentation resulted in significant shifts in organic acid composition, notably reducing malic and citric acids

while increasing fumaric acid, which is associated with an enhanced “mellow and fresh” taste. These finding highlight the potential of *A. cristatus* in modulating tea aroma and taste through fermentation.

Keywords: Fuzhuan Brick Tea, Fermentation, Polyphenols, Green Tea, Robusta Coffee, Secondary Metabolite, *Camellia senensis*, *Coffea cenaphora*, *Saccharomyces cerevisiae*, *Aspergillus cristatus*

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ABBREVIATIONS AND SYMBOLS

α	Alpha
β	Beta
γ	Gamma
µg	Microgram
mg	Milligram
g	Gram
kg	Kilogram
µl	Microliter
ml	Milliliter
L	Liter
µg/kg	Microgram per kilogram
mg/kg	Milligram per kilogram
g/kg	Gram per kilogram
mg/L	Milligram per liter
g/L	Gram per liter
CFU/g	Colony-forming unit per gram
CFU/ml	Colony- forming unit per milliliter
ACE	Absorptive column extraction
ATP	Adenosine triphosphate
BVCs	Bacterial volatile compounds
CA	Catechin
CF	Caffeine
CGA	Chlorogenic acids
CP	Coffee pulp
DHS	Dynamic headspace
DNA	Deoxyribonucleic acid
DM	Diabetes mellitus
EGCG	Epigallocatechin gallate

ABBREVIATIONS AND SYMBOLS

EGC	Epigallocatechin
EC	Epicatechin
ECG	Epicatechin gallate
°C	Degrees Celsius

CHAPTER 1

INTRODUCTION

1.1 Background and Important of the Research Problem

Besides water and soft drinks, coffee and tea are considered the most consumed beverages. The color and physiology of these two beverages have come from active compounds of plants, *Coffea* sp. and *Camellia sinensis*. Phytochemicals found in coffee and tea encompass numerous polyphenolic compounds and phenolic acid, which are also commonly found in vegetables and fruits. The biological properties of coffee and tea are closely linked to their polyphenol content, including flavonoids, catechins, and tannins, as well as caffeine. Phenolic compounds exert various health-promoting properties, ranging from free radical scavenging to antiviral and anticancer effects (LIczbiński & Bukowska, 2022). Coffee beans and tea leaves are produced in at least 50 countries in the world.

Brazil is a top producer of coffee, while China is a top producer and export tea beverage. In 2022, Thailand has a coffee plantation area of 242,465 rai, yielding 18,689 tons which generates income for farmers of approximately 5.5 billion baht per year (Meesaeng, 2022). The production of Arabica coffee in Thailand is cultivated only in the Northern part mainly Chiang Rai and Chiang Mai provinces (Office of Agricultural Economics, 2023). Most Robusta coffee are cultivated in the Southern part of Thailand, especially Chumphon and Ranong provinces. However, the plantation of Robusta is also found in other parts of Thailand including the Northern part of Thailand (Office of Agricultural Economics, 2023).

Robusta coffee has always been known as low quality compared to Arabica coffee which result in the price of Robusta coffee beans (66.75 baht/kg) is lower than Arabica coffee (142.30 baht/kg; Office of Agricultural Economics, 2023). Since, Arabica coffee requires high altitudes and lower temperatures (18-21 ºC). Recently, the yield and quality of Arabica coffee decreased significantly due to current climatic conditions. On the other hand, Robusta coffee can grow in lower altitudes and habitats

with slightly higher temperatures (22-30 ºC). Moreover, Robusta coffee produce of a higher crop yield, elevated caffeine levels, lower sugar content, increased levels of soluble solids, and enhanced resistance to pests and diseases when compared with Arabica coffee (Goldem-berg et al., 2015). Thus, Robusta coffee that is resistant to disease and high temperatures could be a more sustainable option than Arabica coffee. Even though, hash flavor of Robusta coffee makes it less attractive to coffee drinkers; however, several physicochemical, microbiological, agricultural, cultural, processing, storage-related conditions can influence the quality of the beverage. The fermentation method has contributed to the production of coffee with different sensory profiles due to the production of primary and secondary metabolites by microorganism (de Jesus Cassimiro et al., 2023). The role of aroma precursors and flavor formed during the fermentation of coffee and the roasting process have been extensively observed in research studies (Laukalēja & Krūma, 2018). The re-fermentation of unfermented Robusta coffee with kefir is reported to improve volatile profiles since Robusta is a low-grade coffee (Afriliana et al., 2019). Moreover, the amount of oxygen (O2) varies depending on the method used and impacts the quality of the beverage. In anaerobic conditions, lactic acid production is intensified, contributing to the perception of acidity and body of coffee beverages. On the other hand, self-induced anaerobic fermentation (SIAF) is a fermentation method in which the anaerobic condition is gradually formed by microbial metabolism that uses the remaining O2 for its metabolic reactions, releasing carbon dioxide (CO2), volatile and non-volatile compounds. Besides, the SIAF method positively impacts the fermentative performance of aerobic fermentation and yeasts during the processing of natural and pulped coffee (de Jesus Cassimiro et al., 2023).

Since the genetic makeup of Robusta coffee beans is more complex than Arabica coffee beans. In addition, delayed or extended fermentation may lead to inconsistent fermentation contributing to notes in the sensory attributes and unreliable. More importantly, most of the research is carries out with Arabica coffee and it is limited to Robusta coffee. Thus, the first objective of this study is to investigate the quality of Robusta coffee after microbial fermentation as well as conditions during fermentation.

The second part of the study is focusing on another frequently consumed beverage which is tea. As an extract made from the leaves of *C. sinensis* has high concentrations of flavonoids and other antioxidants with posted beneficial properties (Khan & Mukhtar, 2013). The high mountain terrain in Northern Thailand such as Chiang Mai, Chiang Rai, Mae Hong Son, and Phayao is the perfect environment for tea cultivation (Chinwong et al., 2021). According to Office of Agricultural Economics, (2023) in 2020 of Thailand had a tea plantation area of 129,566 rai and yielded 102,914 tons. Type of tea based on processing or harvested leaf development which are black (fermented), green (non-fermented), and oolong (semi-fermented). The different processes of drying and fermentation determine its chemical composition (Khan & Mukhtar, 2013).

Fuzhuan brick tea (FBT) and Pu-erh tea are a type of microbial fermented and unique brick-shaped tea. FBT is raw black matured tea leaves while Pu-erh tea uses sun-dried green tea leaves. The general steps for manufacturing this kind of tea include panning, rapid pile-fermentation, rolling, drying, softening with steam, pilling, partitioning, tea-brick pressing, fungal fermentation, drying, and packing (Xiao et al., 2020). The health-promoting function of this tea, such as its anti-obesity, hypolipidemic, and anti-proliferation could be attributed to the participation of microorganisms found in solid-state fermentation. Several microorganisms such as *Candida albicans*, *Candida famata* (*Debaryomyces hansenii*), *Aspergillus niger*, and *Aspergillus sydowii* have the potential to reduce caffeine content through inoculated and converting caffeine to theophylline (Zhou et al., 2019). Moreover, the fungus secretes various extracellular enzymes e.g. Polyphenol oxidase (PPO), cellulase, pectinase, and proteinase to degrade and transfer polysaccharides, polyphenols, protein, and cellulose which results in producing aroma, taste, and health-benefit compounds.

The bitterness and astringency of green tea are closely associated with the catechin content. To enhance the taste of autumn green tea, efforts have been made to reduce catechin levels. Zhang et al., (2016) improved the sweetness and overall acceptability of green tea infusion by hydrolyzing (-)-epigallocatechin-3-gallate (EGCG) and (-)-epicatechin-3-gallate (ECG) with tannase. These methods effectively reduced bitterness and astringency in autumn green tea and there is a need for further development of techniques process of this post-fermented to enhance its overall quality,

encompassing taste, and aroma. *Aspergillus cristatus*, a fungus commonly found in Fuzhuan brick tea and plays a significant role in the fermentation process of this post-fermented dark tea. This fungus secretes extracellular enzymes during fermentation, which influence the metabolism of chemical compounds in tea leaves and consequently, impact the quality of Fuzhuan brick tea (Xiao et al., 2020). The suggest the potential to of *A. cristatus* is to biotransform phenolic compounds and reduce catechin content suggesting its potential to mitigate the bitterness and astringency of autumn green tea. The second objective of this study, is application of the *A. cristatus* in the fermentation of green tea from locals and investigate its impact on the tea's quality with a focus on taste improvement.

In conclusion, this study aims to incorporate specific microbial strains in pre-fermentation of coffee and post-fermentation of tea to improve the cup quality of coffee and tea. Self-induce anaerobic (SIAF) and anaerobic fermentation methods, the yeast culture technique and compounds in fermented coffee and tea were investigated.

1.2 Research Objectives

1.2.1 Aims to develop a yeast culture technique for use in the fermentation process of Robusta and Arabica coffee.

1.2.2 Aims to identify coffee processing methods and oxygen conditions that enhance coffee quality, particularly for Robusta coffee.

1.2.3 To investigate fungal fermentation in green tea and its effects on compounds associated with tea quality and health benefits.

1.3 The Importance of Research

Coffee is considered as another important economic crop in Thailand and generates a lot of income for farmers. However, since Arabica coffee (*Coffea Arabica L.*) is mostly susceptible to diseases and insects, the yield of Arabica coffee per hectare in Thailand has suffered and has impacted Arabica coffee farmers, especially in the upper Northern region. Robusta coffee (*Coffea Robusta*), it has a high yield and can be

grown widely, but it is not as popular as Arabica coffee due to the quality of Robusta coffee. Therefore, in order to find alternative for farmers in the North to grow more Robusta coffee, the research project under this program aims to develop affordable yeast culture technique and use it in the coffee fermentation process to be suitable for commercial production.

To enhance the taste of autumn in green tea, effort have been made to reduce catechin levels improved the sweetness and overall acceptability of green tea infusion by hydrolyzing EGCG and ECG with tannase. These methods effectively reduced bitterness and astringency in autumn in green tea and there's a need for further development of techniques to enhance its overall quality, encompassing taste, and aroma. Since, *A. cristatus* shows promise in altering phenolic compounds and decreasing catechin levels, indicating its ability to mitigate the bitterness and astringency of autumn green tea potentially.

1.4 Research Hypothesis

Inoculation of yeast during dry processing of Robusta and Arabica coffee using whole fruits under SIAF and anaerobic resulted in influenced production of chemical compounds especially volatile and amino acid compounds.

Inoculation of fungal in green tea leaves will influence the formation of different chemical composition, which will enhance flavor and aroma of tea drink.

1.5 Scope of Research

1.5.1 Sixty kilograms of uniform Arabica and Robusta coffee beans were harvested from the North of Thailand and used for fermentation by dry processing. Fermentations were performed under SIAF and anaerobic conditions with the starter culture of *Saccharomyces cerevisiae* SafAle S-33. The quality of fermented Arabica and Robusta coffee were analyzed by GC-MS, LC-MS, and sensory test.

1.5.2 Ten kilograms of green tea leaves were inoculated with a specific strain of fungi and the quality of fermented tea will be evaluated by HPLC, GC-MS, LC-MS, and sensory test.

1.6 Research Limitations

Coffee fruits do not ripen at the same time and will ripen gradually. In addition, if unripe fruits are mixed in the fermentation process, it could affect the taste of the coffee. Thus, the harvesting method is important and requires meticulous care. The best way to harvest coffee fruit is hand-picked one by one, so only ripened fruit will be harvested and prevent damage to the coffee cherries. However, this method is time-consuming and requires extensive labor; therefore, hand-picked the whole cluster is applied for this project. Moreover, coffee cherries can only be harvested once a year (November – March). So, it is critical to plan experiments to work within a limited timeframe. Also, an efficient method to select only ripened coffee fruits for fermentation is equally important.

Microorganisms played a key role in fermenting tea and coffee, as microbial fermentation is essential to achieve specific flavors and chemical changes in these products. Fermentation with microorganisms such as certain types of bacteria or fungi, transforms the natural compounds in tea and coffee, enhancing flavor, aroma, and nutritional value. However, a challenge in this process was avoiding contamination by other, unwanted microorganisms that could interfere with the controlled fermentation. If these unwanted microbes enter the process, they may introduce undesirable flavors or spoil the product, affecting quality and consistency. Therefore, maintaining a pure microbial environment was essential to achieving the desired results in the study.

1.7 Terminology Definition

C. Arabica	*Coffea Arabica*
C. cenaphora	*Coffea cenaphora*
S. cerevisiae	*Saccharomyces cerevisiae*

C. sinensis	*Camellia sinensis*
A. cristatus	*Aspergillus cristatus*
HPLC	High Performance Liquid Chromatography
GC-MS	Gas Chromatography and Mass Spectrometry
LC-MS	Liquid Chromatography and Mass Spectrometry
HS-SPME	Headspace Solid-phase Microextraction
SPME-GC-MS	Solid Phase Micro Extraction
SIAF	Self-induced Anaerobic Fermentation
PCA	Principal Component Analysis
PLS-DA	Partial Least Squares Discriminant Analysis
YEPD	Yeast Extract Peptone Dextrose Agar
PDA	Potato Dextrose Agar

CHAPTER 2

LITERATURE REVIEW

2.1 Previous Related Studies

Aswathi, et al. (2024), considered the impact of *Saccharomyces cerevisiae* MTCC 17, fermentation on the chemical composition and sensory quality of pulped natural/honey-processed Robusta coffee. Fermented over 192 hours, microbial activity influenced key metabolites, including sugars, organic acids, and alkaloids, as identified through ^{1}H NMR and GC-MS analysis. The treated coffee exhibited enhanced chemical properties and the presence of additional aroma compounds contributing to sweet, fruity, and caramel notes. Sensory evaluation showed a slight improvement in quality scores. This research demonstrating its potential for flavor and quality enhancement.

Xiao, et al. (2020), investigates the role of microorganisms in the fermentation and quality of Pingwu Fuzhuan brick tea, identifying *Eurotium cristatum* (strain PW-1) as the primary fungus responsible for fermentation. Compared to natural fermentation, PW-1 inoculation accelerated the biotransformation of phenolic compounds, improving the tea's infusion color and taste. The fermentation process also increased the proportion of velvety and sweet-tasting amino acids over 16 days. Alcohols were the most abundant volatiles, with comparable content in both natural and inoculated samples. Statistical analyses, including OPLS-DA and HCA, confirmed significant differences between naturally fermented and PW-1 inoculated teas. Given its positive impact on fermentation efficiency and tea quality.

This study aims to enhance the cup quality of coffee and tea by incorporating specific microbial strains in the pre-fermentation of coffee and post-fermentation of green tea. This chapter provides a biophysical review of coffee, yeast, green tea, and fungi cell biology, covering various aspects such as cell structure, metabolism, type of fermentation, phenolic compound, organic acid, and measurement of quality after fermentation.

2.2 Coffee in the Fermentation

Coffee is one of the most popular beverages and one of the larger traded commodities globally. Millions of people consume coffee daily due to its aromatic flavor and the stimulating effect of caffeine, along with other beneficial ingredients. The two main type of coffee, Arabica coffee (*Coffea arabica*) and Robusta coffee (*Coffea canephora*), are cultivated in tropical and subtropical regions. Major coffee producing countries include Brazil, Vietnam, Colombia, and Indonesia (Klingel et al., 2020). Coffee is a significant plantation crop from the family Rubiaceae, subfamily Cinchonoideae members of the Rubiaceae, family are predominantly tropical or subtropical, consisting of around 400 general and 4,800 - 5,000 species. Botanically, coffee belongs to the genus Coffea within the Rubiaceae family. The subgenus Coffea includes over 80 species, primarily fond in Africa and Madagascar. Among these, *C. Arabica* and *C. cenaphora* are the most commercially important and widely cultivated varieties worldwide (Patil et al., 2022). These biological differences between Arabica and Robusta contribute to their distinct characteristics in coffee production, flavor profile, and growing conditions.

2.2.1 The Main Type of Coffee

2.2.1.1 *C. arabica*, commonly known as Arabica coffee, is a small tree or bush that is notable for its compact growth and tetraploid nature, with 2n = 44 chromosomes. The plant exhibits dense branching, with dark green, leathery leaves. At each node, cluster buds emerge in the axils of the leaf (Figure 2.1). Once pollinated, it takes about 8 to 9 months for the fruit to form, eventually becoming dark red in color, containing about 10 to 20 berries per cluster. Arabica coffee plants are highly valued for their smooth, sweet flavor, and are typically grown at higher altitudes (Patil et al., 2022).

Figure 2.1 Arabica coffee tree; (a) tree carrying fruits, (b) flowers, and (c) mature fruits

2.2.1.2 Robusta coffee, derived from the species *C. canephora*, is a diploid species with 2n = 22 chromosomes. It is generally larger than the Arabica plant, with oblong-shaped fruits. The flowers of Robusta are white, fragrant and tend to form in larger clusters compared to Arabica (Figure 2.2). Robusta coffee is known for its resilience to pests and harsher growing conditions. The fruits of Robusta take about 10 to 11 months to mature, about two months longer than Arabica, making it a slightly longer process for fruit development. Robusta beans tend to have a stronger, more bitter taste, with higher caffeine content, making them popular in blends and instant coffee (Patil et al., 2022).

Figure 2.2 Robusta coffee tree; (a) tree, (b) flowers, (c) mature fruits

2.2.2 Structure of Coffee Tree and Cherry

2.2.2.1 Leaves are the shiny, waxed leaves of the coffee plant that typically grow along the main stem and are usually green. In some species or varieties, young leaves may have bronze tips, turning green or dark green as they mature, but they do not become brown unless stressed or fallen. Arabica coffee leaves have a lifespan of about 8 months, while Robusta coffee leaves last between 7 and 10 months. Arabica coffee leaves can grow up to 15 cm long, though Robusta coffee leaves may reach 30 cm, and those of *Coffea liberica* can grow as large as 50 cm (Klingel et al., 2020).

2.2.2.2 Flowers of coffee plants produce white, multi-flowered, cymose inflorescences, with an adult coffee tree yielding around 30,000 - 40,000 flowers annually, particularly in Arabica coffee. Robusta coffee likely produces even more flowers. These flowers are protected from dehydration and insects by collecters, which secrete a glue-like substance. As the flowers begin to wilt, they are plucked to prevent any negative impact on the coffee cherry yield. Once dried, coffee flowers can be brewed into a tisane and are known to contain high levels of phenolic compounds, as well as approximately 1.00 g of caffeine and 1.00 g of trigonelline per 100 g of dry

weight in Robusta coffee. However, due to limited research, toxicological data on coffee flowers is not yet available (Klingel et al., 2020).

2.2.2.3 The structure of a coffee cherry is composed of several layers (Figure 2.3). The outermost layer, the epicarp (skin), starts with green and changes to red (or yellow) as the cherry ripens. Beneath this is the soft, sweet mesocarp (pulp), which surrounds the robust endocarp (parchment). The parchment encloses two seeds, known as coffee beans, which are further protected by a thin layer called the silver skin or tegument. Each layer of the cherry plays a crucial role in the development of the coffee bean, influencing the flavor and quality of the final product (Klingel et al., 2020).

2.2.3 Coffee Harvesting

Harvesting is essential for selecting mature fruits from coffee plants. The process begins with clearing loose soil, leaves, weeds, leftover fruits from previous harvests, and other debris. Three primary harvesting techniques are commonly used: manual, semi- mechanized techniques, and mechanized method

2.2.3.1 The manual technique is harvesting the coffee cherries by hand-picking (Aswathi & Murthy, 2024).

2.2.3.2 Semi-mechanized techniques combine both manual (hand-picking or selective harvesting and mechanized) operations. Research indicates that semi-mechanized harvesting reduces costs by 27% compared to manual methods and boosts efficiency by over 30% (Aswathi & Murthy, 2024).

2.2.3.3 The mechanized technique is increasingly preferred, even on sloped terrain, due to its much higher operational capacity compared to semi-mechanized and manual method, though it requires additional steps like sweeping and transferring during harvest and postharvest processes (Aswathi & Murthy, 2024).

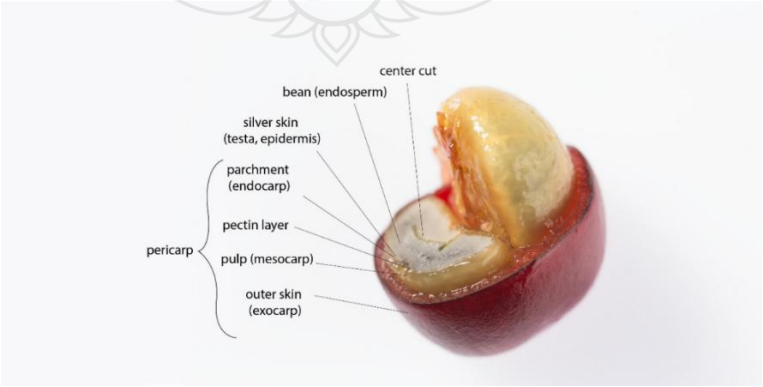


Figure 2.3 Cross section of coffee cherry with its different layer

2.2.4 Coffee Processing

Three main processing methods for coffee can be distinguished: dry (natural), wet (washed), and honey. Each method produces coffee with distinct flavor profiles. Dry processed beans typically yield a fuller flavor and higher body in the resulting coffee, while wet methods produce a cleaner taste profile and the honey processing method combines elements of both dry and wet processing. Various reviews have discussed how these processing methods impact the composition and sensory qualities of coffee brews.

2.2.4.1 The natural or dry processing method is a simple and reliable way to convert coffee cherries into green beans, often resulting in high-quality coffee. In this method, coffee cherries are typically sun-dried in open spaces, spread evenly to a thickness of approximately 8 mm to reduce moisture content (Figure 2.4). Regular raking is necessary to ensure even drying and produce a consistent, high-quality result. Cherries are deemed dry once they produce a characteristic rattling sound (Aswathi & Murthy, 2024). Sun drying is widely practiced by small-scale producers and cooperatives in developing countries, while some industrial firms may use mechanical drying techniques. During sun drying, coffee cherries are typically spread on wire mesh tables, where they remain for around two weeks. The method is particularly popular in regions with minimal rainfall and abundant sunlight, such as Indonesia, Ethiopia, Brazil, and Yemen. Under optimal conditions, drying takes around 12-15 days, although rates depend on factors lie initial humidity, ambient temperature, moisture content, later thickness, and stirring frequency (Murthy & Naidu, 2012).

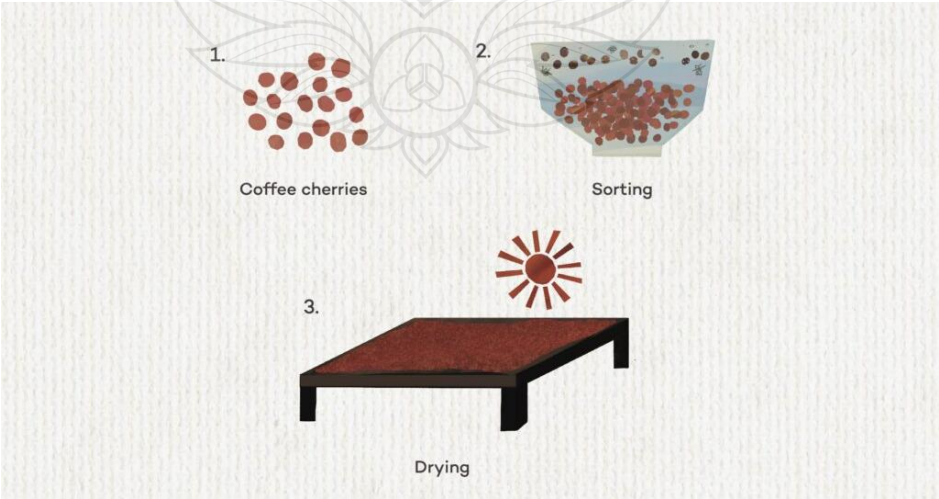


Figure 2.4 Schematic representation of the natural or dry processing

2.2.4.2 The wet processing method is used to remove mucilage from coffee beans through fermentation, washing, and drying, producing what is known as parchment or washed coffee (Figure 2.5). This method requires reliable pulping equipment and ample water. During fermentation, microorganisms like lactic acid bacteria, yeasts, and certain molds play a key role by producing pectinolytic enzymes that break down the pectin in mucilage, creating secondary metabolites. Fermentation occurs in large tanks over 24-72 h, significantly enhancing the coffee's quality, flavor profile, and acidity. The process is complete when beans feel gritty, not sticky, after which they are washed to remove fermentation by-products (Aswathi & Murthy, 2024).

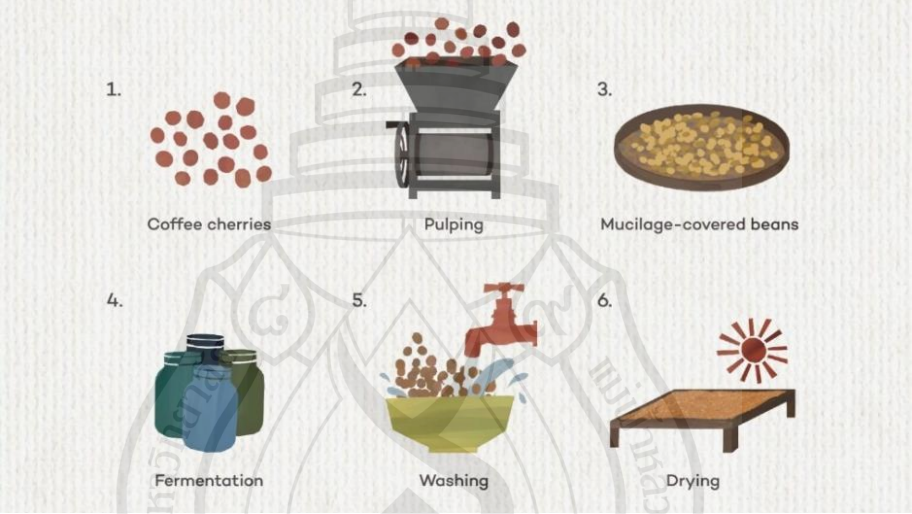


Figure 2.5 Schematic representation of the wet or wash processing

2.2.4.3 Honey processing or pulped natural, process is an innovative semi-dry processing technique that combines characteristics of both natural and washed coffees (Figure 2.6). In this method, coffee cherries are dried after de-pulping, allowing the mucilage to dry with fermentation. Key steps include controlled raking and heaping to prevent spoilage and ensure even fermentation (Aswathi et al., 2024).

Figure 2.6 Schematic representation of the honey processing

2.2.5 Conditions of Coffee Fermentation

2.2.5.1 Aerobic fermentation is the oldest and simplest method of coffee processing, where fermentation and drying occur simultaneously (Figure 2.7). This traditional method allowed natural fermentation to occur, driven by environmental factors and the microbiota present on the coffee fruit. Oxygen availability plays a crucial role in this process, as it influences microbial activity, with aerobic-microorganisms helping to break down the mucilage and other compounds during fermentation (Ferreira et al., 2023).

Figure 2.7 Shows the aerobic fermentation

2.2.5.2 Anaerobic fermentation is microorganisms metabolize sugars in the absence of oxygen, producing energy and byproducts such as ethanol, carbon dioxide (CO_2), glycerol, organic acids, volatile alcohol, and esters (Figure 2.8). This

fermentation method is utilized in countries like Brazil and Thailand to enhance the sensory profiles of coffee, allowing for the creation of high-quality, consistent specialty coffees across different seasons on an industrial scale. One technique associated with anaerobic fermentation is carbonic maceration, inspired by wine processing. It involves the solid-state fermentation of whole, unruptured coffee cherries in an anaerobic environment filled with CO_2 within a sealed container. This process promotes unique flavor development and enhances to complexity of the coffee's sensory profile, contributing to distinctive taste characteristics (Ferreira et al., 2023).

Figure 2.8 Shows the anaerobic fermentation

2.2.5.3 Self-induced anaerobic fermentation (SIAF) is a modern coffee fermentation technique first described by da Mota et al. (2020), which uses closed cylindrical polyethylene bioreactors for solid-state fermentation (Figure 2.9). Unlike traditional methods, SIAF does not require the addition of water and involves both aerobic and anaerobic stages. Initially, aerobic conditions prevail, but as microorganisms consume oxygen and produce carbon dioxide (CO_2), anaerobic conditions develop. The bioreactors, typically ranging from 20 to 200 liters, are equipped with an airlock valve that allows CO_2 to escape while preventing oxygen from entering. SIAF can be applied to both natural and pulped coffee cherries, with the-bioreactors being manually managed for homogenization and feeding. This technique enhances the fermentative activity of lactic acid bacteria (LAB) and yeast, promoting the production of various metabolites. (Pereira et al., 2022).

Figure 2.9 Shows the self-induced anaerobic fermentation (SIAF)

2.2.6 Relationship Between Chemical and Coffee

2.2.6.1 The chemical component of coffee beans is complex, with carbohydrates making up the largest portion, about 60% of the total weight of raw beans. In addition to carbohydrates, coffee beans contain proteins, fats, tannins, caffeine, minerals, and other trace elements. The composition of these ingredients depends on the coffee's variety, origin, and harvest season. During roasting, these components undergo chemical reactions that contribute to the unique flavors and colors found in different types of coffee beans (Saud & Salamatullah, 2021).

2.2.6.2 Volatile components are key to the flavor of coffee, as raw beans lack the characteristics of aroma. During roasting, chemical reactions break down compounds in the beans, generating over 800 volatile aroma components. These are formed mainly through reactions between acids, phenols, sugar, and organic compounds, contributing to the coffee's flavor profile. Common volatile substances include sulfur, nitrogen, and oxygen-containing compounds like furan, thiophene, pyrazine, and pyridine, as well as phenols and terpenes (Saud & Salamatullah, 2021).

2.2.6.3 Alkaloids content; caffeine (1,3,7-trimethlxanthine) is the main alkaloid in coffee, contributes to its bitter taste and is widely found in tea, cocoa, and coffee. Also trigonelline, which is a pyridine derivative found in coffee, makes up about 1% of both Arabica and Robusta coffee beans. However, it undergoes significant degradation during the roasting process, with only 0.1-0.2% of it remaining after dark roasting. One of the thermal degradation products of trigonelline during roasting in N-methylpyridine. In addition to caffeine and rigonelline, coffee contains other bioactive

compounds such as theobromine, theophyllibetine, which are found in coffee leaves. These compounds contribute to the overall health benefits of coffee (Saud & Salamatullah, 2021).

2.2.6.4 Phenolic acids and their derivatives, including p-hydroxybenzoic acid, vanillic acid, p-coumaric acid, ferulic acid, and chlorogenic acid (CGA), along with several caffeine acid derivatives like caffeoylquinic acid, and 3-O-feruloylquinic acid. Chlorogenic acid is the primary phenolic compound in coffee and during roasting, degrades thermally, leading to the formation of bitter phenolic compounds and contributing to the characteristic color and astringency of coffee (Saud & Salamatullah, 2021).

2.2.6.5 Flavonoids content are kind of active ingredients widely existing in natural plants, with antioxidant, anticancer, anti-inflammatory, and antibacterial activities. Small seed coffee contains flavonoids such as catechin, epicatechin, and quercetin (Saud & Salamatullah, 2021).

2.2.6.6 Terpenoids content contains a variety, primary p-kauri-type and coffeol diterpense, with the highest concentration of coffeol and 16-O-methylcoffeol. The latter has been used as a marker to differentiate small and medium coffee beans. Recent studies have identified new terpenoids, including mascarosides and kauri diterpenoid glycosides, from green coffee beans. Additionally, interpenes like dammarane-type caffruones have been isolated from Yunan coffee fruits (Saud & Salamatullah, 2021).

2.2.6.7 Flavor substances such as cell wall polysaccharides, lipids, proteins, sucrose, chlorogenic acid, caffeine, and trigonelline are involved in forming coffee's flavor through the Maillard reaction, Strecker degradation, and caramelization during roasting. Coffee has 28 characteristic flavor substances, categorized into five groups: (1) Aldehydes and ketones contribute to caramel and sweet flavors (e.g., iso-butyral, vanillin); (2) Sulfur compounds create sulfur and roasting odors (e.g., 2-furfuryl mercaptan, dimethyl trisulfide); (3) Pyrazines are linked to soil aromas (e.g., 2-ethyl-3,5-dimethylpyrazine); (4) Phenols and aldehydes are associated with smoky or phenolic aromas (e.g., guaiacol, acetaldehyde); and (5) Furan ketones are related to pungent tastes (e.g., 3-hydroxy-4,5-dimethyl-2-furanone) (Saud & Salamatullah, 2021).

2.3 Cell Structure and Function of Yeast

2.3.1 Morphotypes of Yeasts

The smallest replicative unit of a eukaryotic cell is the nucleus, which depends on the cytoplasm for essential functions necessary for replication. Consequently, the basic functional unit of an organism is the cell, leading to various fundamental morphotypes derived from the relationship between cells and nuclei. The first morphotype is the syncytium, characterized by a single cytoplasm containing multiple nuclei, which is rare in yeasts. An example is the strictly hyphal species *Ashbya gossypii*, which forms true septate hyphae or filaments. In its hyphae, the replicative cycles of individual nuclei are independent of cell growth and cytoplasmic processes, allowing each nucleus to undergo its own cycle regardless of neighboring nuclei. This nuclear autonomy prompts questions about the molecular mechanisms controlling nuclear division. The second morphotype features a typical one nucleus-one cell arrangement, where nuclear division is closely linked to cell division through the cell cycle machinery (Figure 2.10). This coupling is particularly evident during vegetative replication cycles and the first cell division following mating and zygote formation in many yeast species (Knop, 2011).

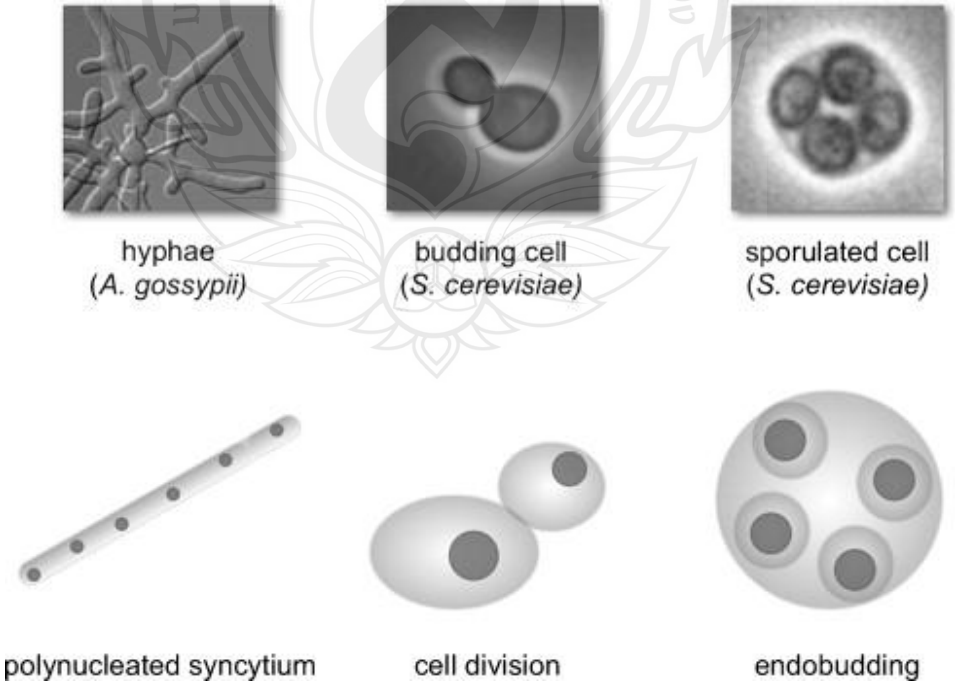


Figure 2.10 Shows the basic cellular morphotypes of yeast cells

2.3.2 Cell Division Budding

Yeasts are unicellular fungi that reproduce primarily through budding or fission, with many species in the Saccharomycetaceae family exhibiting an ellipsoid or spherical shape. A distinguishing feature of yeasts is asymmetric cell division, where a- mature mother cell forms a smaller daughter cell through budding. This process, regulated by cytokinesis, ensure the proper segregation of cellular content between the mother and daughter cells. *Saccharomyces cerevisiae* serves as a key model organism for studying the mechanism, contributing to a deeper understanding of cellular division and its regulatory processes (Knop, 2011).

2.3.3 Life Cycles of Yeasts

The life cycles of yeast species typically alternate between haploid and diploid stages, with mating and meiosis/sporulation events separating them (Figure 2.11). Cellular decisions to undergo morphogenic transitions, such as mating and meiosis, are influenced by both external factors (like nutrient availability) and the internal status of the cell. These transitions are initiated during the G1 phase of the cell cycle, as only cells in G1 are capable of undergoing the necessary changes for processes like mating and meiosis (Knop, 2011).

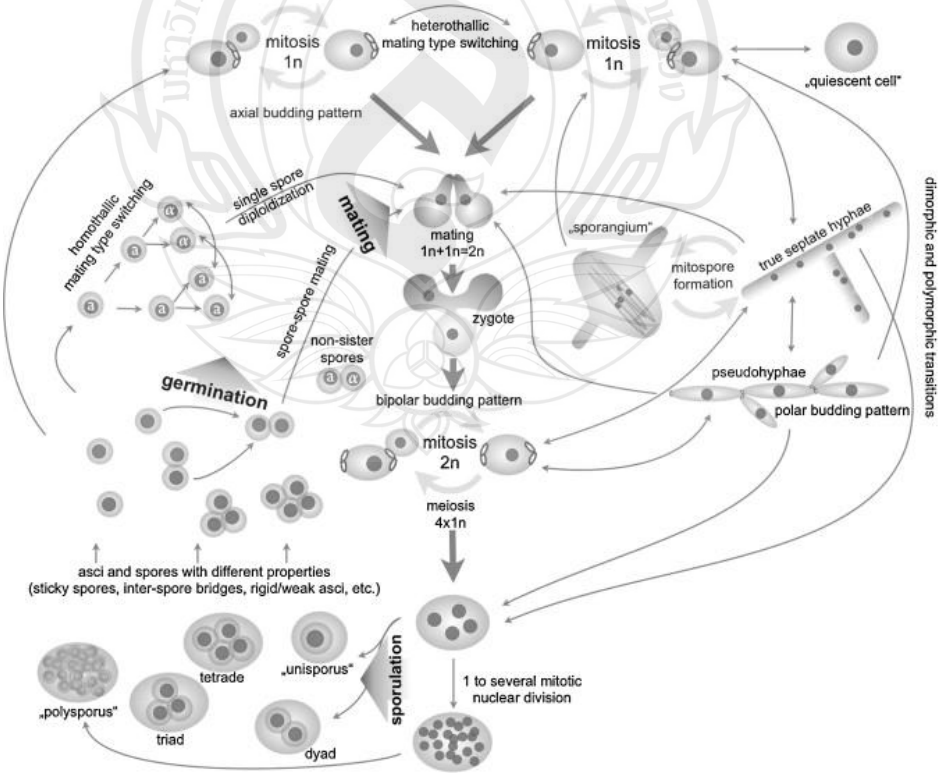


Figure 2.11 Schematic representation of life cycle of the yeast cells

2.3.4 Yeast Starter Culture

Yeasts have been known for their significant pectinolytic activity and their essential role in coffee demucilaging. Research has shown that pectinolytic yeasts, such as *Saccharomyces marxianus*, *S. bayanus*, *S. cerevisiae* var. *ellipsoideus*, and *Schizosaccharomyces* spp., contribute to breaking down the mucilage layer of Robusta coffee. Yeasts are involved from the beginning of the fermentation process, as they naturally reside on the coffee cherry's surface. Their presence aids in natural fermentation, and adding a mixture of selected pectinolytic yeasts to the fermentation process further accelerates mucilage degradation. In particular, a combination of *Saccharomyces* species has been shown to completely break down pectic substances within 7 to 8 hours (Ruta & Farcasanu, 2021).

2.4 Green Tea in Fermentation

Tea is the second most consumed beverage globally, following water, and is primarily made from the leaves, buds, or stems of the Camellia genus, particularly *Camellia sinensis*. The *C. sinensis* is one of the most popular sources of antioxidants and the most widely consumed beverage after water. It is classified into three main types green tea, oolong tea, and black tea based on antioxidant levels and fermentation processes. Tea leaves are steamed at high temperatures after harvesting to inactivate polyphenol oxidizing enzymes, which results in preserving the vitamins and enchancing antioxidant content. Green tea is renowned for its high antioxidant levels and is used for anti-aging and neuroprotective effects, as well as for treating or preventing various diseases, including cancer, cardiovascular conditions, and obesity.

2.4.1 Botanical Classification of Green Tea

The *Camellia* genus includes over 90 species distributed from Nepal to Taiwan and Japan in East Asia, with *C. sinensis*, being the most widely known. Green tea is especially popular in East Asia, particularly in China and Japan, while black tea is favored in Western countries. The use of tea leaves dates back over 3,000 years, originating in southwest China, where they were initially chewed and consumed similarly to coffee. Sealy (1958) estimated that the tea plant likely originated in the

Yunnan district of China, though this has not been definitively confirmed. Additionally, a wild type of the *Assamica* variety of *C. sinensis* was discovered in India in 1,835, with subsequent findings in Thailand and Burma (Shrivastava et al., 2018).

Figure 2.12 Green tea; (a) flower (b) leaves tea (c) plantation

2.4.1.1 Flowers of the tea plant are bisexual and white, emitting a fragrant scent. They can occur solitary or in pairs on leaf axils, with pedicels that are 6-10 mm long and curve downward. The flowers have 5-6 rounded, mucilaginous sepals with membranous margins that feature lashes, and 5-8 broadly obovate petals. The numerous stamens have their outer filaments fused to a tube. The ovary is superior, tomentose, and 3-locular, with a single style that is apical and 3-lobed. The flowers are scented and can appear singly or in clusters of two to four shown in Figure 2.12 (a) (Shrivastava et al., 2018).

2.4.1.2 Leaves of the tea plant are alternate and have a petiole measuring 3-7 mm in length. The leaf blade is thinly leathery, elliptic, or obovate, ranging from 5 to 12 cm long and 1.8 to 4.5 cm wide. The apex is either mucronate or blunt-pointed, while the base is cuneate. The leaf margins are serrated, and the surface may be glabrous or slightly hairy, with about eight pairs of lateral veins that are visible. The leaves are bright green, shiny, and often have a hairy underside, as shown in Figure 2.12 (b) (Shrivastava et al., 2018).

2.4.1.3 Cultivation of *C. sinensis*, the tea plant native to East Asia, Southeast Asia, and the Indian Subcontinent, is now cultivated in tropical and subtropical regions globally. It thrives in sunny climates with hot temperatures and ample rainfall, requiring a growing season of at least eight months. There are three primary growth spurts: spring (late March to early May), early summer (June to early July), and late summer to fall (mid-July to October). The plant prefers acidic soils, with sandy loam being ideal due

to its ability to drain well while retaining essential nutrients shown in Figure 2.12 (c). *C. sinensis* can tolerate annual precipitation between 70 and 310 cm, average temperatures of 14 to 27 °C, and a soil pH ranging from 4.5 to 7.3 (Shrivastava et al., 2018).

2.4.2 Type of Tea

2.4.2.1 Green tea is partially fermented. It is rich in various beneficial compounds, offering numerous positive effects on human health (Vishnoi et al., 2018).

2.4.2.2 Black tea comprises approximately 72% of the world's total tea production. Black tea undergoes full fermentation, which oxidizes most of the EGCG antioxidants. However, it still retains a significant amount of polyphenol antioxidants, such as flavonoids, which help eliminate harmful toxins from the body (Vishnoi et al., 2018).

2.4.2.3 White tea is made from buds and young tea leaves collected just before they fully open, white tea is minimally processed through steaming and drying. This method preserves the highest levels of antioxidants and the lowest caffeine content compared to green, black, and Oolong teas (Vishnoi et al., 2018).

2.4.2.4 Red tea or oolong tea is a partially fermented tea, oolong combines the flavor and health benefits of both green and black teas. It is rich in antioxidants that help protect healthy skin cells and slow down the aging process (Vishnoi et al., 2018).

2.4.2.5 Dark tea or Pu’erh tea is a sourced from a large-leaf variety of the tea plant, Pu’erh tea is processed similarly to black tea but is unique in that it is aged 50 to 100 years after being picked (Vishnoi et al., 2018).

2.4.2.6 Yellow tea or huángchá in Chinese, yellow tea is a lightly fermented and rare variety unique to China. Its pleasant mellow taste and recognized health benefits, such as anti-oxidation, anti-inflammation, and anti-cancer properties, have contributed to its growing popularity. Although yellow tea and green tea share a similar initial production process, yellow tea undergoes additional steps, particularly a unique “sealed yellowing” procedure. This process increases the oxidation level and eliminates the grassy smell characteristic of green tea while retaining its health benefits (Xu et al., 2018).

The six main types of tea all come from the same plant, but their chemical composition and flavor differences result from variations in processing. Key steps that

influence these differences include oxidation, halting the oxidation process, shaping the tea, and drying it that shows in Figure 2.13.

Figure 2.13 The type of tea

2.4.3 Chemical Composition of Green Tea

Green tea, derived from *Camellia sinensis*, is a potent source of disease-fighting antioxidants called polyphenols, mainly catechins, which make up 10% to 20% of its dry weight. Key catechins include epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), and epicatechingallate (ECG). In addition to polyphenols, green tea contains proteins (15-20%), essential amino acids (1-4%), carbohydrates (5-7%), minerals and trace elements (5%), lipids, and alkaloids (3-4%) shown in Figure 2.14. This diverse chemical composition contributes to the numerous health benefits attributed to green tea, including antioxidant, anti-inflammatory, and potential disease-preventing properties (Hazimeh et al., 2023).

2.4.3.1 Catechin is the one of antioxidant potential of tea beverages is largely attributed to their significant catechin content, which are phenolic compounds beneficial to human health. Green tea primarily contains four main catechins: (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG), with EGCG being the most active and abundant. The polyphenolic content in green tea exhibits a greater capacity for scavenging free radicals than vitamin C alone. Catechins, particularly from tea, have

shown exceptional antioxidant activity due to their ability to neutralize free radicals and enhance detoxification enzymes like glutathione peroxidase, catalase, and glutathione reductase. Research has demonstrated that catechins possess greater antioxidant capacity than glutathione, vitamin C, and flavonoids, underscoring their crucial role in maintaining cellular redox homeostasis (Kochman et al., 2020).

2.4.3.2 Caffeine is a key component of tea beverages, contributing to their unique flavor and enhancing their antioxidant potential. Its concentration can vary based on several factors, including the time of harvest and the age of the leaves; generally, older leaves contain lower levels of caffeine. Additionally, the caffeine content is influenced by the tea variety, environmental conditions during growth, and the brewing method used. Caffeine's beneficial effects are linked to its ability to neutralize reactive oxygen species and boost antioxidant enzyme activity, as well as increase total glutathione levels. Regular intake of caffeine may help reduce persistent oxidative stress, which can lower the risk of diseases associated with free radicals. Furthermore, caffeine has been shown to inhibit the secretion of pro-inflammatory cytokines, highlighting its potential anti-inflammatory effects (Kochman et al., 2020).

2.4.3.3 Phenolic acids are secondary metabolites found in plants, known for their significant antioxidant and anti-inflammatory properties. They also exhibit neuroprotective and hypoglycemic effects and have been associated with the inhibition of cancer cell growth and prevention of metastasis. Additionally, some phenolic acids can modulate lipid and carbohydrate metabolism, supporting the regulation of metabolic disorders. One of the most prevalent phenolic acids found in various foodstuffs is chlorogenic acid, highlighting its importance in both nutrition and health (Kochman et al., 2020). Phenolic acids are essential dietary components characterized by the presence of one carboxylic acid group. They fall into two sub-groups based on their chemical structure: hydroxybenzoic acids (derived from benzene) and hydroxycinnamic acids (derived from cinnamic acid). Common hydroxycinnamic-acids include chlorogenic, ferulic, caffeic, p-coumaric, and sinapic acids, while vanillic, p-hydroxybenzoic, protocatechuic, and syringic acids are examples of hydroxybenzoic acids. These compounds are synthesized through the phenylpropanoid pathway and exist in various forms, including free, soluble, conjugated, and insoluble forms (Mihaylova et al., 2024).

2.4.3.4 Flavonoids are classified into seven structural classes: flavanones, flavones, isoflavones, flavonols, flavanols, and anthocyanins. They represent a crucial subgroup of natural phenols, significantly contributing to the color and aroma of various fruits (like berries, grapes, and apples) and vegetables (such as onions and cabbage). Their biosynthesis primarily occurs through the phenylpropanoid pathway, with most flavonoids found in leaves, flowers, fruits, and seedlings (Mihaylova et al., 2024).

2.4.3.5 Content of Quercetin is a phytochemical known for its antioxidant and neuroprotective properties. It has been shown to play a role in normalizing carbohydrate metabolism by inhibiting glucose absorption in the gastrointestinal tract, regulating insulin secretion, and improving insulin sensitivity in tissues. Furthermore, when combined with epigallocatechin gallate (EGCG), quercetin may enhance the anticarcinogenic effects of both compounds, potentially providing greater protective benefits against cancer (Kochman et al., 2020). Quercetin and other flavonoids are known for their diverse biological activities, primarily due to their antioxidant properties (Malaguti et al., 2013).

2.4.3.6 Flavonols are consumed in human nutrition in both glycoside and aglycone forms. In Western populations, the estimated daily intake of flavonols ranges from 20 to 50 mg. Among these, quercetin is the most prominent, accounting for approximately 13.82 mg per day. This widespread consumption highlights the significance of flavonols, particularly quercetin, in contributing to the overall antioxidant intake in the diet properties (Malaguti et al., 2013).

2.4.3.7 Rutin is a polyphenolic compound recognized for its potent antioxidant properties. Its synergistic interaction with ascorbic acid (vitamin C) may enhance cardiovascular protection by strengthening blood vessels. Additionally, rutin exhibits antidiabetic and anti-inflammatory effects, helping to prevent complications associated with diabetes. Its combined antioxidant and anti-inflammatory actions suggest potential benefits in preventing conditions related to free radicals or inflammation, including various neurodegenerative diseases (Kochman et al., 2020).

2.4.3.8 Ascorbic acid, or vitamin C, is recognized as one of the most potent water-soluble natural antioxidants, associated with minimal toxicity and found in various dietary foods and plants. It effectively combats reactive species such as superoxide radical anions, hydrogen peroxide (H_2O_2), hydroxyl radicals, and singlet

oxygen radicals, and can neutralize reactive nitrogen species (RNS) in aqueous solutions. Rich sources of ascorbic acid include citrus fruits, kiwi, cherries, melons, tomatoes, and leafy vegetables like broccoli, cauliflower, and cabbage. Additionally, tocopherols (vitamin E) are prominent antioxidants primarily found in nuts, seeds, and vegetable oils (Prasanth et al., 2019). Vitamin C is a potent exogenous antioxidant that plays a crucial role in enhancing the immune defense of the body. As an essential micronutrient, it must be consumed daily in adequate amounts to support overall health and well-being. Its antioxidant properties help protect cells from oxidative stress, contributing to various physiological functions and promoting a healthy immune response (Kochman et al., 2020).

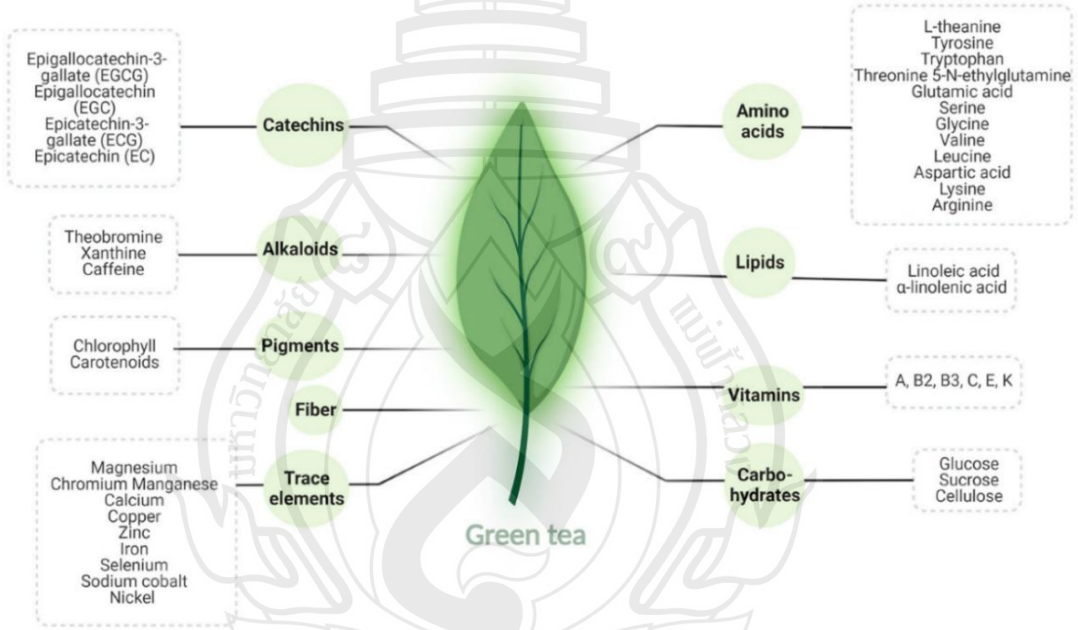


Figure 2.14 Schematic representation of chemical composition of green tea

2.4.4 Volatile Compounds of Green Tea

2.4.4.1 Aldehydes make up the largest proportion of volatile compounds in most green, oolong, and black teas. Thirty-four aroma-active aldehydes have been identified, contributing primarily to citrus-like and green flavors, though some add bitter-almond, malty, honey-like, fatty, nutty, or metallic notes. For example, (E,E)-2,4-heptadienal, found in high-grade Jingshan green tea, is responsible for its fatty and flowery aroma, while aldehydes like 2-methylpropanal and hexanal dominate the malty and green notes in oolong teas. Key aldehydes such as 2-methylbutanal and (E,E)-2,4-

decadienal have been identified as major contributors to Darjeeling black tea's aroma. In raw pu-erh tea, vanillin is the primary aldehyde, imparting a vanilla-like aroma, while cis- and trans-4,5-epoxy-(E)-2-decenal, with a metallic scent, are found in green teas like Longjing. Aldehydes in tea are formed through two main pathways: lipoxygenase-mediated lipid oxidation and Strecker degradation. For instance, hexanal and (E)-2-nonenal originate from linolenic acid oxidation, while octanal comes from oleic acid. Strecker aldehydes, such as 2-methylbutanal and 3-methylbutanal, result from amino acid degradation. During pan-firing and drying, these aldehydes may reduce due to enzyme inactivation, evaporation, or conversion to corresponding alcohols. Differences in tea varieties, processing methods, and extraction techniques contribute to the diversity of aldehydes in tea (Zhai et al., 2022).

2.4.4.2 Ketones are the second-largest group of odorants in tea, with 23 aroma-active ketones identified, contributing to flowery, green, woody, mushroom-like, and fruity aromas. Among them, β-ionone, derived from carotenoids during fermentation or heating, is a key contributor to violet-like aromas due to its high concentration relative to its odor threshold. Recent research revealed that β-ionone is formed during tea withering through a dehydration-induced biosynthesis pathway (Zhai et al., 2022).

2.4.4.3 Alcohols represent the third largest group of volatiles detected in tea, with 21 alcohols. These alcohols, which include both straight-chain and branched types, often derive from the reduction of Strecker aldehydes or the hydrolysis of glycoside precursors. In Chinese congou black teas like Tanyang, Yixing, Dianhong, and Keemun, alcohols account for approximately 50% of the total volatiles. Among these, monoterpene alcohols such as geraniol and linalool are prominent, with geraniol contributing a rose-like and citrus-like aroma, while linalool offers a citrus-like and flowery note. Other notable alcohols include 1-octen-3-ol, recognized for its mushroom-like aroma and significant presence in Longjing green tea, and (Z)-3-hexenol, which has a lettuce-like aroma and forms during lipid degradation or glycoside hydrolysis. Additionally, 2-phenylethanol, known for its flowery and honey-like scent, is an important aroma-active compound in many green teas. The amounts of these alcohols increase during the withering process but decrease during higher temperature processes like pan-firing and drying (Zhai et al., 2022).

2.4.4.4 Esters play a significant role in contributing aroma to tea, with a total of 18 aroma-active esters identified, including four lactones: butyrolactone, γ-hexalactone, γ-nonalactone, and γ-octalactone. These compounds are associated with sweet, fruity, and flowery odor profiles, exhibiting high flavor dilution (FD) factors, which are part of modern molecular sensory science methodologies in flavor analysis. For instance, γ-nonalactone and γ-octalactone are known to impart strong coconut-like aromas in green teas. Additionally, (Z)-methyl epi-jasmonate, which has a noble orchid-like scent, has been identified as a primary aroma driver in premium-grade Chinese green tea (Zhai et al., 2022).

2.4.4.5 Phenols represent a significant category of aroma compounds, contributing clove-like, smoky, and phenolic characteristics, particularly in dark teas such as pu-erh tea. Research has identified 14 phenolic compounds, including 3,4-dimethoxyphenol, 2,6-dimethoxyphenol, 2-ethylphenol, 4-ethylphenol, 4-ethyl-2-methoxyphenol, 2-methoxyphenol, and trans-methyl isoeugenol, which show notable changes between raw and ripened pu-erh teas. These variations suggest that the origins of these phenols may be linked to the sequential activities of microorganisms and enzymes during the unique fermentation process that pu-erh tea undergoes. In green teas, six phenols-3-ethylphenol, 4-ethylphenol, eugenol, 3-methylphenol, 4-methylphenol, and 2-methoxyphenol have been identified as aroma-active compounds with high flavor dilution (FD) factors. However, due to the influence of the tea infusion matrix on odor release, these phenols do not significantly contribute to the- overall aroma of green tea. This highlights the complexity of aroma perception in tea, where certain compounds may have high odor activity but do not translate into dominant aroma characteristics in the final brewed beverage (Zhai et al., 2022).

2.4.4.6 Acids are another important group of aroma compounds in tea, contributing various sensory notes. Among the acids identified, butanoic acid, decanoic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, pentanoic acid, and phenylacetic acid have been recognized as typical aroma-active compounds in different types of tea. These acids impart a range of aromas, including sweaty, soapy, musty, fruity, beeswax-like, and honey-like notes. Phenylacetic acid, in particular, has been identified as a key odorant responsible for the beeswax-like and honey-like aroma in Jingshan green tea. In contrast, other acids such as acetic acid, hexanoic acid, nonanoic acid, octanoic acid,

and propanoic acid have been detected in ripened pu-erh tea, though they are not recognized as aroma-active in raw pu-erh tea (Zhai et al., 2022).

2.4.4.7 Linalool oxides are prominent in many teas, imparting woody and floral flavors, while cis- and trans-pyranoid linalool oxides are specifically found in pu-erh tea (Zhai et al., 2022).

2.4.5 Type of Microbial Fermentation in Tea

2.4.5.1 Bacteria fermentation in tea (Figure 2.15), recent advances in Kombucha fermentation research recent studies on tea fermentation have predominantly focused on kombucha. The fermentation process of kombucha induces notable changes in sugar and acid content, imparting a unique flavor to the tea. Additionally, the production of aromatic compounds enhances the tea's aroma, while alterations in phenolic substances boost its antioxidant capacity. Kombucha fermentation results in significant changes in various parameters, including antioxidant potential, pH, acetic acid, alcohol, and sugar content. The fermentation process also generates beneficial ingredients like organic acids, minerals, vitamins, amino acids, and polyphenols, contributing to the health-promoting properties of kombucha (Hu et al., 2022).

2.4.5.2 Yeast fermentation in tea (Figure 2.15), using yeast to ferment tea enhances both the biochemical activity and sensory quality of the tea. During the fermentation process, complex biochemical reactions lead to the production of flavor compounds such as ethanol, acids, and esters, improving the overall taste and aroma of the tea. Research has shown that *Dabaryomyces hansenii* fermentation of black tea reduces caffeine and tannins, enhancing its nutritional and medicinal properties. This makes fermented tea more beneficial than traditional black tea.

In studies on Pu-erh tea, yeast fermentation increased the levels of beneficial compounds like tea polyphenols, theaflavins, and catechins, while decreasing amino acids, caffeine, flavonoids, thearubigins, and theabrownin. These changes directly affect the taste, color, and aroma of the tea. The decrease in theanine reduced bitterness, while the rise in the flavonoids improved the tea's sensory qualities, contributing to better color, aroma, and taste. This demonstrates that yeast plays a significant role in enhancing the quality of fermented Pu-erh tea (Hu et al., 2022).

2.4.5.3 Fungal fermentation in tea (Figure 2.15), enhances its quality through processes like degradation, oxidation, and methylation induced by mold metabolites. Researchers have used molds such as *Streptomyces*, *Aspergillus niger*, and *Mucor* in studies, finding that these microbes trigger beneficial biochemical transformations. These reactions improve the tea's flavor, aroma, and nutritional profile, offering potential for producing high-quality, fermented teas with enhanced sensory and health benefits. Studies have shown that tea fermented with different microbial strains, including *Streptomyces*, *Aspergillus*, and *Eurotium cristatum*, leads to significant changes in polyphenol content and other tea components. Fermentation by *Streptomyces bacillaris* and *Streptomyces cinereus* increased total phenol content, while *Aspergillus niger* and *Aspergillus fumigatus* enhanced polyphenol and purine alkaloid concentrations. Fermentation with molds increased caffeine content, while yeast fermentation reduced it. *E. cristatum* secreted enzymes like amylase and oxidase, catalyzing transformations that improved tea flavor, increased aromatic components, and enhanced beneficial compounds like flavonoids and free amino acids (Hu et al., 2022).

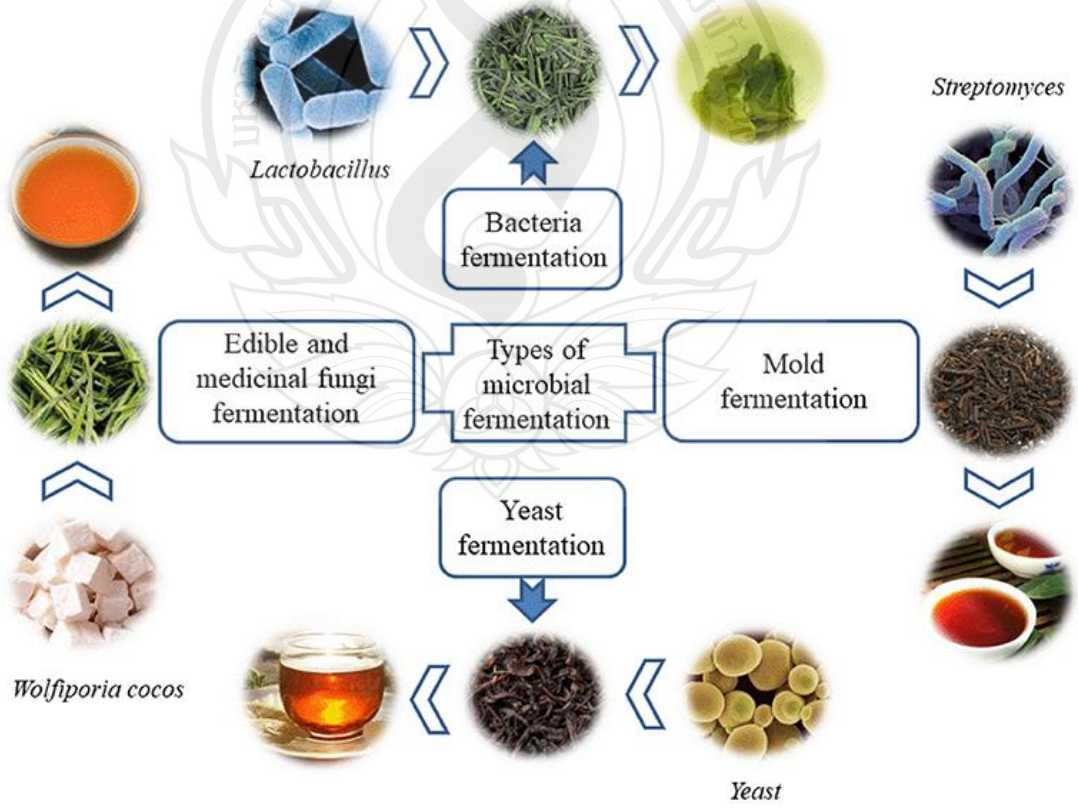


Figure 2.15 Type of microbial fermentation in tea

2.5 Fungal in Fermentation

Aspergillus cristatus, or "golden flower," (also named *Eurotium cristatum*) is the primary strain involved in the microbial fermentation of Fu brick tea. It has several beneficial biological characteristics, including enzyme production, antimicrobial properties, immune regulation, antitumor effects, fat reduction, and weight loss benefits. Its probiotic nature allows *A. cristatus* to be combined with various tea substrates to create different fermented teas. During fermentation, it secretes extracellular enzymes, such as hydrolytic enzymes and oxidoreductases, which metabolize and transform chemical components in tea through oxidation, degradation, and condensation, thereby significantly influencing tea quality. *A. cristatus* is a crucial strain in the flowering process of Fu brick tea, impacting its quality. Initially isolated from the "golden flower" in dark teas, it has undergone numerous identification studies. Originally named *Aspergillus chevalieri* in 1983, it was later renamed A. cristatus due to classification conflicts and finally designated as *A. cristatus*. This evolution in nomenclature reflects the complexities in identifying the rich microbial flora associated with the golden flower fungus.

2.5.1 Colony Characters

According studies of Chen et al. (2017), the colony characteristics of *Aspergillus cristatus* were observed across various media at 25 °C for 7 days. On medium, colonies appeared moderately deep with slightly sulcate margins, mycelium ranging from sulfur yellow to orange, and a velvety to floccose texture, with sporulation varying from absent to dense. Conidia were dark green, with no soluble pigments or exudates, and a reverse coloration of straw, sulfur yellow, or fulvous shows in Figure 2.16.

2.5.2 Micromorphology of Gold Fungal

The micromorphology of *A. cristatus* features eurotium-like ascomata that are cleistothecial, superficial, and yellow, ranging from 100 to 200 μm in size. The asci are 8-spored and globose to subglobose in shape. Ascospores are hyaline, appearing globose to subglobose in surface view, with verruculose to rugulose bodies measuring 4.5-6 × 4-6 μm, and lenticular in side view with a furrow and crests measuring 1.2-1.5

μm. Conidiophores have smooth stipes, are hyaline or light brown, and measure 300-500 × (6-) 8-12 μm. The vesicles are globose to subglobose, 35-51 μm wide, and fertile over two-thirds to the entire surface. Phialides are flask-shaped, measuring 5.5-9 × 3.5-6 μm, while the conidia are globose, subglobose to ellipsoidal, and tuberculate, measuring 4-6.5 × 3.5-5 μm shows in Figure 2.24 (Chen et al., 2017).

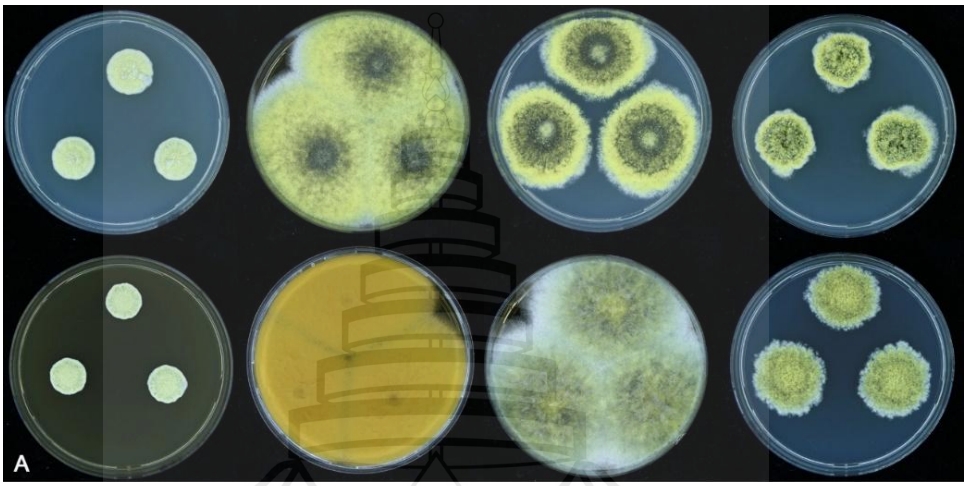


Figure 2.16 The colony characters of *A. cristatus*

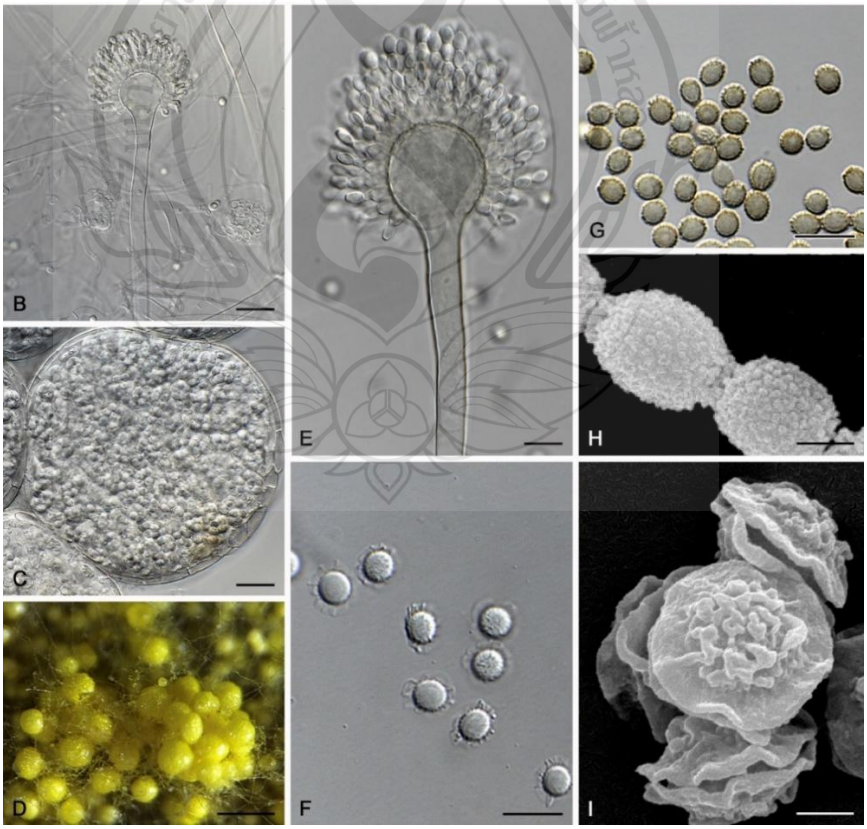


Figure 2.17 The micromorphology of *A. cristatus*

2.5.3 Enzymes Produced

A. cristatus not only meets its growth requirements but also plays a vital role in transforming and degrading various substances in tea through its production of extracellular enzymes such as polyphenol oxidase, cellulase, and protease. These enzymes convert macromolecular compounds like pectin and cellulose into smaller molecules, enhancing the unique qualities and flavors of tea. For example, polyphenol oxidase reduces phenolic compounds, thereby decreasing the bitterness of fermented tea (Zhang & Zhang, 2024).

2.5.4 Effect on Tea

2.5.4.1 Effect on tea taste; the amino acid and polyphenol content in tea are key indicators of tea quality and flavor. During fermentation, *A. cristatus* consumes tea amino acids as a nitrogen source, leading to a decrease in amino acid content, which affects the sensory quality of the tea. *A. cristatus* produces various extracellular enzymes, such as polyphenol oxidase, cellulase, pectinase, and protease, which transform tea components. Studies have shown that fermentation with *A. cristatus* increases flavonoids and polysaccharides while decreasing polyphenol content. For instance, in experiments with mulberry leaf black tea and lychee grass tea, *A. cristatus* improved flavor quality by producing active metabolites and facilitating the oxidation and degradation of tea components. The oxidation of phenolic compounds by polyphenol oxidase reduces bitterness, significantly enhancing the sensory quality of the fermented tea (Zhang & Zhang, 2024).

2.5.4.2 Effect on tea aroma; The fermentation of tea by *A. cristatus* produces various volatile compounds, including ketones, alcohols, and aldehydes, which alter the aromatic profile of the tea. Fermentation significantly increased nine volatile components in Jinhua loose tea, contributing to its unique fungal fragrance. In fermented green tea extract, revealing new volatile aroma components, we observed a decrease in alcohols and an increase in aldehydes and ketones during the fermentation of ginkgo biloba leaves, attributing these changes to oxidation processes (Zhang & Zhang, 2024).

2.5.4.3 Effect on tea color, during the blooming process, *A. cristatus* produces extracellular enzymes that enhance the formation of theabrownine, a compound that imparts a brown-red color and contributes to the flavor of fermented

tea. Bo et al. found that after the blooming process, Jinhua white tea exhibited significantly improved sensory qualities, including a vibrant orange-yellow color, mellow flavor, reduced bitterness, and a distinctive aroma. The Shoumei loose tea's color transitioned from yellow to orange-yellow as microbial activity increased and polyphenols oxidized, resulting in a bright orange-red hue. Zhang and Zhang, (2024), observed similar results in mulberry leaf green tea, where fermentation with *A. cristatus* led to a bright yellow-brown color and the development of a unique fungal aroma, enhancing the overall quality of the tea.

2.6 Determination Chemical and Quality of Fermentation in Coffee and Tea

2.6.1 High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a widely used technique for separating and analyzing various substances (Figure 2.18). It works by passing a liquid sample through a column packed with a stationary phase under high pressure, enabling effective separation based on the interaction between sample components and the stationary phase. Key components of an HPLC system include a pump to generate high pressure, an injector for sample introduction, a column for separation, and a detector for analyte identification. Success in HPLC measurement depends on factors like mobile and stationary phase selection, flow rate, and column temperature, all of which influence separation quality and detection sensitivity.

The principle behind HPLC (high-performance liquid chromatography) relies on the distribution of the analyte between a mobile phase (eluent) and a stationary phase (column packing material). As the sample moves through the stationary phase, its molecules interact with the packing material based on their chemical structure, leading to varying retention times. This differential retention allows distinct components to be separated as they are eluted at different times. After separation, a detector, often a UV detector, identifies each analyte as it exits the column, with data recorded in a chromatogram. A typical HPLC system includes a solvent reservoir, pump, injection valve, column, detector, and data processing unit. The pump delivers the solvent at a

high, steady pressure, ensuring minimal detector signal noise and drift, while the injection valve introduces the sample into the eluent (Meyer, 2010).

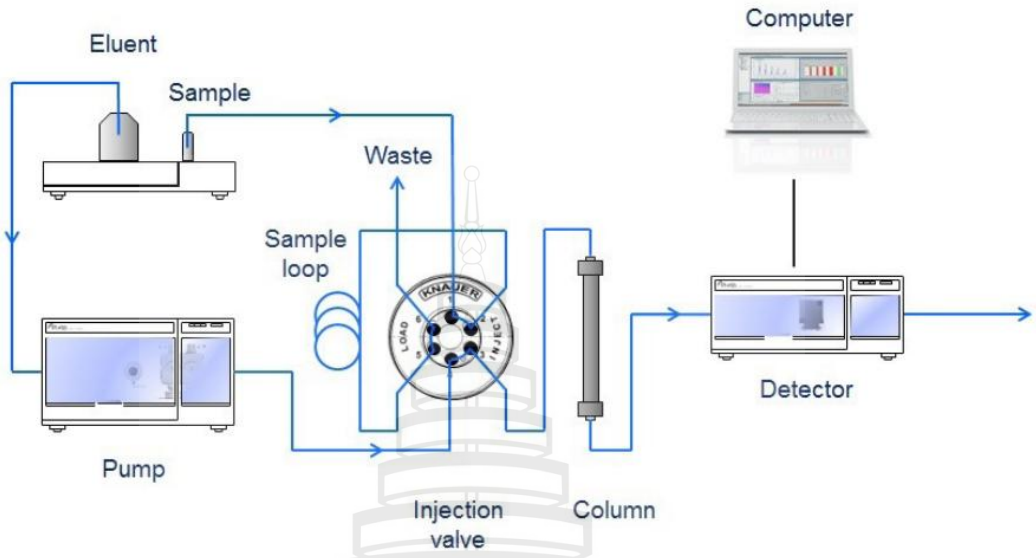


Figure 2.18 Schematic representation of HPLC

2.6.2 Gas Chromatography – Mass Spectrometry (GC-MS)

Gas chromatography–mass spectrometry (GC-MS) combines gas-liquid chromatography and mass spectrometry to analyze and identify substances within complex samples (Figure 2.19). It is widely used in various fields, including drug detection, environmental analysis, forensic investigations, and airport security for detecting trace substances in luggage or on individuals. Its ability to provide highly specific identification, often called the gold standard for forensic analysis, allows GC-MS to positively confirm the presence of particular compounds, reducing the risk of false positives associated with less specific tests (Sahil et al., 2011).

2.6.3 Liquid Chromatography – Mass Spectrometry (LC-MS)

In an LC/MS/MS analytical workflow, a liquid chromatography (HPLC) system first separates and concentrates sample components (Figure 2.20). These components then enter a triple quadrupole mass spectrometer, where ions are filtered by their mass-to-charge ratios (m/z). The triple quadrupole configuration uses the first quadrupole to isolate molecular ions, the second as a collision cell for fragmenting ions, and the third to isolate these fragments for detection, providing high selectivity, sensitivity, and structural detail. While single quadrupole mass spectrometers are suitable for detecting

specific masses in simpler matrices, triple quadrupole instruments are essential for distinguishing molecules with similar masses and analyzing complex mixtures (Yost & Enke, 1978).

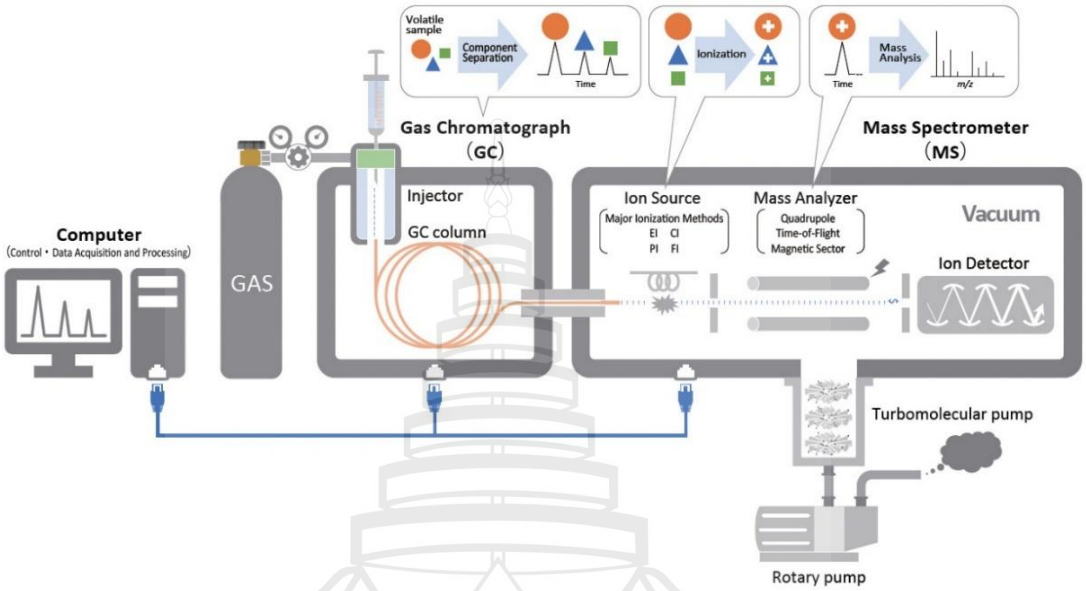


Figure 2.19 Schematic representation of GC-MS

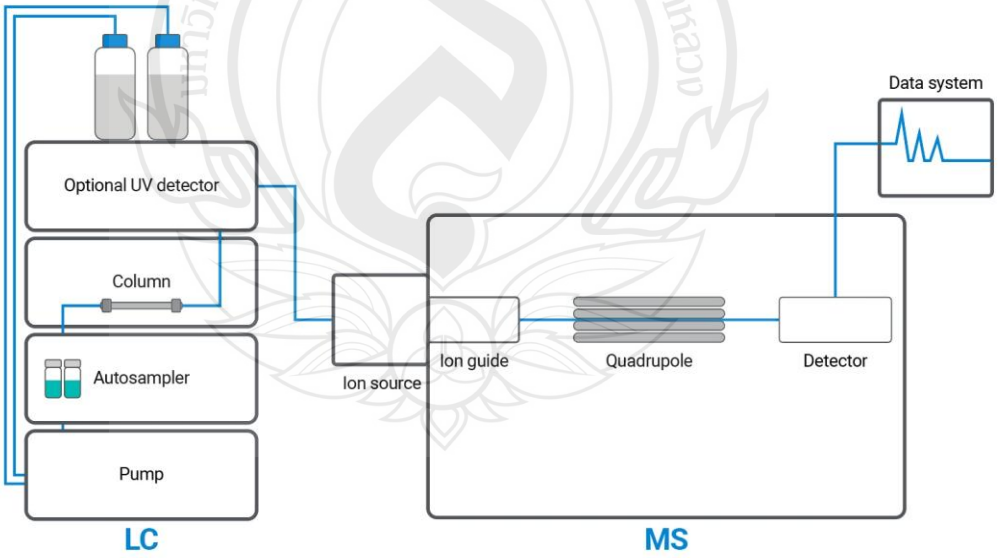


Figure 2.20 Schematic representation of LC-MS

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Fermentation of Coffee

3.1.1 Yeast Culture

Commercial yeast strains *Saccharomyces cerevisiae* SafAle s-33 (Fermentis, Lille, France), which is currently being used in the beverage industry, was grown on yeast extract peptone glucose (YEPG) broth medium (20 g/L glucose (Himedia, Mumbai, India), 10 g/l peptone soy (Gibco, MI, USA), 10 g/l yeast extract (Gibco, MI, USA)). Two cultured methods were used to culture yeast for coffee fermentation. The first method was cultured in 500 ml of YEPG broth medium in a 1,000 ml of Erlenmeyer flask and incubated in a shaker incubator at 30 ºC, 120 rpm for 48 h. The cells were recovered by centrifugation at 8,000 rpm for 10 min. The second method was cultured with 5 L of YEPG broth medium in a 10 L tank of bottle Transparent Carboy (polycarbonate carboys or PC, Nalgene, U.S.A) sealed with wound dressing tape and connected to air filtration sterile and pump (pressure at 100 PSI). The cultures, were cultivated at room temperature (25-28 ºC) for 48 h. The cells were recovered by a separation funnel and suction pump (Figure 3.1)

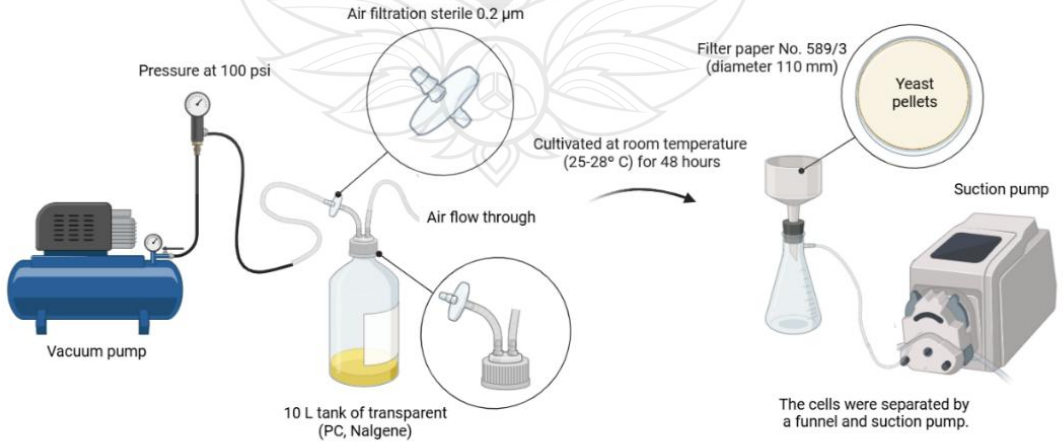


Figure 3.1 Diagram of yeast cultivation and yeast pellet separation

3.1.2 Harvesting and Preparing of Coffee Cherry

Coffee cherries of Robusta (*C. canephora*) and Arabica (*C. arabica*) were collected from a coffee plantation in Chiang Rai province. The coffee cherries were harvested manually from a coffee farm located in Doi Pangkhon, Chiang Rai, Thailand (latitude: 18º56’ 38” S; longitude: 46º59’ 33” W with an elevation of 1,250-1,500 meters above sea level). The Arabica were harvested in November 2023, while cherries of Robusta were harvested in January 2024.

Coffee cherries were washed with water twice to remove dirt or soil, then hand-picked ripe coffee cherries and discarded other contaminants, including rotten or unripe beans, leaves, sticks, etc. Those selected coffee cherries were subjected to surface sterilization using a solution of 5% potassium chloride, for 5 min to reduce contamination from other microorganisms. Some coffee cherries were milled though a coffee cherry milling machine, and the coffee beans and coffee cherry peels were collected separately for the next step (Figure 3.2).

Figure 3.2 Washed and preparing the coffee cherries of Arabica and Robusta

The collected yeast cells were dried in 4 conditions, which were 30, 40, and 50°C in a hot air oven for 24 h and at room temperature for 96 h. The pellets were stored in the freezer (-20 °C) and refrigerator (4 °C) for 25 days. For the viability test, the yeast cells were suspension in phosphate-buffered saline (PBS), then 100 µl of cell suspension was mixed with 100 µl of methylene blue solution (0.1 mg/ml, prepared in 2% sodium citrate dihydrate solution). After cells were incubated at room temperature for 5 min, cells were counted under a microscope by observing at least 200 cells in a single biological replicate. Viable cells appeared colorless, while dead cells were stained with methylene blue. The cell viability was counted before and after storage for 1, 3, 5, 10, and 25 days. The viability percentage was calculated following (Kwolek-Mirek & Zadrag-Tecza, 2014);

$$\text{Viability (\%)} = \frac{\text{Total counted cells} - \text{Total counted dead cells}}{\text{Total counted}} \times 100$$

Before coffee fermentation, yeast solution was prepared by resuspending with sterile water to a concentration of approximately 1×10^7 CFU/ml (de Jesus Cassimiro et al, 2023). In the processing of natural coffee, the whole fruits of Arabic and Robusta coffee were used to ferment with yeast under SIAF and anaerobic conditions.

3.1.2.1 Treatment I: In SIAF conditions, the 8 kg of coffee cherry and 4 L of yeast suspension were added to plastic bags and placed in a polyethylene container and the lid was closed tightly.

3.1.2.2 Treatment II: In anaerobic conditions, the 8 kg of coffee cherry and 4 L of yeast suspension were added to plastic bags and placed in a polyethylene container. The candle was lit in the plastic container and closing the lid tightly to prevent air from circulating into the container.

3.1.2.3 Treatment III: In SIAF_peel conditions, the 5 kg of peeled coffee beans mixed with 3 kg of the outer skin or exocarp and 4 L of yeast suspension were added to plastic bags and placed in a polyethylene container and the lid was closed tightly.

During fermentation, pH was measured every 2 days. The fermentation process stopped when the pH was in the range of pH 2-3. After fermentation, coffee beans were taken to the shelf for the drying stage. The moisture content of the dried coffee beans was every 2 days by the grain moisture meter (Fuzhou Hedao Trade Co. Ltd,). The dry fermented coffee beans with the moisture content in the range of 11-12% were stored in zip-lock bags and kept at room temperature for 15 days before being hulled and roasted the coffee beans.

To observe the yeast surface on coffee cherries, 250-300 dried coffee cherries were randomly collected, and the number of coffee cherries with and with-out yeast attached to the surface of the coffee cherries was counted. The coffee cherries have yeast attached as observed from the white cloudy stains on the surface of the coffee cherries. Then calculate the percentage according to the formula;

$$\text{Yeast surface on coffee cherries (\%)} = \frac{\text{Count of coffee cherries have yeast on surface}}{\text{Count of total of the coffee cherries}} \times 100$$

3.1.3 Determination of Coffee Fermentation

3.1.3.1 The analysis of volatile compounds in ground coffee involved a precise protocol using gas chomatography-mass spectrometry (GC-MS) with a Shimadzu GCMS-QP2010 SE instrument. Initially, a 2.0 g coffee sample was brewed in 5 ml of boiling water within a 20 ml headspace vial, and the solution was equilibrated at 60 ºC for 30 min to facilitate the release of volatiles. A solid-phase microextraction (SPME) fiber coated with divinylbenzene/carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) was used to extract volatiles at 60 ºC over 30 min. Following extraction, volatiles were desorbed into the GC injector at 325 ºC, for 5 min and separated with an RTX-5Sil MS capillary column (30 m × 0.25 mm × 0.25 µm). The temperature program began with an initial temperature of 40 ºC held for 3 min, then increased to 85 ºC at a rate of 3 ºC per min and held for another 3 min, further raised to 160 ºC at the same rate, and finally increased at 10 ºC per min to a maximum of 240 ºC, where it was held for 5 min. Though out the process, helium, with a purity exceeding 99.99%, served as the carrier gas at a flow rate of 1.0 ml per min. The volatiles were identified by referencing the National Institute of Standards and Technology (NIST14s) MS data library, and their relative concentrations were calculated though peak area normalization, expressing the results as the ratio of each constituent's area to the total area, thereby providing a quantitative profile of volatile compounds in the coffee sample (Xiao et al., 2020).

3.1.3.2 The analysis of organic acids in ground coffee was conducted using liquid chomatography-mass spectrometry (LC-MS) with a Shimadzu LCMS-9030 or LCMS-9050 quadrupole time-of-flight (Q-TOF) mass spectrometer. A 0.5 g sample of ground coffee was extracted in 5 ml of 50% methanol at room temperature for 30 min in a sonicator bath. After extraction, the solution was filtered though a 0.22-µm nylon membrane and analyzed using an Inertsil ODS-4 column (4.6 mm × 250 mm × 5 µm). The mobile phase involved a gradient elution with two solvents: eluent A (ultrapure water with 0.1% formic acid) and eluent B (methanol). The gradient was programmed as follows: 5–22% B from 0 to 5 min, maintaining 22% B from 5 to 20 min, gradually increasing to 24% B at 20–35 min, reaching 25% B from 35–45 min, followed by an increase to 40% B at 45–50 min, and finally reaching 45% B at 50–60 min. UV detection was performed at 280 nm, with the column maintained at 30 ºC, a flow rate

of 0.8 ml per min, and an injection volume of 1.0 µL, allowing for precise detection and quantification of organic acids in the coffee sample (Xiao et al., 2020).

3.1.4 Sensory Analysis

The sensory evaluation was conducted by a Q-grader in accordance with the guidelines established by the Specialty Coffee Association of America (SCAA). Coffee samples were roasted within 24 h prior to cupping and were allowed to rest for a minimum of 8 h post-roast. During the cupping process, the roast level was assessed between 30 min and 4 h after roasting. The coffee was ground to the SCAA's standard grind size for cupping and the measurements were taken at room temperature to ensure consistency and accuracy in the sensory analysis (Athinuwat et al., 2024).

3.2 Fermentation of Green Tea

3.2.1 Isolation of Fungi

The compressed FBT tea samples were first loosened using a mortar and pestle. Subsequently, the FBT tea sample was placed on Potato Dextrose Agar (PDA) plates, which were then incubated at 25-28 °C for 3 days. Following incubation, the mycelium that developed from the tea leaves was transferred to a new Sabouraud Dextrose Agar (SGA) plate to isolate and obtain a pure strain of the fungi.

3.2.2 Identification of New Fungal Strain

The isolated strains were cultured on PDA and Czapek's Agar (CA) and a two-point inoculation method was used for morphological and molecular identification. These cultures were incubated at 28 °C to assess their growth at this temperature. The fungi’s reproductive structures were examined using a compound microscope and a stereo microscope to facilitate detailed observation of their characteristics.

DNA extraction from fungi was performed using the Genomic DNA Isolation Kit from BIO-HELEX CO., LTD. (Taiwan). Polymerase chain reaction (PCR) was performed with a set of master mix reagents (Promega Go Taq™, USA). The PCR reaction mixture had a final volume of 25 µl, comprising 12.5 µl of 2X PCR Master Mix, 9.5 µl of nuclease-free water, 1 µl of each primer (ITS1/4, β-tub, and RPB2), and 1.0 µl of the DNA template. The specific fungal primer sets and thermocycling

protocols were followed as described by Efimenko et al. (2021). The resulting amplification products were purified using a PCR clean-up and gel extraction kit from BIO-HELEX CO., LTD. and subsequently visualized on a 1% agarose gel at 100V for 30 min. Samples were submitted for Sanger sequencing to SolGent Co., Ltd. (Daejeon, Korea). The sequences obtained were analyzed using the Basic Local Alignment Search Tool (BLAST) against the National Center for Biotechnology Information (NCBI) database for identification. Subsequent inspection and assembly of the sequences were conducted using BioEdit version 7.1.8. Sequence alignments were performed utilizing the FFT-NSi strategy implemented in MAFFT version 7. Additionally, maximum likelihood (ML) phylogenetic trees were constructed using FigTree version 1.4.4 to visualize the relationships among the identified sequences (Chen et al., 2017).

3.2.3 Microbial Culture

Selected strains were sub-cultured in PDA medium at 28 ºC for 10 days and then inoculated ten pieces (0.5 x 0.5 cm^2) of mycelia agar plugs in 500 ml Erlenmeyer flasks containing 300 ml potato dextrose broth (PDB) medium. Broth cultures were incubated at 28 ºC for 2 weeks under static conditions. After that fungal mycelium was separated by using sheet cloths then pre-freeze by storing in a -80 ºC refrigerator for 1-2 h and then freeze-dried. The temperature of the condenser was approximately -80 ºC and the chamber pressure and approximately 1 mbar. After 24 h, the fungal samples were ground by mortar and pestle.

3.2.4 Compressed Tea Processing

The processed green leaves were mixed with the shoot (appropriate ratio: leave 80% and shoot 20%) and were used to compact into a brick shape. The reprocessing process was done following the step of steam pilling (102 ºC for 35 min), and pile fermentation (80-88 ºC for 2 h). In the first method, tea was compacted by using a hydraulic press along with a mold to shape the tea (during compression, the pressure was controlled to not exceed 100 psi). The tea brick had a diameter of 13 cm and were dried at 95 °C for 24 h. The second method differed by using a manual hydraulic press along with a mold to shape the tea into small circular pellets with a diameter of 1.8 cm. Unlike the first method, the tea was not weighed before compression due to the smaller size of the mold. The compressed tea pellets were then placed in a hot air oven at 60 °C for one day to ensure they were fully dried. The temperature was lowered from the first

method, which used 95 °C, because the pellets in the second method were smaller and thinner. Using a higher temperature could risk burning the tea pellets.

The fungal suspension was prepared at a concentration of 1×10^7 CFU/ml in water sterile and sprayed onto the tea bricks. For the larger pellets, 8 ml of the suspension was sprayed, and the bricks were incubated at 28 °C for 1 week. After this, an additional 10 ml of the fungal suspension was sprayed onto the bricks. The bricks were then wrapped in wax paper, placed in zip-lock bags to maintain moisture, and incubated at 28 °C for another 9 days, totaling 16 days. For the smaller bricks, 1 ml of the fungal suspension was sprayed onto each brick, which was then wrapped in wax paper and placed in a zip-lock bag to retain moisture. The bricks were incubated at 28 °C for 1 week. After incubation, the tea pellets were examined under a stereo microscope, where the growing fungal colonies were identified as golden-colored fungi. Control treatment was done with a spray of sterile water.

3.2.5 Antioxidant Activity

The DPPH radical scavenging assay was conducted using a method adapted from Hasan et al. (2024). A 1.0 g of ground tea sample was extracted with 50 ml of distilled water, brewed for 3 min, and then centrifuged at 11,515 rpm for 15 min at room temperature. Ascorbic acid was used as control and methanol was used as blank. Solutions of 0, 50, 100, and 150 µg/ml of both the control and samples (green tea extracts) were prepared in methanol. 0.1 mM solution of DPPH in methanol was freshly prepared, and 0.5 ml was added with the different concentration of control and tea samples. The mixture were incubated for 30 min at room temperature, and the absorbance of the mixture were measured at 517 nm using 150 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The of inhibition percentage of DPPH free radicals was calculated as follows;

$$\text{Inhibition (\%)} = \frac{[\text{ABS (control)} - \text{ABS (sample)}]}{\text{ABS (control)}} \times 100$$

Note; ABS mean absorbance

$ABS_{control}$ mean absorbance of the control

ABS_{sample} mean absorbance of the test sample

3.2.6 Physicochemical Properties Analysis

3.2.6.1 Instrumental color analysis was conducted using a Hunter Lab colorimeter (ColorQuest XE, USA) to measure the color characteristics of the sample solution (Figure 3.3). A 1.0 ground tea samples was extracted with 50 ml of distilled water, brewed for 3 min, and centrifuged at 11,515 rpm for 15 min at room temperature. The measurements were taken in the CIELAB color space, providing quantitative data on the brightness, red-green, and yellow-blue color properties of the tea sample.

3.2.6.2 Total soluble solid (TSS) content was measured using a digital hand-held pocket refractometer (0-53%), [Atago N1, Tokyo, Japan] (Figure 3.3), followed the method described by Muhialdin et al. (2019).

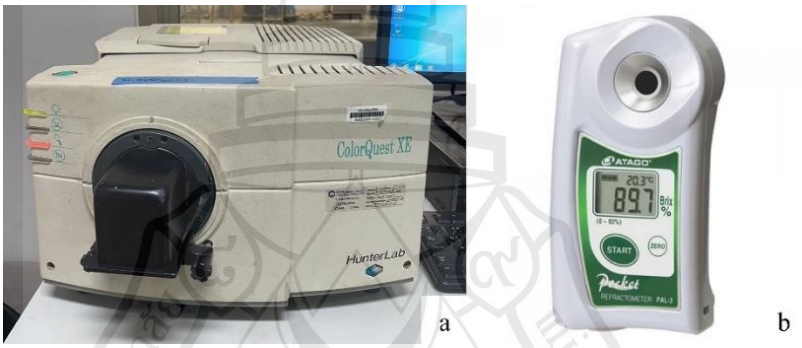


Figure 3.3 (a) instrumental of color analysis, (b) instrumental of total soluble solid (TSS) content

3.2.6.3 The water activity of the dried samples was measured using a water activity meter (Novasina LabMaster-aw Neo, Switzerland) (Figure 3.4). Approximately 0.5 g of each sample was transferred to a sample holder equipped with a relative humidity probe and measured at a controlled temperature of 25 °C. The water activity values were recorded once equilibrium relative humidity was achieved by the method outlined by Topuz et al. (2014).

Figure 3.4 Instrumental of water activity

3.2.6.4 Moisture content was determined by sampling 1,000 g of tea leaves. The sample was accurately weighed and placed in moisture can, which was then placed in hot-air oven set to 105 °C for 24 h. After drying, the sample was removed from the oven and allowed to cool in a desiccator (Figure 3.5). The sample was weighed again, and the moisture content was calculated using the following;

$$\text{Moisture content} = \frac{(\text{Weight before drying} - \text{Weight after drying})}{\text{Weight before drying}} \times 100$$

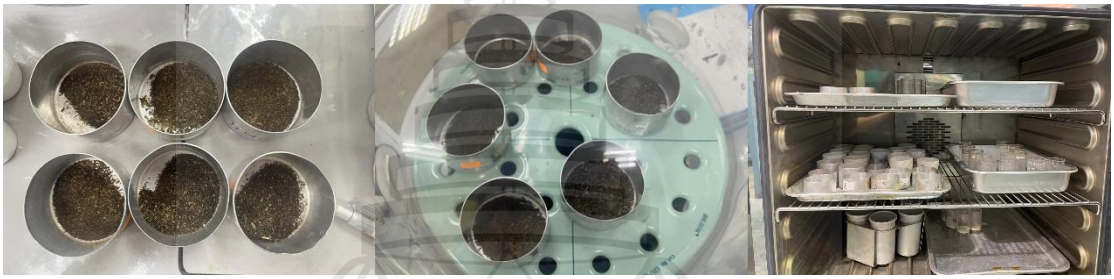


Figure 3.5 Instrumental of moisture content

3.2.6.5 Ash content was determined by weighing a 2.0 ground tea sample and placed in crucibles for pre-fired, which has been fired for 2-3 h. The crucibles were heated on hot plate until no smoke was visible from the sample. Then, the crucibles were transferred to a muffle furnace set to 525 °C and heated for 8-10 h, or until white or gray ash was observed (Figure 3.6). After cooling in a desiccator, the crucibles were weighed again to determine the weight of the ash. The ash content was calculated using the following;

$$\text{Ash content} = \frac{\text{Weight of ash after firing}}{\text{Weight of tea sample before firing}} \times 100$$

Figure 3.6 Instrumental of ash content

3.2.7 Determination of Green Tea Quality

3.2.7.1 The analysis of caffeine, catechin, and gallic acid were performed using high-performance liquid chomatography (HPLC). For sample preparation, 0.5 g of ground tea was extracted with 5 ml of 70% methanol in a sonicator bath at room temperature for 30 min, and then filtered though a 0.22 -µm nylon membrane. The analysis was carried out using a Themo Ultimate 3000 HPLC system, equipped with a diode array detector (Ultimate 3000, Thermo Fisher Scientific, Waltham, MA, USA) and an lnertsil ODS-4 column (4.6 mm × 250 mm × 5 µm, GL Science Inc., Tokyo, Japan). The elution gradient was as follows; 5-22% methanol (eluent B) from 0-5 min, held at 22% B from 5-20 min, increased to 24% B from 20-35 min, 24-25% B from 35-45 min, 25-40% B from 45-50 min, and 40-50% B from 50-60 min. Detection was done at 280 nm, with the column maintained at 30 °C. The flow rate was 0.8 ml/min, and the injection volume was 1.0 µl, with a total run time of 15 min (Xiao et al., 2020).

3.2.7.2 The analysis of volatile compound by gas chomatography-mass spectrometry (GC-MS), a 2.0 g of ground tea was brewed with 5 ml of boiling water in 20 ml headspace vial and equilibrated at 60 °C for 30 min. Volatile compounds were extracted at the same temperature using a 50 µm solid-phase microextraction (SPME) fiber coated with divinylbenzene/carboxen/poly-dimethylsiloxane (VB/CAR/PDMS) from Supelco (Sigma Aldrich, St. Louis, MO, USA). GC-MS analysis was performed using a Shimadzu GCMS-QP2010 SE instrument (Kyoto, Japan), operated at 70 eV over a mass range of 35 to 400 amu. Desorption of volatiles into the GC injector occurred at 325 °C for 5 min. The compounds were separated using an RTX-5Sil MS capillary column (30 m × 0.25 mm × 0.25 µm), with the following temperature program; an initial 40 °C held for 3 min, increased to 85 °C at 3 °C/min and held for 3 min, further raised to 160 °C at 3 °C/min, then ramped to 240 °C at 10 °C/min, holding for 5 min. The interface port and ion source temperatures were set to 280 °C and 230 °C, respectively. Helium (purity > 99.99%) was used as the carrier gas at a flow rate of 1.0 ml/min. Volatile compounds were identified by comparing the data with the NIST14s MS library, and the relative proportions of volatiles were calculated by normalizing the peak area. Results were expressed as the ratio of each constituent's area to the total area (Xiao et al., 2020).

3.2.7.3 The analysis of organic acids in green tea samples was conducted using liquid chomatography-mass spectrometer (LC-MS). A 0.5 g of ground tea was extracted with 5 ml of 50% methanol in a sonicator bath at room temperature for 30 min, and the resulting solution was filtered though a 0.22 -µm nylon membrane. The analysis was performed using either an LCMS-9030 quadrupole time-of-flight (Q-TOF) mass spectrometer (Shimadzu Corporation, Japan) and an lnertsil ODS-4 column (4.6 mm × 250 mm × 5 µm, GL Science Inc., Tokyo, Japan). The elution gradient was as followed; 5-22% methanol (eluent B) from 0-5 min, held at 22% B from 5-20 min, increased to 24% B from 20-35 min, 24-25% B from 35-45 min, 25-40% B from 45-50 min, and 40-45% B from 50-60 min. Detection was performed at a UV wavelength of 280 nm, with the column temperature set at 30 °C, a flow rate of 0.8 ml/min, and an injection volume of 1.0 µl (Xiao et al., 2020).

3.2.8 Sensory Analysis

Tea leave was washed with water for 40 min. One gram of tea leave was sieved in 100 mL of RO water (TDS10, 95 ºC) for 3 min. Sensory evaluation in for fragrance, flavor, and color was conducted with the use of the ranking test. The tea quality was estimated and scored by five professional tea tasters, from Chiang Rai provinces.

3.3 Statistical Analysis

All result were presented as mean ± standard deviations. Statistical significance was determined using one-way ANOVA, followed by Duncan's test ($P < 0.05$) in SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Additionally, advanced statistical analyses, including orthogonal principal components analysis (PCA), partial least squares discriminant analysis (PLS-DA), dendrograms, and heatmaps, were conducted using the MetaboAnalyst package in R (accessible at https://github.com/xia-lab/MetaboAnalystR). Hierarchical clustering analysis (HCA) was performed with the heatmap package within the R environment (http://www.r-project.org/). These analytical tools provided comprehensive insights into the variations and groupings within the tea quality data (Xiao et al., 2020).

CHAPTER 4

RESEARCH RESULTS

4.1 Yeast Coffee Fermentation

4.1.1 Yeast Culture

In the experiment, two different methods of yeast cultivation were compared. The first method, which was cultivating yeast cells in 1 L of Erlenmeyer flask, yielded resulted in 5.12 g per 500 ml of liquid medium or approximately 10 g/L. In contrast, the second method, which was cultivating yeast cells in 10 L of a container, yielded 15.94 g per 5 L of a liquid medium or approximately 3 g/L.

The collected yeast were dried in four different temperature. Unfortunately, the survival rate of yeast cells at high temperatures were only 1.51% to 3.83%. In contrast, the cell which dried at room temperature had a survival rate of 96.22% (Figure 4.1). This indicates that higher drying temperatures adversely affect the viability of yeast cells.

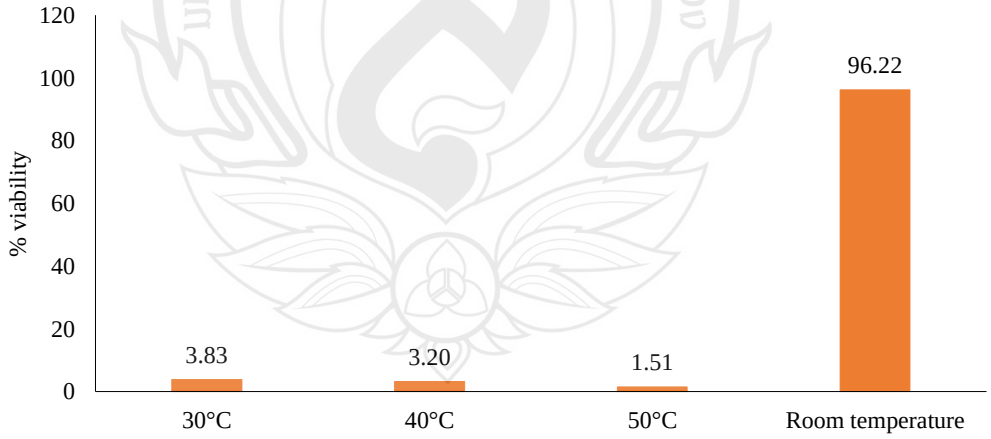


Figure 4.1 The percentage viability of yeast cells dried at different temperature

Eventhough, it require longer hour to dried yeast cell at room temperature, however this method was chosen to dried the rest of yeast cell. Those yeast cells were then assessed for their viability after 25 days storage at 4 and -20 °C. Storage at 4 °C

preserved higher viability (97.56% to 91.64%), while -20 °C resulted in greater cell death (96.77% to 83.39%). The statistically significant differences indicate that 4 °C is more effective for yeast preservation.

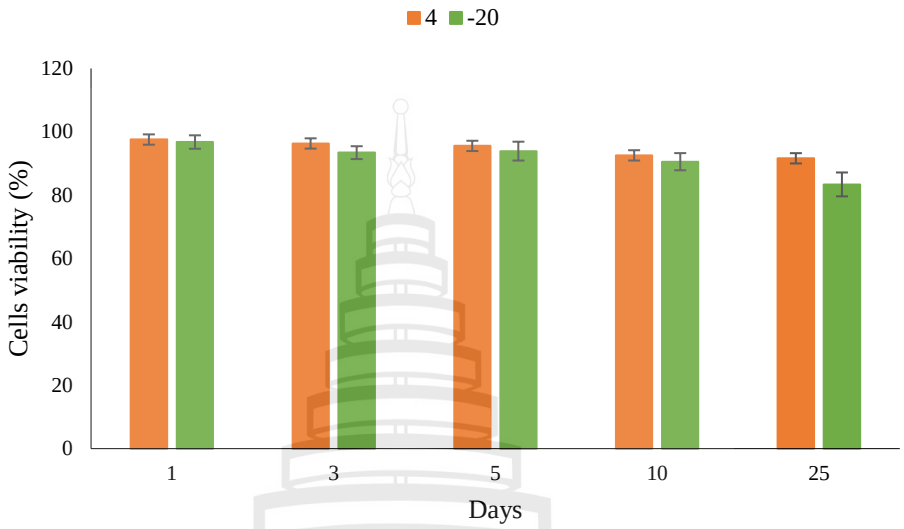


Figure 4.2 The percentage viability of yeast cells stored at different temperatures

4.1.2 Coffee Cherry

In general, Robusta coffee cherries tend to be slender, plump, smaller, and have thicker skin than Arabica. Additionally, fully ripe Robusta cherries are darker red than those of Arabic cherries. Although the hand-picked method was used but pulling them from the clusters resulted in a mixture of cherries in various ripening stages, as shown in the different colors of coffee cherries (Figure 4.3). Thus, after manually collecting 100 kg of ripe coffee cherries, only approximately 80 kg of coffee cherries were used for fermentation, due to the need to remove contaminants including rotten, unripe beans (green, yellow and orange), as well as leaves and branches (Figure 4.3).

Figure 4.3 Harvested and selected cherry coffee to use in yeast fermentation

4.1.3 Coffee Processing

All treatment of Arabica coffee fermentation required a total of 8 days to achieve its optimal pH range of 2-3, while Robusta coffee needed a longer fermentation period of approximately 10 days to reach its ideal pH value, as shown in Table 4.1. This highlights the differences in fermentation duration required for each coffee variety to achieve the desired acidity level.

Table 4.1 The pH in the fermentation container during fermented

Conditions	pH value ± SD					
	0 day	2 days	4 days	6 days	8 days	10 days
Arabica coffee						
SIAF	5.05 ± 0.13	5.10 ± 0.04	4.13 ± 0.03	3.87 ± 0.10	2.72 ± 0.40	n/a
Anaerobic	5.02 ± 0.16	5.00 ± 0.13	4.19 ± 0.03	3.85 ± 0.05	2.90 ± 0.20	n/a
SIAF_peel	4.89 ± 0.06	4.90 ± 0.09	4.17 ± 0.06	3.77 ± 0.05	2.70 ± 0.35	n/a
Robusta coffee						
SIAF	4.37 ± 0.02	4.29 ± 0.04	4.23 ± 0.02	3.90 ± 0.12	3.60 ± 0.06	2.94 ± 0.03
Anaerobic	4.43 ± 0.02	4.34 ± 0.03	4.24 ± 0.02	3.91 ± 0.27	3.58 ± 0.09	2.97 ± 0.04
SIAF_peel	4.42 ± 0.01	4.33 ± 0.07	4.14 ± 0.08	3.93 ± 0.11	3.67 ± 0.16	2.91 ± 0.03

Note Data are shown as the mean. All samples were analyzed in triplicate. (±) mean standard deviation and (-) mean not detected

All the fermented coffee cherry were then sun-dried on SIAF. The moisture content of coffee cherry before the sun-dried was approximately 38-40% (Table 4.2). A total of 12 days of drying to achieve optimal moisture content for storage (10-12%). The moisture percentage in treatment with peeled coffee beans was lower than optimal moisture (5.8-8.8%). The coffee cherries obtained from fermentation in SIAF and anaerobic fermentation after being sun-dried for 12 days showed a noticeable amount of yeast on the surface of some coffee beans. Under anaerobic conditions, approximately 13.36 and 20.58% of the cherries had yeast visibly adhered to the surface of Arabica and Robusta cherries, respectively (Figure 4.4). On the other hand, approximately 5.39 and 6.30% of Arabica and Robusta cherries demonstrated visible yeast on the cherries from SIAF conditions (Figure 4.4). This result indicates that anaerobic fermentation promotes better yeast growth than SIAF fermentation. In addition, when comparing the quantity of yeast after SIAF and anaerobic fermentation

of Arabica and Robusta coffee, it was found that the surface of Robusta coffee exhibited a higher amount of yeast (Figure 4.5).

Table 4.2 The moisture in the dried coffee cherries after fermentation

Conditions	Moisture content (%)						
	0 day	2 days	4 days	6 days	8 days	10 days	12 days
Arabica coffee							
SIAF	41.1 ± 1.19	40.6 ± 0.96	37.0 ± 0.25	33.0 ± 0.50	25.8 ± 0.35	18.0 ± 0.85	10.5 ± 1.04
Anaerobic	42.4 ± 0.80	42.2 ± 2.91	38.3 ± 0.74	32.7 ± 1.04	26.0 ± 1.00	18.3 ± 1.15	10.0 ± 0.87
SIAF_peel	41.4 ± 1.27	40.5 ± 0.46	36.8 ± 0.25	31.7 ± 1.44	26.4 ± 0.55	17.7 ± 1.31	8.8 ± 3.96
Robusta coffee							
SIAF	38.2 ± 0.38	37.6 ± 0.40	33.9 ± 1.81	30.0 ± 0.75	24.6 ± 0.32	16.8 ± 0.68	10.2 ± 0.78
Anaerobic	38.5 ± 0.72	37.0 ± 0.40	32.7 ± 1.10	29.7 ± 0.74	24.5 ± 0.47	16.7 ± 0.68	10.3 ± 0.83
SIAF_peel	38.7 ± 0.74	37.8 ± 0.62	33.1 ± 0.83	27.8 ± 1.44	22.4 ± 0.75	14.7 ± 0.96	5.8 ± 0.72

Note Data are shown as the mean. All samples were analyzed in triplicate. (±) mean standard deviation and (-) mean not detected

Figure 4.4 The pellet of yeast surface on the cherry coffee in anaerobic fermentation; (a, b, and c) yeast surface on the cherry coffee of Arabica coffee, (d, e, and f) yeast surface on the cherry coffee of Robusta coffee

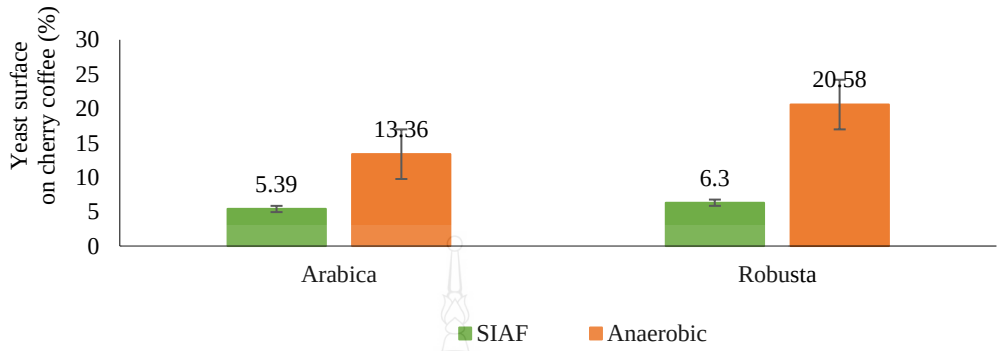


Figure 4.5 The quantity of yeast on coffee cherries after fermentation and dried

4.1.4 Volatile Compounds by GC-MS

A total of 41 volatile compounds were detected in roasted Arabica beans from three condition of fermentation with yeast by SPME/GC-MS. Those 41 compounds were classified by their chemical groups including alcohols, aldehydes, ester, furanones, ketones, organic acid, organic compound, pyrazines, pyridines, pyrroles and others (Table 4.3). The analysis of volatile compounds, which are crucial to the aroma and flavor of coffee, revealed that the three fermentation conditions resulted in statistically significant differences in the volatile content of Arabica and Robusta coffee. Specifically, coffee fermented under SIAF condition (both whole cherries and depulped coffee mixed with pulp) showed a positive volatile content (-1 to 2) for component 1, while anaerobic fermentation was in the negative range (0 to 2) for component 1 (Figure 4.6). Interestingly, Arabica coffee produced from coffee bean mix with coffee pulped fermentation under SIAF condition contained higher amounts of essential volatiles that affect coffee's aroma and flavor compared to other conditions of fermentation. Arabica coffee, produced from coffee bean mix with coffee pulped fermentation under SIAF conditions exhibited higher levels of pyrazine and furfuryl compounds, including pyrazine ethyl, pyrazine 2,6-dimethyl, and furfuryl ethyl, than coffee processed by the other two methods. Pyrazine compounds contribute a nutty aroma to the coffee, reminiscent of almond, hazelnut, peanut, pistachio, and walnut, along with chocolate and cocoa notes. Furfuryl compounds impart a sweet, caramel, and bready scent to the coffee.

Similar to Arabica coffee, the analysis of volatile compounds critical to aroma and flavor indicated that the three fermentation conditions led to statistically significant differences in the volatile content of Robusta coffee. SIAF and anaerobic fermentation conditions influenced yeast growth differently, with SIAF conditions (SIAF and SIAF_Peel) showed a positive volatile content (0 to 2) for component 1, while anaerobic fermentation was in the negative range (1 to 2) for component 1. However, it is noteworthy that the three fermentation conditions, yield distinct differences in volatile compounds of Robusta coffee (Figure 4.7). A total of 48 volatile compounds were detected in roasted coffee bean from three condition of Arabica cherries fermentation with yeast by SPME/GC-MS. Those 48 compounds were classified by their chemical groups including alcohols, aldehydes, ester, furanones, ketones, organic acid, organic compound, pyrazines, pyridines, pyrroles and others (Table 4.7). Robusta coffee produced from whole-cherry fermentation under SIAF conditions contained higher levels of essential volatile compounds that affect coffee's aroma and flavor than coffee fermented anaerobic, similar to Arabica coffee. Both of SIAF fermentation for whole Robusta coffee cherries exhibited higher levels of pyrazine and furfuryl compounds. Interestingly, the method combining coffee cherry fermentation with pulp under SIAF conditions was the only one in which furan and furfuryl compounds were detected. These compounds provide a caramel aroma to coffee fermented with yeast.

Table 4.3 Volatile classes identified by headspace GC-MS of roasted Arabica coffee at different conditions

Compounds	**GC peak (%) ± SD**		
	SIAF	**Anaerobic**	**SIAF_Peel**
Alcohol			
Ethanol	34.20 ± 20.12	27.18 ± 4.20	17.62 ± 25.74
2,3-Butanediol	1.92 ± 0.16	2.51 ± 0.24	-
2-Furanmethanol	100.00 ± 0.00	100.00 ± 0.00	76.28 ± 47.45
Aldehydes			
Butanal, 3-methyl-	2.79 ± 0.65	4.05 ± 0.41	2.68 ± 0.29
Furfural	75.32 ± 5.17	80.84 ± 8.95	91.37 ± 5.75
Furfuryl ethyl ether	1.44 ± 0.28	45.42 ± 87.72	-
Furfuryl formate	-	4.16 ± 0.35	4.20 ± 0.33
Esters			
2-Furancarboxaldehyde, 5-methyl-	52.84 ± 5.25	52.55 ± 2.15	53.39 ± 4.36
2-Furanmethanol, acetate	14.16 ± 0.82	15.87 ± 0.88	12.32 ± 0.98

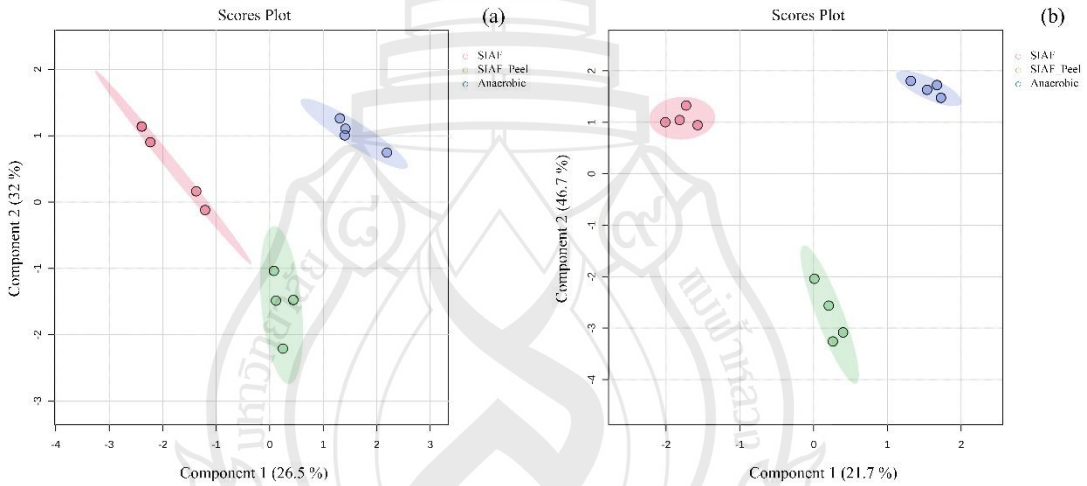
Table 4.3 (continued)

Compounds	GC peak (%) ± SD		
	SIAF	Anaerobic	SIAF_Peel
Furanones			
3(2H)-Furanone, dihydro-2-methyl-	8.11 ± 1.30	9.97 ± 0.83	9.56 ± 1.20
3(2H)-Furanone, 2-(1-hydroxy-1-methyl-2 oxopropyl)-2,5-dimethyl	3.44 ± 0.14	3.64 ± 0.13	3.66 ± 0.22
Furaneol	4.41 ± 0.70	3.95 ± 0.72	4.64 ± 0.65
Furaneol	4.41 ± 0.70	3.95 ± 0.72	4.64 ± 0.65
Ketones			
2,3-Pentanedione	6.69 ± 1.08	9.06 ± 0.94	9.26 ± 1.05
1,2-Cyclopentanedione, 3-methyl-	-	2.45 ± 0.31	1.90 ± 1.31
Organic acids			
Acetic acid	58.72 ± 9.43	60.32 ± 4.09	50.29 ± 2.25
2-Butenoic acid, 3-methyl-	0.97 ± 0.34	1.66 ± 0.64	1.90 ± 0.52
Organic Compounds			
2-Propanone, 1-hydroxy-	25.75 ± 1.94	28.01 ± 1.28	27.56 ± 1.59
2-Propanone, 1-(acetyloxy)-	17.18 ± 1.58	18.98 ± 0.64	15.53 ± 1.38
2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	2.72 ± 0.25	-	-
Flamenol	-	-	1.50 ± 0.17
Pyrazines			
Pyrazine	2.44 ± 0.56	2.73 ± 0.81	2.19 ± 0.31
Pyrazine, methyl-	34.88 ± 2.84	39.01 ± 1.09	37.79 ± 3.60
Pyrazine, 2,5-dimethl-	38.91 ± 15.96	47.90 ± 0.65	46.95 ± 0.14
Pyrazine, ethyl-	-	-	15.81 ± 1.11
Pyrazine, 2,6-dimethyl-	-	-	54.20 ± 0.12
Pyrazine, 2,3-dimethyl-	4.42 ± 1.78	5.74 ± 0.60	5.06 ± 0.95
Pyrazine, 2-ethyl-6-methyl-	7.10 ± 1.80	9.07 ± 0.56	7.07 ± 1.72
Pyrazine, 2-ethyl-5-methyl-	10.77 ± 3.17	14.17 ± 0.96	12.62 ± 2.39
Pyrazine, 2-ethyl-3-methyl-	2.87 ± 1.35	4.23 ± 0.32	2.60 ± 1.00
Pyridine			
Pyridine	11.57 ± 1.04	14.09 ± 1.41	10.91 ± 1.13
Pyrroles			
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	1.82 ± 0.95	3.08 ± 0.21	2.47 ± 0.92
1H-Pyrrole-2-carboxaldehyde, 1-ethyl-	0.56 ± 0.17	0.61 ± 0.19	0.43 ± 0.01
Others			
Acetoin	2.37 ± 0.37	3.18 ± 0.17	2.73 ± 0.18
1-Hydroxy-2-butanone	2.55 ± 0.23	2.65 ± 0.15	2.58 ± 0.14
Oxirane, (propoxymethyl)-	1.56 ± 0.25	1.87 ± 0.26	1.73 ± 0.30
4-Cyclopentene-1,3-dione	1.52 ± 0.45	1.75 ± 0.29	1.75 ± 0.33

Table 4.3 (continued)

Compounds	GC peak (%) ± SD		
	SIAF	**Anaerobic**	**SIAF_Peel**
3-Buten-2-ol, 4-(2,6,6-trimethyl-2 cyclohexen-1-yl)-, (3E)	-	0.74 ± 0.02	0.63 ± 0.06
4-Acetyl-1-methylcyclohexene	0.65 ± 0.09	-	0.72 ± 0.09
3-Acetyl-1-methylpyrrole	0.48 ± 0.17	0.73 ± 0.27	0.83 ± 0.10
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	1.89 ± 0.31	1.93 ± 0.21	1.50 ± 1.07
1-Hydroxy-2-butanone	2.55 ± 0.23	2.65 ± 0.15	2.58 ± 0.14

Note Data are shown as the mean. All samples were analyzed in triplicate. (±) mean standard deviation and (-) mean not detected.



Note SIAF mean self-induce anaerobic fermentation of coffee cherries with yeast, SIAF_Peel mean self-induce anaerobic fermentation of coffee pulped with yeast, and anaerobic mean anaerobic fermentation with cherries coffee with yeast.

Figure 4.6 Principle component of the volatile group after fermented with yeast using the three different conditions; (a) Arabica coffee, (b) Robusta coffee

Table 4.4 Volatile classes identified by headspace GC-MS of roasted Robusta coffee at different conditions

Compounds	GC peak (%) ± SD		
	SIAF	Anaerobic	SIAF_Peel
Alcohol			
Ethanol	24.38 ± 5.35	35.79 ± 19.00	23.36 ± 24.91
2,3-Butanediol	1.86 ± 0.13	2.41 ± 0.32	0.63 ± 1.25
2-Furanmethanol	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Aldehydes			
Butanal, 3-methyl-	2.77 ± 0.80	3.60 ± 0.50	3.14 ± 1.05
Furfural	75.26 ± 6.34	81.41 ± 8.36	84.68 ± 7.64
Furfuryl ethyl ether	1.39 ± 0.32	1.58 ± 0.10	44.25 ± 88.50
Furfuryl formate	-	3.06 ± 2.06	4.25 ± 0.34
Esters			
2-Furancarboxaldehyde, 5-methyl-	52.60 ± 6.40	52.76 ± 2.21	54.70 ± 2.23
2-Furanmethanol, acetate	13.90 ± 0.78	15.31 ± 0.28	13.25 ± 2.69
Furanones			
3(2H)-Furanone, dihydro-2-methyl-	7.74 ± 1.31	9.58 ± 0.67	9.49 ± 1.08
3(2H)-Furanone, 2-(1-hydroxy-1-methyl-2 oxopropyl)-2,5-dimethyl	3.39 ± 0.11	3.61 ± 0.12	3.62 ± 0.17
Furaneol	4.56 ± 0.77	3.80 ± 0.59	4.83 ± 0.38
Allyl acetate	23.19 ± 2.22	29.06 ± 6.58	34.90 ± 2.21
Ketones			
2,3-Pentanedione	6.42 ± 1.14	8.34 ± 0.65	9.27 ± 1.07
1,2-Cyclopentanedione, 3-methyl-	-	1.73 ± 1.15	2.63 ± 0.36
Organic acids			
Acetic acid	58.36 ± 11.52	58.86 ± 2.14	54.93 ± 7.18
2-Butenoic acid, 3-methyl-	0.87 ± 0.34	1.42 ± 0.53	1.91 ± 0.52
2-Methoxy-4-vinylphenol	2.01 ± 0.10	1.41 ± 0.06	2.00 ± 0.39
Maltol	3.77 ± 0.74	4.39 ± 0.66	-
Organic Compounds			
2-Propanone, 1-hydroxy-	25.27 ± 2.07	27.38 ± 0.57	28.15 ± 1.91
2-Propanone, 1-(acetyloxy)-	16.50 ± 1.01	19.06 ± 0.64	16.39 ± 2.16
2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	2.74 ± 0.30	0.66 ± 1.33	-
Flamenol	-	-	1.15 ± 0.78
Pyrazines			
Pyrazine	2.47 ± 0.68	2.61 ± 0.83	2.44 ± 0.32
Pyrazine, methyl-	34.58 ± 3.40	38.13 ± 1.91	37.15 ± 2.78
Pyrazine, 2,5-dimethl-	35.82 ± 18.02	47.91 ± 0.65	47.28 ± 0.59
Pyrazine, ethyl-	-	-	11.53 ± 7.71
Pyrazine, 2,6-dimethyl-	-	-	40.62 ± 27.08

Table 4.4 (continued)

Compounds	GC peak (%) ± SD		
	SIAF	Anaerobic	SIAF_Peel
Pyrazine, 2,3-dimethyl-	3.69 ± 1.23	5.79 ± 0.68	5.10 ± 1.02
Pyrazine, 2-ethyl-6-methyl-	6.45 ± 1.53	8.87 ± 0.25	7.36 ± 2.13
Pyrazine, 2-ethyl-5-methyl-	9.69 ± 2.84	13.80 ± 0.40	12.85 ± 2.67
Pyrazine, 2-ethyl-3-methyl-	2.37 ± 1.12	4.15 ± 0.19	2.92 ± 1.45
Pyrazine, 3-ethyl-2,5-dimethyl-	5.98 ± 0.66	5.29 ± 0.43	5.51 ± 0.85
Pyrazine, 3,5-diethyl-2-methyl-	0.68 ± 0.03	0.47 ± 0.15	0.08 ± 0.16
Pyridine			
Pyridine	11.34 ± 1.14	13.70 ± 1.70	11.53 ± 1.89
Pyrroles			
Pyridine	11.34 ± 1.14	13.70 ± 1.70	11.53 ± 1.89
Pyrroles			
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	1.46 ± 0.76	2.97 ± 0.10	2.51 ± 0.97
1H-Pyrrole-2-carboxaldehyde, 1-ethyl-	0.55 ± 0.21	0.58 ± 0.17	0.50 ± 0.14
1H-Pyrrole, 1-(2-furanylmethyl)-	1.09 ± 0.06	1.06 ± 0.02	1.12 ± 0.05
Ethanone, 1-(1H-pyrrol-2-yl)-	1.49 ± 0.40	1.30 ± 0.16	1.53 ± 0.15
Others			
Acetoin	2.42 ± 0.43	2.91 ± 0.48	2.87 ± 0.34
1-Hydroxy-2-butanone	2.52 ± 0.28	2.63 ± 0.15	2.62 ± 0.15
Oxirane, (propoxymethyl)-	1.52 ± 0.30	1.77 ± 0.23	1.71 ± 0.26
4-Cyclopentene-1,3-dione	1.58 ± 0.53	1.65 ± 0.35	1.66 ± 0.26
3-Buten-2-ol, 4-(2,6,6-trimethyl-2 cyclohexen-1-yl)-, (3E)	-	0.56 ± 0.37	0.66 ± 0.06
4-Acetyl-1-methylcyclohexene	0.64 ± 0.11	0.16 ± 0.33	0.52 ± 0.35
3-Acetyl-1-methylpyrrole	0.43 ± 0.17	0.68 ± 0.26	0.85 ± 0.08
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	2.02 ± 0.22	1.78 ± 0.24	1.41 ± 0.96
1-Hydroxy-2-butanone	2.52 ± 0.28	2.63 ± 0.15	2.62 ± 0.15

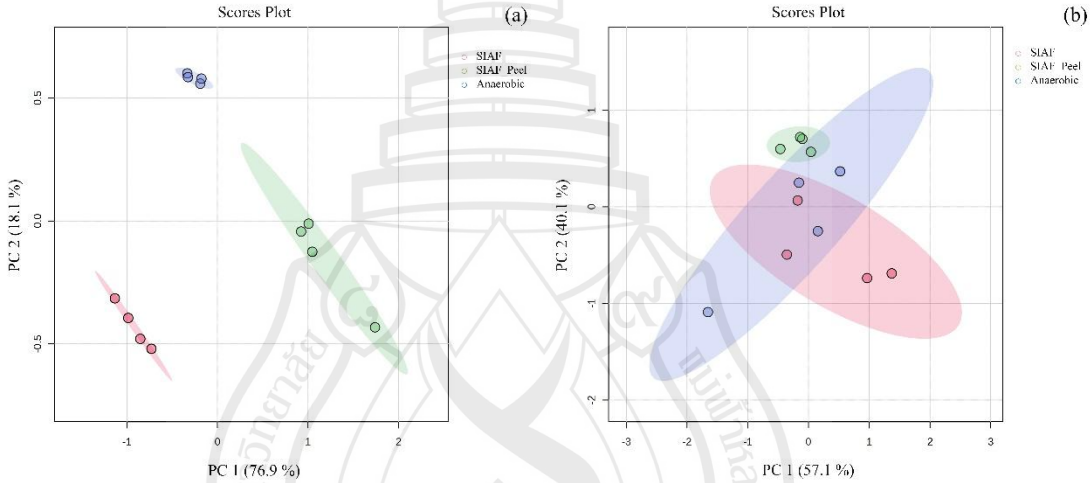
Note Data are shown as the mean. All samples were analyzed in triplicate. (±) mean standard deviation and (-) mean not detected.

4.1.5 Organic Acid by LC-MS

The organic acids present in roasted Arabica coffee are quinic, citric, lactic, tartaric, malic, fumaric, melaic, and succinic acid. While fumaric, citric, malic, melaic, lactic, succinic, and quinic acids are detected in roasted Robusta coffee.

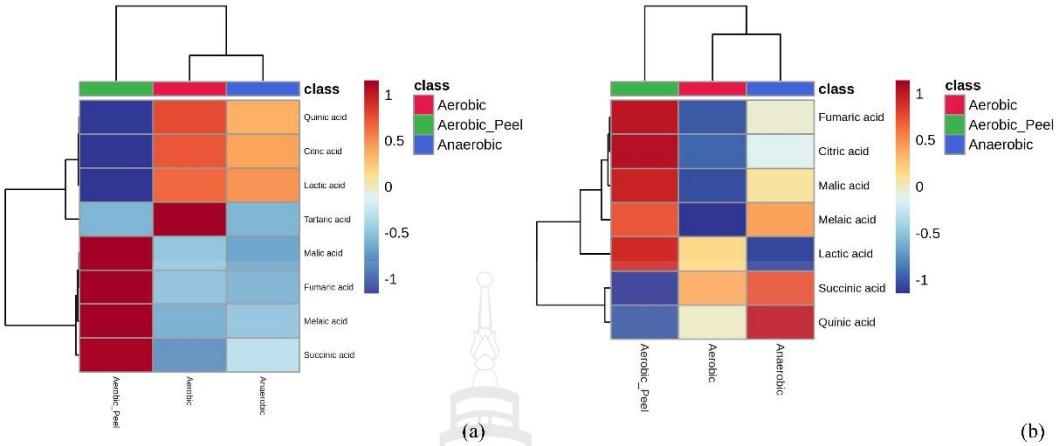
In Arabica coffee fermented under SIAF conditions, the levels of essential amin acids were significantly higher compared to anaerobic fermentation (Figure 4.7).

Specifically, SIAF fermented with whole-cherry coffee had higher levels of tartaric acid than other methods, giving the coffee a flavor profile reminiscent of grapes, cherries, cranberries, and tamarind. Conversely, coffee fermented with depulped cherries and mixed with pulp in SIAF conditions exhibited higher levels of malic acid, imparting an apple-like flavor to the coffee (Figure 4.8). Similarly, in Robusta coffee, SIAF fermentation resulted in significantly higher levels of essential amino acid compared to anaerobic fermentation (Figure 4.7). In particular, Robusta coffee fermented with whole cherries and pulp together under SIAF conditions contained higher levels of malic acid than other methods, resulting in an apple-like flavor profile (Figure 4.8).



Note SIAF mean self-induce anaerobic fermentation of coffee cherries with yeast, SIAF_Peel mean self-induce anaerobic fermentation of coffee pulped with yeast, and anaerobic mean anaerobic fermentation with cherries coffee with yeast.

Figure 4.7 Principle component of the organic acid after fermented with yeast using the three different conditions; (a) Arabica coffee, (b) Robusta coffee



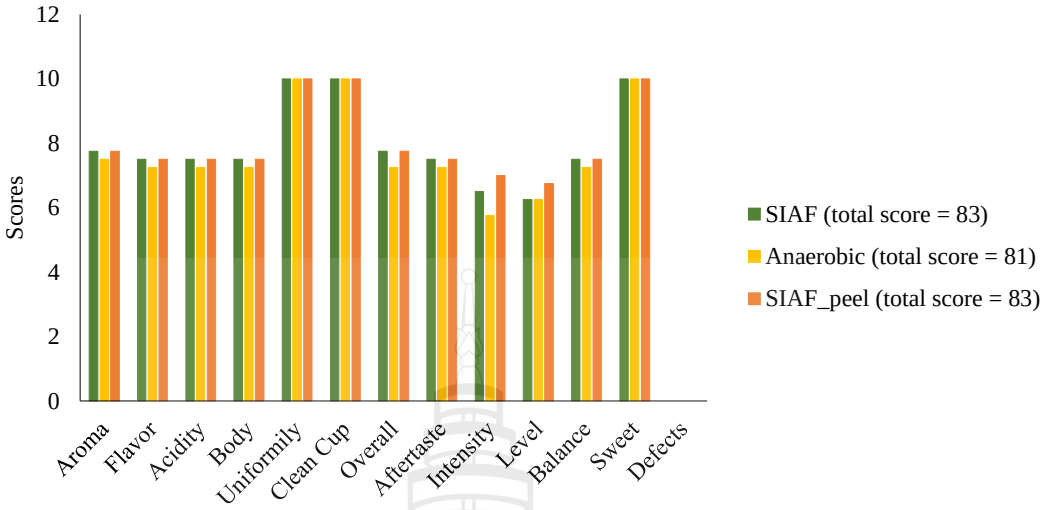
Note SIAF mean self-induce anaerobic fermentation of coffee cherries with yeast, SIAF_Peel mean self-induce anaerobic fermentation of coffee pulped with yeast, and anaerobic mean anaerobic fermentation with cherries coffee with yeast.

Figure 4.8 Shown the heatmap of organic acid after fermented with yeast using the three different conditions; (a) Arabica coffee, (b) Robusta coffee

4.1.6 Sensory Analysis

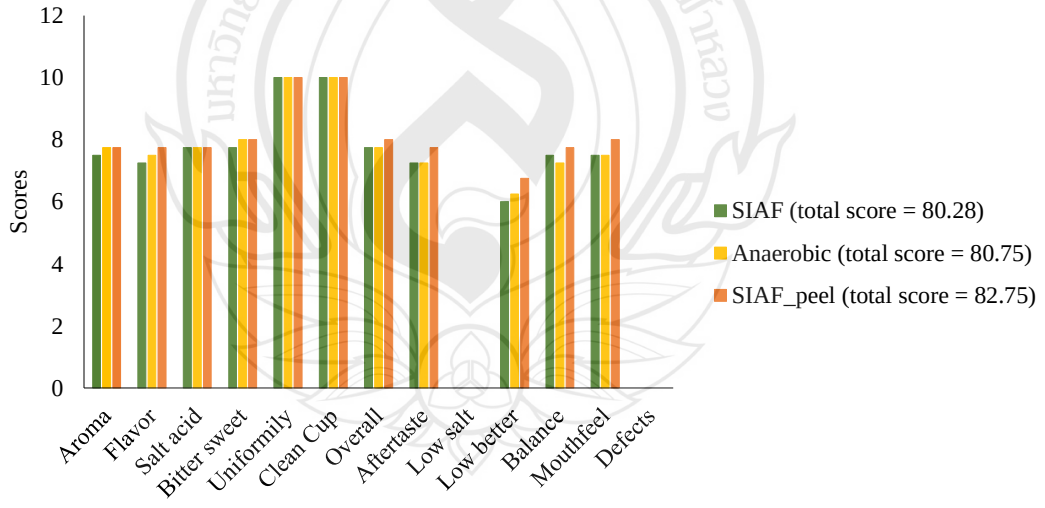
The analysis of volatile compounds and organic acid content using GC-MS and LC-MS aligned with sensory test results. The sensory test by a Q-grader followed the guidelines created by the Specialty Coffee Association of America (SCAA). The sample was roasted within 24 h of cupping and followed to rest for at least 8 h. The roast level for cupping was measured between 30 min and after roasting using the coffee ground to the SCAA standard grind for cupping and measured at room temperature.

Arabica coffee fermented under both SIAF conditions, scored a total 83 which is slightly, higher than the anaerobic fermentation (scored 81; Figure 4.9). Additionally, the SIAF conditions of Robusta coffee cherries with pulp yielded the highest quality coffee, consistent with the sensory test results. Specifically, the SIAF conditions of Robusta coffee cherries with pulp scored the highest at 82.75, followed by anaerobic fermentation, which scored 80.75, and SIAF whole-cherry fermentation, which scored 80.25 (Figure 4.10).



Note SIAF mean self-induce anaerobic fermentation of coffee cherries with yeast, SIAF_Peel mean self-induce anaerobic fermentation of coffee pulped with yeast, and anaerobic mean anaerobic fermentation with cherries coffee with yeast.

Figure 4.9 The result of sensory test in Arabica coffee beans from three difference fermentation



Note SIAF mean self-induce anaerobic fermentation of coffee cherries with yeast, SIAF_Peel mean self-induce anaerobic fermentation of coffee pulped with yeast, and anaerobic mean anaerobic fermentation with cherries coffee with yeast.

Figure 4.10 The result of sensory test in Robusta coffee beans from three difference fermentation

4.2 Green Tea Fermented with Fungal

4.2.1 Morphological and Molecular Identification

The phylogenetic tree shows that the fungus strain BT01 was clustered in the same group of *Aspergillus* sp. and *Aspergillus cristatus* (Figure 4.11). After incubation in media for 3 days, colonies of BT01 on CA and PDA had diameters of 16 and 14 mm, respectively (Figure 4.12). The morphology of the colonies on both media was similar. They had a sub-circular shape with a central bulge and were granular-shaped due to the abundant production of ascomata. They were slightly sulcate, with a gold-yellow color, then changed to sand color when mature. Yellow pigments were produced and abundant, no exudates were seen, and colonies on PDA had a coarser surface than those on CA. Micromorphological observation of a fungus revealed unique features of *Aspergillus* species. The fungus displayed smooth conidiophore stalks, translucent and divided hyphae with asymmetric branching, round-to-round vesicles that were fertile, and flask-shaped phialides arranged in a single layer. Conidia were observed budding from mature phialides, with some forming chains.

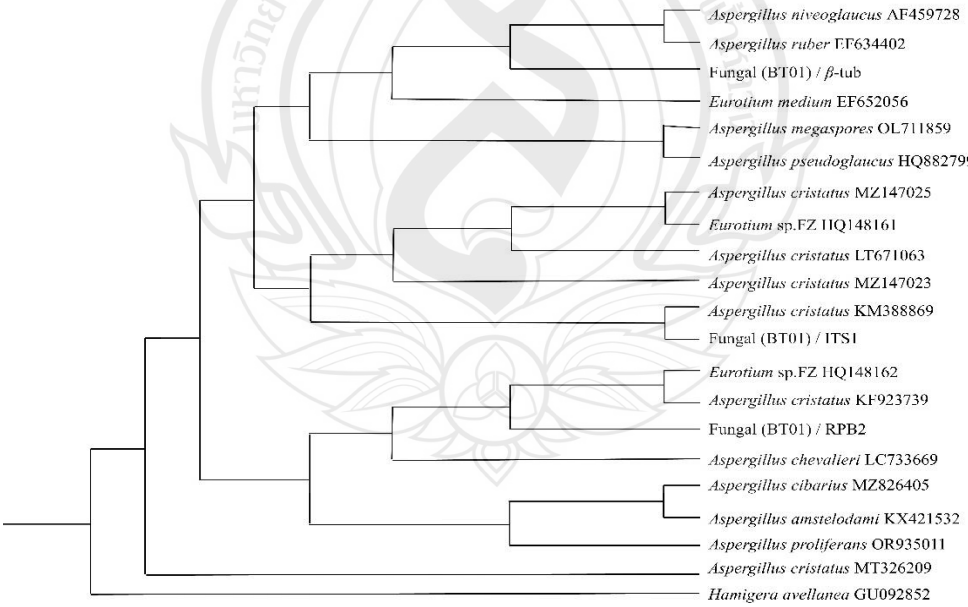


Figure 4.11 Phylogenetic tree of BT01 isolated from Pingwu Fuzhuan brick, the tree is rooted with *Hamigera avellanea* NRRL 1938^{T}

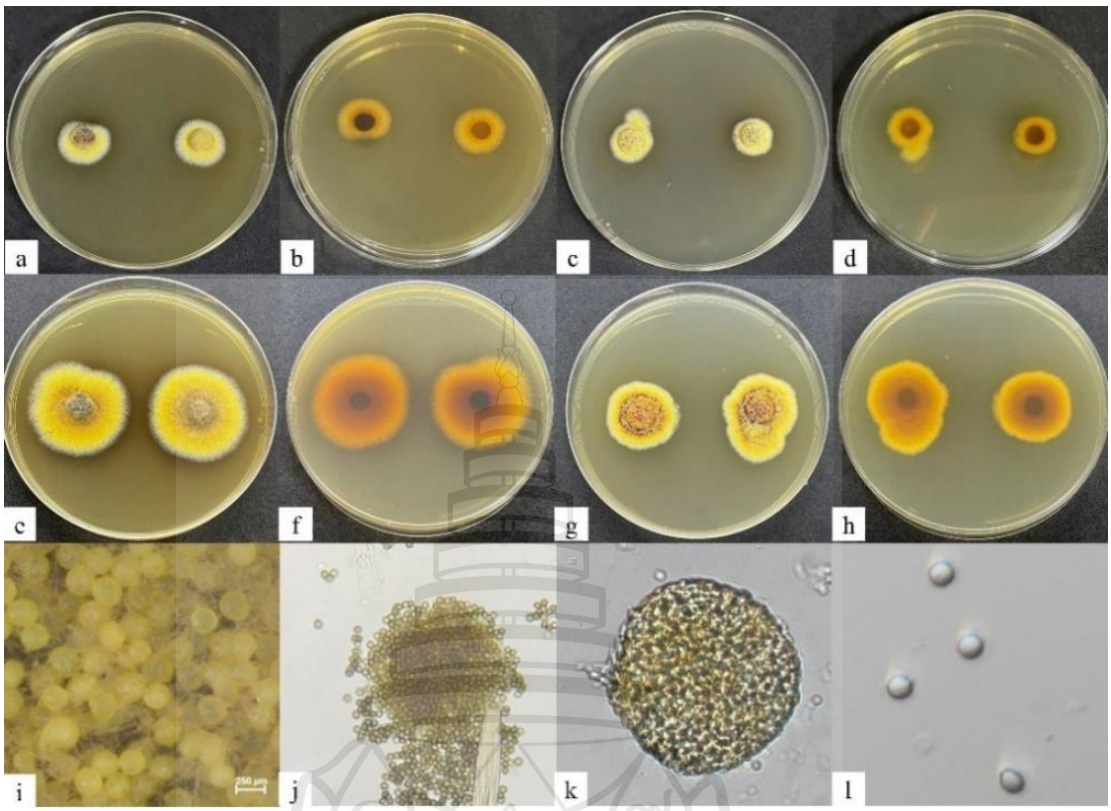


Figure 4.12 Morphological features of fungus strain BT01; (a, b, c, and d) colonies after 3 days at 28 °C on CA and PDA, (e, f, g, and h) colonies after 7 days at 28 °C on CA and PDA, (i) ascomata under stereo microscope, (j, k, and l) conidia, ascomata, and ascospore under compound microscope.

4.2.2 Phenolic Compounds by HPLC

Tea bricks were successfully inoculated with *A. cristatus* as the mycelium was observed on tea bricks (Figure 4.13). The content of catechin (CT), caffeine (CF), and gallic acid (GA) was quantified after 16 days of fermentation (Table 4.5). The content of CF was increased approximately 1.5 times in inoculated tea bricks compared to un-inoculated tea. In contrast, CT and GA content were significant decreased after post-fermented with BT01 (Table 4.5).

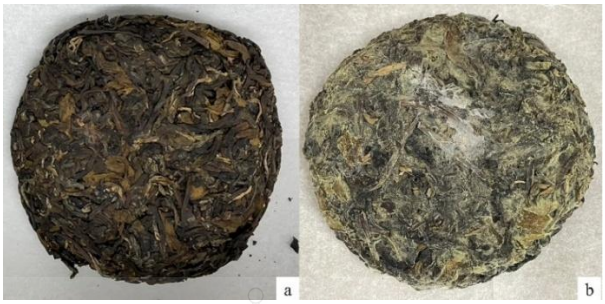


Figure 4.13 Tea bricks after incubated for 16 days; (a) tea brick inoculated with sterile water (control), (b) tea brick inoculated with *A. cristatus* (BT01)

Table 4.5 The content of caffeine, catechins, and gallic acid in tea brick after fermented with *A. cristatus* by HPLC at different fermentation stages

Brick tea	Concentration (mg/kg)		
	Caffeine**	**Catechins***	**Gallic acid***
Control	5.57 ± 0.41^{a}	3.49 ± 0.35^{c}	15.58 ± 1.59^{b}
BT01	8.22 ± 0.40^{b}	2.44 ± 0.08^{a}	10.77 ± 0.16^{a}

Note Control = tea brick inoculated with sterile water, BT01 = tea brick inoculated with sterile water. Each value is expressed as the mean SD. The results were statistically analyzed with a one-way ANOVA. a, b, c. The correlation coefficients between the variables used in constructed and the constituents that were correlated with the total quality were listed. *and** indicate significantly different values $P < 0.05$ ($^{*}P < 0.05$).

4.2.3 Volatile Compounds by GCMS

An analysis using GCMS-SPME identified 88 volatiles in tea across all treatments. The volatile compounds of both tea bricks that were inoculated with *A. cristatus* were clustered under the negative value while volatile compounds in control treatments were grouped in the positive value of component 1 (Figure 4.14). This data suggested that post fermentation with *A. cristatus* led to distinct variations in overall volatile compounds. Notably, linalool and methyl salicylate were identified as key volatile present in the tea sample inoculated with *A. cristatus* but absent in the control. Previously, linalool was reported to be an important contributor to tea aroma (Khan & Mukhtar, 2013).

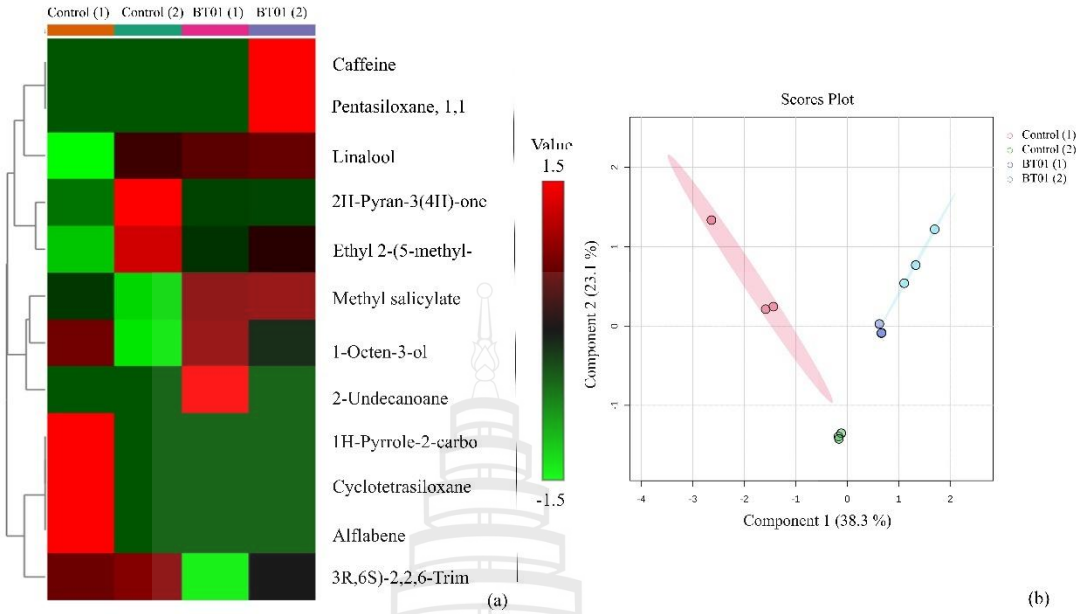


Figure 4.14 Orthogonal partial least squares discriminant analysis (PLS-DA) of different fermented tea samples based on volatile compounds; (a) heatmap, (b) PLS-DA scores

4.2.4 Organic Acid by LCMS

Similar to volatile compounds, post-fermentation with *A. cristatus* resulted in a change of organic acids in fermented tea brick. As shows in Figure 4.15, the overall organic acids in tea inoculate with *A. cristatus* and control exhibited at positive and negative values in PC 2, respectively. Particularly, tea brick that were inoculate with BT01 show higher amount of fumaric acid while lower amount of malic and citric acid compared to un-inoculate tea brick (Figure 4.15).

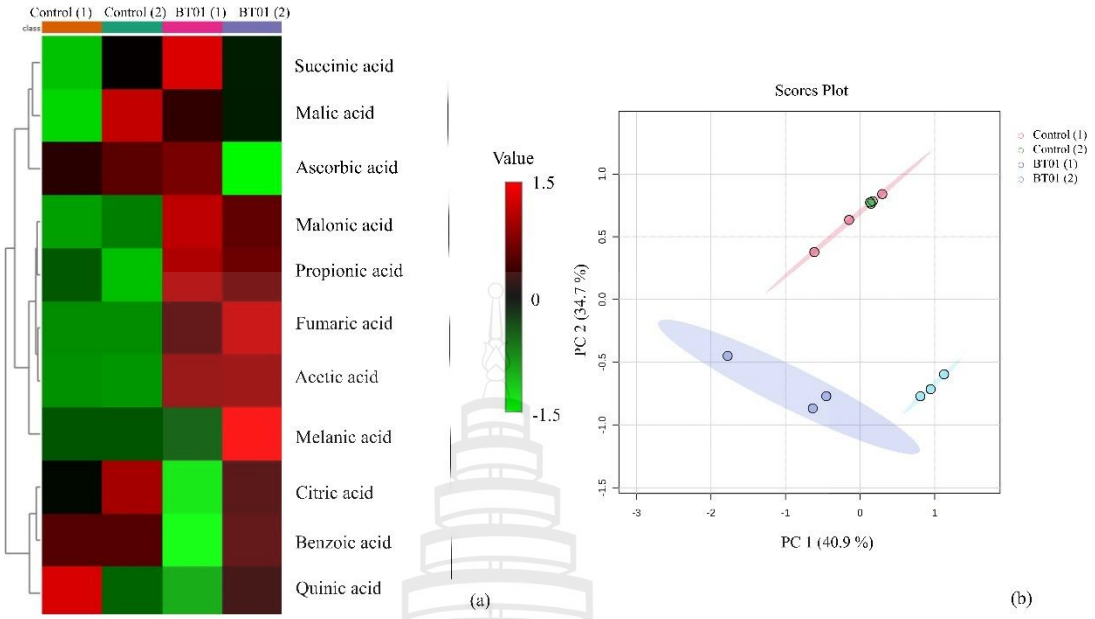


Figure 4.15 Orthogonal partial least squares discriminant analysis (PLS-DA) of different fermented tea samples based on organic acid; (a) heatmap, (b) PCA scores

4.2.5 Moisture Content and Water Activity

The moisture content, ash content, and water activity levels in both the control and BT01 of green tea samples are summarized in Table 4.6. The moisture content in the bud of each variety showed no significant difference, with values ranging from 5.74 to 6.80 g per 100 g in the control, and 7.93 to 8.68 g per 100 g in BT01, aligning with findings from the National Tea Research Institute in Pakistan.

4.2.6 Ash Content

Ash content, indicating the total mineral content in green tea powder, ranged between 5.70 and 5.76 g per 100g. Ash content in tea reflects the tea's mineral profile and includes physiological ash, derived directly from plant tissue. The ash composition varies with leaf maturity, as water-soluble potash and phosphoric acid decrease in order leaves. Acid-insoluble ash content helps assess contamination levels introduced during manufacturing.

4.2.7 Color Analysis

The color values (L, a, and b) of the green tea powders, representing lightness, redness, and yellowness, varied between the control and BT01 samples, as shown in Table 4.6. For the control, the values were L = 36.44, a = 1.34, and b = 5.61, while for BT01, the values were L = 34.38, a = 2.78, and b = 4.28. These differences indicate that the BT01 sample was slightly darker (lower L value), more red-toned (higher a value), and had less yellowness (lower b value) than the control sample. Statistical analysis showed that all color values of the green tea powders were significantly different between the two samples ($P < 0.05$), suggesting that processing or formulation variations impacted the final color of the powders.

Table 4.6 Shows moisture content, water activity, ash, and color analysis of green tea powder

Chemical content	Control	BT01
Moisture content (%)	6.37 ± 0.32	8.40 ± 0.23
Dry matter (%)	93.63 ± 0.32	91.60 ± 0.23
Ash wwb (%)	5.34 ± 0.06	5.28 ± 0.02
Ash dwb (%)	5.70 ± 0.08	5.76 ± 0.01
Water activity (%)	0.52 ± 0.00	0.56 ± 0.00
Color values		
*L**	36.44^{c} ± 0.67	34.38^{c} ± 0.31
*a**	1.34^{a} ± 0.54	2.78^{a} ± 0.23
*b**	5.61^{b} ± 0.12	4.28^{b} ± 0.28

Note The results were statistically analyzed with a one-way ANOVA. a, b, c. The correlation coefficients between the variables used in constructed and the constituents that were correlated with the total quality were listed. *and** indicate significantly different values $P < 0.05$ (*$P < 0.05$).

4.2.8 Antioxidant Activity

The total phenolic content in the green tea powders ranged from 35.68 to 59.67 g GAE per 100 g in the control and from 53.10 to 95.99 g GAE per 100 g in the BT01 sample, as shown in Table 4.7.

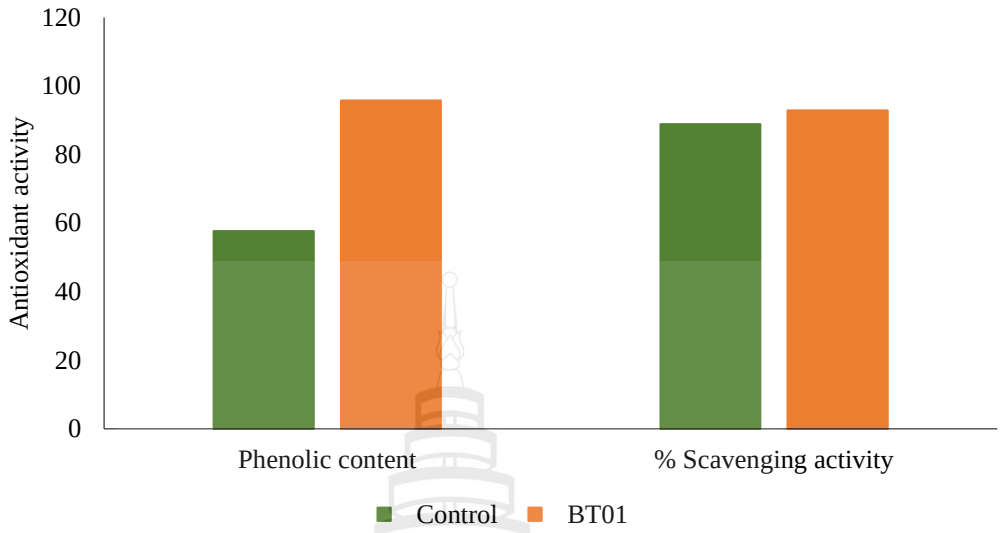


Figure 4.16 Total phenolic content, and free radical scavenging activity (DPPH) of green tea powders

4.2.9 Sensory Test

The brewed BT01 samples exhibited a clear, golden color (Figure 4.16), accompanied by flavor notes of honey, mushroom, seaweed, earthy undertones, and caramel. The predominant characteristics of these post-fermented teas included, which enhance their distinct profile.

Figure 4.17 Green tea leave was brew with RO water (TDS10, 95 ºC)

CHAPTER 5

CONCLUSION AND DISCUSSION

5.1 Conclusion

In this study, the first objective is aims to develop a yeast culture technique for use in the fermentation process of Robusta and Arabica coffee. The experiments shown that yeast cultivation can be adapted using commonly available equipment, reducing the need for expensive laboratory tools. This makes the method to convenient and suitable for farmers and roaster, enabling them to effectively apply yeast cultivation in coffee fermentation. Additionally, storing fresh yeast at 4 °C, using a refrigerator, simplifies management an prolongs viability. While cultivating yeast in a polyethylene tank yields less biomass compared to laboratory, it remains a more practical and cost-effective approach for farmers. Overall, these findings contribute to reducing the low-cost of yest used in coffee fermentation while making the process more efficient and accessible.

The second objective is aims to identify coffee processing methods and oxygen conditions that enhance coffee quality, particularly for Robusta coffee, analyzing chemical composition and cup quality. The findings indicate that SIAF fermentation generally produces higher-quality coffee than anaerobic processes. Specifically, SIAF fermentation of Robusta cherries with pulp resulted in the highest quality scores of 82.75, while anaerobic conditions slightly lowered scores across both coffee types. Moreover, using *S. cerevisiae* as a starter culture, fermentation impacted the production of organic acid and volatile compounds, with a total of 41 and 48 volatiles identified in Arabica and Robusta, respectively. The results underscore the potential of controlled fermentation to enhance coffee quality, particularly for Robusta coffee under SIAF conditions, as confirmed by sensory tests in collaboration with a local coffee enterprise.

The last objective is to investigate fungal fermentation in green tea and its effect on compounds related to tea quality and health benefits. Post-fermentation with *A.*

cristatus introduced notable changes in the chemical composition and sensory qualities of green tea. The inoculated tea brick, BT01 and BT02, exhibited increased caffeine content approximately 1.5 times higher than control samples and a unique volatile profile with 88 identified compounds, including key aroma-contributing volatiles like linalool and methyl salicylate. These volatiles, along with variations in organic acid and reduced malic and citric acids, contributed positively to the tea's mellow and rich flavor. The moisture, ash, color, and phenolic content demonstrated significant differences between the inoculated and control samples. BT01 had a slightly higher total phenolic content, reflecting enhanced antioxidant properties.

5.2 Discussion

Fermentation is a crucial post-harvest process that enhances the flavor, aroma, and overall sensory quality of coffee. Self-induced anaerobic fermentation (SIAF) is an innovative coffee fermentation which utilizes a closed environment without added water (da Mota et al., 2020). This process unfolds in two stages: aerobic and anaerobic, with anaerobic conditions gradually forming as microorganisms consume oxygen and produce CO_2. SIAF enhances the activity of yeast, improving sensory qualities and intensifying fruity flavors (Ferreira et al., 2023). While the operational costs of this method might be higher, they are compensated by the increased market value that specialty coffees can command. According da Silva Vale et al., (2022), longer fermentation times 48 and 72 h resulted in higher production of key metabolites and a wider range of sensory profiles compared to 24 h fermentation. Previous studies, highlights fermentation methods and coffee varieties with high-quality scores according to the SCA evaluation. Key findings include: 87.09 score for Self-induced anaerobic fermentation (SIAF), open environment fermentation of depulped coffee (Pereira et al., 2022); 85.15 for solid state anaerobic fermentation of natural (Junior et al., 2021); 87.25 using the SIAF method with *Bourbon Amarelo* and *Torulaspora delbrueckii* inoculum (da Mota et al., 2020); and 91.5 for submerged fermentation with aerobic and aerobic conditions. Similarly, this study was observed the impact of different fermentation conditions on the volatile compounds found in roasted Arabica

and Robusta coffee beans. The study uses SPME/GC-MS to detect and classify 41 volatile compounds in Arabica coffee and 48 in Robusta coffee, focusing on the compounds responsible for coffee's aroma and flavor. The SIAF fermentation of both Arabica and Robusta coffees fermented under SIAF (both whole cherries and depulped coffee mixed with pulp), showed higher levels of volatiles that are essential for aroma and flavor.

During coffee fermentation and roasting, volatile compounds such as pyrazine, esters, pyrroles, and pyridine are produced, with pyrazines being primary group. Pyrazines are essential to coffee flavor due to their low sensory threshold concentrations, contributing nutty, chocolate, caramel, and roasted notes (Liu et al., 2019; da Silva et al., 2021). Furfuryl compounds (e.g., furfuryl ethyl) impart a caramel, sweet, and bready scent, enhancing the overall flavor profile (Bressani et al., 2018). In this study, the SIAF and inoculating with *S. cerevisiae*, distinct flavors were produced, allowing coffee producers to create unique sensory profiles. Notably, SIAF_Peel condition resulted in the presence of pyrazine, ethyl and pyrazine, 2-6-dimethyl compounds, which were absent in the other conditions (SIAF and anaerobic fermentation). These compounds contribute to distinct flavor attributes, suggestion the fermentation conditions significantly influence the chemical composition and sensory profile of coffee. These findings reinforce the potential for controlled fermentation techniques to enhance coffee quality and diversify flavor profiles.

The analysis of volatile compounds and organic acids using GC-MS and LC-MS matched the sensory tests done by a Q-grader following SCAA guidelines. For Arabica, SIAF and SIAF_Peel conditions received a slightly higher score (83) compared to anaerobic fermentation (81). For Robusta, SIAF_Peel fermentation had the highest score (82.75), followed by anaerobic fermentation (80.75) and SIAF fermentation (80.25). These results highlight that SIAF_Peel fermentation produced the best flavor profiles for Arabica and Robusta coffee. Fermenting coffee by mixing beans with pulped coffee enhances both aroma and flavor, benefiting both Arabica and Robusta varieties. This method also affects organic acid levels, likely due to the nutrients found in the coffee husks. The pulp, rich in sugars and other nutrients, serves as an energy source for yeast, improving fermentation efficiency and influencing organic acid production. According to Jitjaroen et al., (2023), the pulp or mucilage is a

clear gel-like layer in the middle of the coffee cherry (mesocarp) composed of glucose, protein, starch, fiber, carbohydrates, oils, and pectin. It serves as a nutritional source for microorganisms, with its composition varying based on factors such as coffee variety and cherry ripeness. Higher mucilage content enhances the flavor and aroma of coffee, contributing to improved quality.

After 16 days of fermentation, the content of caffeine was found to increase by approximately 1.5 times in inoculated tea brick compared to the un-inoculated tea. This indicates that the fungus effectively colonized the tea bricks, likely contributing to changes in the chemical composition during fermentation. The increased caffeine content implies that the fermentation with *A. cristatus* might have played a role in altering the chemical composition of the tea, potentially enhancing or transforming certain bioactive compounds like caffeine, which could affect the flavor, aroma, and health properties of the tea. A sensory test was conducted to evaluate the brewed BT01 tea samples, which displayed a clear, golden color and a unique flavor profile. Key flavor notes identified included: honey, mushroom, seaweed, earthy undertones, and caramel. The predominant characteristics of BT01 tea, derived from post-fermentation, contributed to its distinct flavor profile. The combination of earthy and sweet notes like caramel and honey provided a complex and rich sensory experience. This sensory profile, combined with the golden color, makes BT01 tea appealing and unique, highlighting the impact of fermentation in enhancing both the visual appeal and taste qualities of the tea.

Linalool have previously been reported as a significant contributor to the aroma of tea, supporting the idea that its presence in the tea sample inoculated with *A. cristatus* might enhance the overall fragrance profile of the tea, making it more appealing in terms of floral and sweet notes (Khan & Mukhtar, 2013). In this study, the GC-MS-SPME analysis revealed 88 volatile compounds, with distinct differences in volatile profiles between tea bricks inoculated with *A. cristatus* and the control group. Suggesting that fermentation with *A. cristatus* led to significant changes in the tea's volatile composition. Key volatiles, such as linalool and methyl salicylate, were present in the *A. cristatus* inoculated tea but absent in the control group, with linalool, known for its floral aroma, being a major contributor to the tea's fragrance. This finding is consistent with the study by Zhang & Zhang, (2024), which highlights *A. cristatus* as a

fermentation starter that plays a crucial role in transforming and metabolizing tea constituents through processes such as oxidation, degradation, and condensation. These processes enhance both the sensory attributes and the biological functionality of tea. As a result, *A. cristatus* emerges as a significant probiotic agent with considerable potential for development.

The changes observed in volatile compounds, post-fermentation with *A. cristatus* also led to alterations in the organic acids in tea inoculated with this fungus, when compared to the control group. Organic acids, especially malic acid, lactic acid, and citric acid, are key components influencing the sour taste of tea. A balanced concentration of these acids enhances the richness of the taste, while excessive levels can negatively impact the overall quality. Chen et al., (2017) reported that fumaric acid plays a significant role in contributing to the "mellow and fresh" taste characteristic of black tea. Therefore, the reduced levels of malic acid and citric acid, alongside increased fumaric acid concentrations, positively impact the flavor profile of tea fermented with *A. cristatus*.

5.3 Suggestion

This analysis highlights how fermentation techniques can enhance coffee and tea flavor profiles through specific chemical changes. SIAF fermentation in coffee, especially when whole cherries or pulp are used, amplifies nutty, caramel, and fruity notes by increasing organic acids like malic and tartaric acids. Similarly, fungal fermentation with *A. cristatus* in green tea creates a unique volatile profile and adjusts organic acid levels, balancing the tea's acidity and enriching its flavor. These methods allow for precise control over aroma and taste complexity, underscoring their potential for producing high-quality, distinctively flavored beverage.

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APPENDIX A

MEASUREMENT OF THE PH VALUE AND MOISTURECONTENT IN COFFEE FERMENTATION WITH YEAST

1. The pH value of the Arabica and Robusta coffee fermentation was measured in triplicate.

Table A1 The pH value of Arabica coffee during fermentation at 0, 2, 4, 6, and 8 days under three different fermentation methods

Tank	pH value				
	0 day	**2 days**	**4 days**	**6 days**	**8 days**
1.1	5.11	4.31	4.15	3.98	2.76
1.2	4.90	4.35	4.15	3.82	2.30
1.3	5.15	4.28	4.09	3.80	3.10
2.1	5.20	4.50	4.22	3.80	3.10
2.2	4.96	4.47	4.17	3.84	2.89
2.3	4.90	4.26	4.19	3.90	2.70
3.1	4.92	4.39	4.23	3.72	2.45
3.2	4.87	4.23	4.11	3.80	3.10
3.3	4.81	4.23	4.16	3.80	2.56

Table A2 The pH value of Robusta coffee during fermentation at 0, 2, 4, 6, and 8 days under three different fermentation methods

Tank	pH value					
	0 day	**2 days**	**4 days**	**6 days**	**8 days**	**10 days**
1.1	4.39	4.25	4.25	3.78	3.65	2.95
1.2	4.35	4.32	4.22	4.01	3.54	2.91
1.3	4.37	4.30	4.21	3.9	3.61	2.97
2.1	4.44	4.36	4.25	4.12	3.54	2.93
2.2	4.45	4.30	4.24	4.00	3.69	3.00
2.3	4.41	4.35	4.22	3.60	3.52	2.98
3.1	4.42	4.40	4.22	4.03	3.86	2.90
3.2	4.43	4.33	4.06	3.95	3.60	2.89
3.3	4.42	4.27	4.14	3.82	3.56	2.94

2. The moisture content of the Arabica and Robusta coffee fermentation was measured in triplicate.

Table A3 The moisture content of Arabica coffee after fermentation and during dried process at 0, 2, 4, 6, 8, 10 and 12 days under three different fermentation methods

Tank	Moisture content						
	0 day	**2 days**	**4 days**	**6 days**	**8 days**	**10 days**	**12 days**
1.1	40.6	40.2	36.8	32.5	25.4	18.9	11.7
1.2	42.5	41.7	37.0	33.0	26.0	17.2	10.2
1.3	40.3	39.9	37.3	33.5	26.0	18.0	9.7
2.1	42.5	45.5	39.1	33.5	27.0	19.0	9.8
2.2	41.6	39.9	38.0	33.0	25.0	19.0	11.0
2.3	43.2	41.3	37.7	31.5	25.9	17.0	9.3
3.1	42.5	40.6	37.1	32.5	26.1	19.1	8.6
3.2	40.0	40.9	36.6	32.5	26.0	17.5	5.0
3.3	41.6	40.0	36.8	30.0	27.0	16.5	12.9

Table A4 The moisture content of Robusta coffee after fermentation and during dried process at 0, 2, 4, 6, 8, 10 and 12 days under three different fermentation methods

Tank	Moisture content						
	0 day	**2 days**	**4 days**	**6 days**	**8 days**	**10 days**	**12 days**
1.1	37.8	37.2	35.6	29.3	24.4	17.3	9.8
1.2	38.5	38.0	34.1	30.0	25.0	16.0	11.1
1.3	38.4	37.6	32.0	30.8	24.5	17.0	9.7
2.1	38.9	37.1	32.2	30.5	24.3	16.2	10.0
2.2	39.0	37.4	34.0	29.4	24.1	16.5	9.6
2.3	37.7	36.6	32.0	29.1	25.0	17.5	11.2
3.1	37.9	37.1	33.8	29.5	23.3	15.8	5.2
3.2	39.0	38.3	33.4	27.0	22.0	14.3	5.6
3.3	39.3	38.0	32.2	27.0	22.0	14.0	6.6

APPENDIX B

THE VIABILITY OF YEAST CELLS AND COUNTING THE YEAST SURFACE ON COFFEE CHERRY

3. The viability of yeast cells at different temperatures in triplicate.

Table B1 The viability of yeast cells at different temperatures

Temperature	Viability of yeast cells					
	Replication 1		Replication 2		Replication 3	
	Total	Dead	Total	Dead	Total	Dead
25 °C	102	3	91	0	107	9
30 °C	143	137	116	112	156	150
40 °C	184	177	103	99	105	103
50 °C	121	119	117	116	148	145

4. The counting yeast pellet surface on coffee cherries of Arabica and Robusta coffee after fermentation under two different fermentation methods in triplicate.

Table B2 The counting yeast pellet surface on coffee cherries of Arabica and Robusta coffee

Coffee cherry	Yeast pellet surface on coffee cherries					
	Replication 1		Replication 2		Replication 3	
	Total	TR7	Total	TR7	Total	TR7
Arabica coffee						
SIAF	227	6	297	28	316	13
Anaerobic	310	12	317	36	318	79
Robusta coffee						
SIAF	236	15	251	12	258	20
Anaerobic	253	42	288	51	277	76

Note TR7 is the yeast surface on coffee cherry

APPENDIX C

DETERMINATION QUALITY OF COFFEE FERMENTATION WITH YEAST

5. The volatile compounds by GC-MS after fermentation processing and roasted.

Table C1 Volatile classes identified by headspace GC-MS of Arabica coffee at different conditions after roasted

Volatile compounds	Replication of SIAF				Replication of anaerobic				Replication of SIAF_Peel			
	1	2	3	4	1	2	3	4	1	2	3	4
Hydrazinecarboxamide	1.92	1.51	2.56	1.11	7.25	2.27	0.98	2.82	4.47	5.10	0.69	1.92
Ethanol	28.48	26.33	18.32	63.66	32.11	23.38	24.02	29.21	56.11	6.24	1.89	6.24
Acetic acid	69.08	46.18	59.81	59.81	61.40	57.51	56.73	65.64	50.85	50.85	52.37	47.10
Butanal, 3-methyl-	3.65	2.10	2.55	2.85	3.84	3.92	3.79	4.66	2.94	2.69	2.27	2.83
2-Propanone, 1-hydroxy-	27.63	24.37	23.80	27.19	28.14	27.38	26.79	29.74	29.80	27.02	26.05	27.37
2,3-Pentanedione	7.69	6.09	5.48	7.51	8.41	9.11	8.33	10.37	10.00	8.49	8.23	10.31
Acetoin	2.91	2.10	2.24	2.23	3.30	3.16	2.95	3.30	2.94	2.51	2.72	2.76
Pyrazine	2.63	3.06	1.73	2.35	3.83	2.28	1.98	2.82	2.54	2.29	2.09	1.82
Pyridine	12.50	11.29	10.22	12.26	16.15	13.30	13.08	13.84	12.20	10.48	9.58	11.39
1-Hydroxy-2-butanone	2.78	2.56	2.23	2.62	2.80	2.44	2.64	2.71	2.78	2.47	2.53	2.55
2,3-Butanediol	1.79	1.79	2.01	2.10	2.75	2.18	2.60	2.50	0.00	0.00	0.00	0.00
Oxirane, (propoxymethyl)-	1.82	1.51	1.23	1.68	2.03	1.50	1.87	2.08	1.50	1.69	1.56	2.16
3(2H)-Furanone, dihydro-2-methyl-	9.10	7.62	6.49	9.23	10.36	8.85	9.87	10.78	9.72	9.29	8.17	11.07
Pyrazine, methyl-	35.78	30.75	37.22	35.76	37.41	39.83	39.53	39.27	39.83	34.80	34.71	41.82
Furfural	75.26	68.93	81.60	75.49	73.17	90.30	86.66	73.22	88.40	88.51	88.57	100.00
2-Furanmethanol	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	5.10

Table C1 (continued)

Volatile compounds	Replication of SIAF				Replication of anaerobic				Replication of SIAF_Peel			
	1	2	3	4	1	2	3	4	1	2	3	4
2-Propanone, 1-(acetyloxy)-	17.40	16.70	15.41	19.20	18.82	19.87	18.35	18.87	17.29	15.47	13.91	15.45
4-Cyclopentene-1,3-dione	0.98	1.80	1.96	1.34	1.36	1.86	2.04	1.72	1.53	2.00	1.40	2.06
2-Butenoic acid, 3-methyl-	0.67	1.26	0.67	1.26	1.11	1.11	2.21	2.21	1.39	2.48	1.54	2.19
Furfuryl ethyl ether	1.66	1.46	1.04	1.61	1.42	1.63	1.64	177.00	0.00	0.00	0.00	0.00
Furfuryl formate	0.00	0.00	0.00	0.00	3.65	4.41	4.18	4.38	4.61	3.80	4.19	4.19
Pyrazine, 2,5-dimethyl-	46.22	15.01	46.22	48.18	48.15	46.94	48.37	48.15	47.07	47.07	46.82	46.82
Pyrazine, ethyl-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.84	15.84	14.43	17.14
Pyrazine, 2,6-dimethyl-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	54.09	54.09	54.30	54.30
Pyrazine, 2,3-dimethyl-	5.09	3.20	2.77	6.62	5.06	5.45	6.02	6.41	4.71	5.26	4.00	6.25
3(2H)-Furanone, 2-(1-hydroxy-1-methyl-2 oxopropyl)-2,5-dimethyl	3.50	3.28	3.39	3.60	3.54	3.51	3.77	3.73	3.79	3.46	3.48	3.91
2-Furancarboxaldehyde, 5-methyl-	46.38	52.26	59.17	53.53	55.42	50.30	51.79	52.67	57.88	53.94	54.31	47.42
2-Furanmethanol, acetate	14.66	13.11	13.92	14.93	15.51	15.26	15.52	17.17	12.78	11.34	11.70	13.47
Pyrazine, 2-ethyl-6-methyl-	7.75	6.83	4.76	9.04	9.03	8.51	8.90	9.84	4.65	7.59	7.34	8.70
Pyrazine, 2-ethyl-5-methyl-	12.83	8.93	7.30	14.00	14.01	13.20	13.98	15.49	9.13	13.38	13.41	14.54
Pyrazine, 2-ethyl-3-methyl-	3.60	2.10	1.41	4.37	4.18	3.91	4.14	4.68	1.14	3.03	2.81	3.41
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	2.34	1.10	0.95	2.90	3.08	2.86	3.03	3.36	1.11	2.78	2.80	3.17
1,2-Cyclopentanedione, 3-methyl-	0.00	0.00	0.00	0.00	2.33	2.33	2.24	2.91	2.19	2.48	2.94	0.00
2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	2.50	2.65	3.07	2.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3-Buten-2-ol, 4-(2,6,6-trimethyl-2 cyclohexen-1-yl)-, (3E)	0.00	0.00	0.00	0.00	0.76	0.76	0.72	0.72	0.68	0.57	0.68	0.57
4-Acetyl-1-methylcyclohexene	0.53	0.65	0.75	0.65	0.00	0.00	0.00	0.00	0.79	0.64	0.64	0.79
1H-Pyrrole-2-carboxaldehyde, 1-ethyl-	0.52	0.35	0.77	0.59	0.70	0.33	0.69	0.71	0.42	0.42	0.44	0.44
Furaneol	4.40	3.88	5.39	3.95	3.33	3.33	4.57	4.57	4.44	5.11	5.20	3.81
Ethanone, 1-(1H-pyrrol-2-yl)-	1.39	1.15	1.93	1.22	1.35	1.12	1.50	1.60	1.30	1.62	1.60	0.98
3-Acetyl-1-methylpyrrole	0.33	0.33	0.63	0.63	0.35	0.74	0.98	0.83	0.91	0.91	0.74	0.74
Pyrazine, 3-ethyl-2,5-dimethyl-	5.22	6.44	6.27	5.87	4.96	4.97	5.34	6.74	5.39	4.84	5.05	5.18

Table C1 (continued)

Volatile compounds	Replication of SIAF				Replication of anaerobic				Replication of SIAF_Peel			
	1	2	3	4	1	2	3	4	1	2	3	4
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	1.92	2.27	1.87	1.51	1.83	1.69	2.08	2.13	0.00	1.90	1.60	2.50
Pyrazine, 3,5-diethyl-2-methyl-	0.66	0.66	0.71	0.39	0.29	0.59	0.59	0.32	0.00	0.00	0.00	0.00
1H-Pyrrole, 1-(2-furanylmethyl)-	1.12	1.12	1.02	1.02	1.07	1.07	1.06	1.06	1.12	1.13	1.17	1.17
Flamenol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.75	1.43	1.42	1.39
2-Methoxy-4-vinylphenol	1.95	1.95	2.12	1.32	1.46	1.42	1.42	1.72	1.67	2.10	2.51	1.58
Hydrazinecarboxamide	6.91	8.57	6.43	1.19	1.95	8.59	2.14	12.65	1.60	1.90	0.37	4.47
Ethanol	33.48	47.62	46.34	40.24	47.98	45.72	47.87	63.49	27.67	24.22	16.91	12.29
Acetic acid	53.45	59.76	57.51	57.56	66.26	63.52	66.95	67.39	54.27	57.21	52.20	43.79

Table C2 Volatile classes identified by headspace GC-MS of Robusta coffee at different conditions after roasted

Volatile compounds	Replication of SIAF				Replication of anaerobic				Replication of SIAF_Peel			
	1	2	3	4	1	2	3	4	1	2	3	4
Butanal, 3-methyl-	3.12	4.90	4.33	3.47	3.94	4.24	4.42	7.85	4.82	6.00	2.61	4.82
Allyl acetate	20.53	25.76	23.96	22.50	25.75	25.27	26.30	38.90	32.98	36.81	32.98	36.81
2-Propanone, 1-hydroxy-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.90	14.82	24.90	14.82
2,3-Pentanedione	4.71	6.57	6.65	6.33	5.50	6.20	6.39	9.38	9.21	10.49	6.98	4.13
Acetoin	2.43	3.05	2.89	2.56	2.70	2.75	2.61	3.84	2.63	3.42	2.37	1.30
Pyrazine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.10	2.46	2.15	2.01
Pyridine	11.99	13.93	13.13	12.06	9.39	9.50	11.46	13.64	10.66	11.21	8.59	8.36
1-Hydroxy-2-butanone	0.00	0.00	0.00	0.00	1.85	1.90	1.69	1.69	2.34	2.66	2.24	1.69
2,3-Butanediol	4.25	3.14	4.00	3.38	4.03	3.81	3.49	4.76	0.00	0.00	0.00	0.00
Oxirane, (propoxymethyl)-	1.48	2.00	1.84	1.26	1.37	1.68	1.33	2.68	2.17	2.40	2.40	0.94
3(2H)-Furanone, dihydro-2-methyl-	6.99	9.45	8.64	6.53	6.98	8.31	6.86	12.47	11.92	13.60	7.57	5.10
Pyrazine, methyl-	36.00	45.76	45.63	42.78	41.84	44.58	37.58	57.47	45.41	48.83	34.08	36.47

Table C2 (continued)

Volatile compounds	Replication of SIAF				Replication of anaerobic				Replication of SIAF_Peel			
	1	2	3	4	1	2	3	4	1	2	3	4
Furfural	48.60	58.23	61.77	61.24	66.06	68.03	61.83	77.51	100.00	92.57	82.52	85.51
2-Furanmethanol	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.66	100.00	100.00	100.00
2-Propanone, 1-(acetyloxy)-	15.74	18.82	18.02	16.30	14.64	15.55	14.18	20.62	16.59	19.05	14.26	13.97
4-Cyclopentene-1,3-dione	0.00	0.00	0.00	0.00	0.69	0.71	0.69	0.48	1.14	1.26	1.14	1.26
2-Butenoic acid, 3-methyl-	3.57	0.39	3.57	3.55	3.88	7.59	11.19	0.52	4.83	5.24	7.62	5.31
Furfuryl ethyl ether	2.52	1.04	2.52	1.04	0.00	0.00	0.00	0.00	2.47	2.45	2.47	2.45
Furfuryl formate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.03	2.11	2.11	4.03
Pyrazine, 2,5-dimethyl-	48.37	59.82	57.70	59.82	48.38	60.98	54.66	75.76	60.62	69.12	51.53	60.62
Pyrazine, ethyl-	19.69	24.61	22.51	20.80	15.44	23.27	21.20	28.22	23.16	26.72	19.68	14.33
Pyrazine, 2,3-dimethyl-	5.63	8.17	6.76	6.05	3.56	7.83	6.23	8.58	7.17	7.95	2.11	5.09
2-Furancarboxaldehyde, 5-methyl-	47.39	45.12	47.88	49.41	42.53	43.46	48.02	53.07	54.15	58.93	53.88	64.28
2-Furanmethanol, acetate	14.24	17.83	17.73	17.24	15.36	15.46	14.75	19.69	14.14	15.66	12.30	13.37
Pyrazine, 2-ethyl-6-methyl-	11.44	14.63	14.53	13.70	14.68	15.45	14.71	19.46	12.45	14.14	10.96	9.14
Pyrazine, 2-ethyl-5-methyl-	19.82	23.83	23.59	23.08	24.42	24.89	24.80	30.08	19.71	21.61	18.64	17.65
Pyrazine, 2-ethyl-3-methyl-	5.26	6.69	6.71	6.66	7.20	7.72	7.02	9.55	5.92	6.72	5.64	5.00
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	3.20	4.24	4.41	4.08	3.96	4.27	4.12	5.06	3.85	3.56	3.37	2.59
1H-Pyrrole-2-carboxaldehyde	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.61	3.06	3.61	3.06
1,2-Cyclopentanedione, 3-methyl-	2.60	2.19	2.52	2.65	2.02	1.82	2.27	1.84	1.78	2.90	3.45	2.90
2-Butanone, 4-cyclopentylidene-	0.14	0.26	0.47	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1H-Pyrrole-2-carboxaldehyde, 1-ethyl-	0.39	0.44	0.69	0.52	0.53	0.92	1.00	1.17	0.37	0.43	0.37	0.31
Furaneol	4.24	4.24	3.71	3.71	3.82	4.52	5.16	4.52	7.26	6.13	7.26	6.13
Ethanone, 1-(1H-pyrrol-2-yl)-	2.15	1.92	2.19	2.15	2.15	2.38	2.18	2.18	0.75	1.01	3.51	2.91
3-Acetyl-1-methylpyrrole	0.63	0.53	1.22	1.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pyrazine, 3-ethyl-2,5-dimethyl-	11.77	11.51	11.37	12.59	13.93	12.88	14.80	13.83	8.56	9.86	11.61	12.46
Pyrazine, 3-ethyl-2,5-dimethyl-	11.77	11.51	11.37	12.59	13.93	12.88	14.80	13.83	8.56	9.86	11.61	12.46
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	1.98	2.08	1.03	2.10	2.11	1.63	2.57	2.17	1.09	1.47	2.16	2.27

Table C2 (continued)

Volatile compounds	Replication of SIAF				Replication of anaerobic				Replication of SIAF_Peel			
	1	2	3	4	1	2	3	4	1	2	3	4
Maltol	3.64	3.64	3.00	4.78	4.66	3.40	4.82	4.66	0.00	0.00	0.00	0.00
5H-5-Methyl-6,7 dihydrocyclopentapyrazine	1.23	1.10	1.23	1.10	1.17	1.38	1.17	1.38	0.69	0.69	1.35	1.35
Pyrazine, 2,3-diethyl-5-methyl-	0.36	0.49	0.52	0.72	0.70	0.45	0.44	0.34	0.19	0.19	0.41	0.41
Pyrazine, 3,5-diethyl-2-methyl-	1.69	1.51	1.65	2.04	2.02	1.64	1.58	1.42	0.97	0.90	0.94	1.94
2-Isoamyl-6-methylpyrazine	0.00	0.00	0.00	0.00	0.31	0.31	0.53	0.53	0.00	0.00	0.00	0.00
Benzoic acid, 2-hydroxy-, ethyl ester	4.54	3.00	2.56	4.00	4.70	3.48	3.95	3.13	0.00	0.00	0.00	0.00
Phenol, 4-ethyl-2-methoxy-	1.76	0.88	0.83	0.88	1.40	0.78	0.82	0.63	1.57	1.58	1.57	1.58
2-Methoxy-4-vinylphenol	12.80	8.55	7.80	9.74	10.99	9.46	11.92	8.30	6.13	6.15	14.21	17.23
Furfural	48.60	58.23	61.77	61.24	66.06	68.03	61.83	77.51	100.00	92.57	82.52	85.51

6. The organic acid by LC-MS after coffee fermentation processing and roasted.

Table C3 Organic acid by LC-MS of Arabica coffee at different conditions after roasted

Organic acid	Replication of SIAF (ppm)				Replication of anaerobic (ppm)				Replication of SIAF_Peel (ppm)			
	1	2	3	4	1	2	3	4	1	2	3	4
Citric acid	2682.8	2611.3	2454.4	2546.2	2460.5	2571.4	2587.1	2304.4	2107.0	2087.5	2215.7	1814.8
Fumaric acid	7815.2	7779.6	7817.2	7799.9	7799.2	7795.5	7787.2	7805.8	7910.6	7904.1	7907.0	7962.7
Lactic acid	221.8	185.7	129.6	183.6	116.5	164.4	158.5	109.9	21.3	3.6	15.6	19.2
Malic acid	458.3	513.7	575.9	647.5	495.2	557.9	577.2	439.0	936.4	933.2	998.7	739.2
Melaic acid	8000.7	7980.4	8026.0	8017.8	8018.8	8014.3	8001.3	8029.7	8124.9	8132.0	8129.6	8183.0
Quinic acid	6079.1	5816.3	5516.5	5487.4	5324.0	5545.9	5814.2	5256.1	4864.7	4608.9	5106.7	4182.1
Succinic acid	5566.3	5566.6	5596.0	5616.7	5614.4	5597.1	5596.3	5620.6	5669.7	5671.5	5654.3	5697.5
Tartaric acid	472.2	472.7	472.5	472.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table C4 Organic acid by LC-MS of Robusta coffee at different conditions after roasted

Organic acid	Replication of SIAF (ppm)				Replication of anaerobic (ppm)				Replication of SIAF_Peel (ppm)			
	1	2	3	4	1	2	3	4	1	2	3	4
Citric acid	1787.8	1650.3	1411.1	1452.7	1712.5	1855.0	1545.4	1615.7	1798.1	1886.8	1816.4	1890.3
Fumaric acid	7843.5	7789.6	7868.5	7894.1	7864.1	7859.1	7911.1	7885.6	7918.1	7899.4	7947.1	7893.7
Lactic acid	12.7	5.7	22.1	38.6	0.6	16.3	40.5	13.6	27.5	15.0	26.6	25.1
Malic acid	177.5	117.0	26.3	17.9	204.7	209.4	143.9	94.6	258.8	427.6	347.2	355.1
Melaic acid	8008.8	8015.7	8074.5	8095.1	8076.1	8118.7	8128.6	8101.1	8125.9	8115.3	8111.6	8114.0
Quinic acid	4335.4	4355.9	4066.2	4138.4	4383.4	4735.0	4135.1	4262.7	4174.1	4053.8	4209.0	3882.5
Succinic acid	5570.2	5606.4	5633.5	5606.1	5615.4	5591.4	5627.0	5600.5	5597.9	5590.4	5576.4	5574.2

7. Cupping scored of sensory test in Arabica and Robusta coffee beans at three difference fermentation.

Table C5 Scored of sensory test in Arabica coffee beans

Arabica	Aroma	Flavor	Acidity	Body	Uniformily	Clean Cup	Overall	Aftertaste	Intensity	Level	Balance	Sweet	Defects	Total
SIAF	7.75	7.50	7.50	7.50	10.00	10.00	7.75	7.50	6.50	6.25	7.50	10.00	0.00	83.00
Anaerobic	7.50	7.25	7.25	7.25	10.00	10.00	7.25	7.25	5.75	6.25	7.25	10.00	0.00	81.00
SIAF_Peel	7.75	7.50	7.50	7.50	10.00	10.00	7.75	7.50	7.00	6.75	7.50	10.00	0.00	83.00

Table C6 Scored of sensory test in Robusta coffee beans

Robusta	Aroma	Flavor	Salt acid	Bitter sweet	Uniformily	Clean Cup	Overall	Aftertaste	Low salt	Low better	Balance	Mouthfeel	Defects	Total
SIAF	7.50	7.25	7.75	7.75	10.00	10.00	7.75	7.25	0.00	6.00	7.50	7.50	0.00	80.25
Anaerobic	7.75	7.50	7.75	8.00	10.00	10.00	7.75	7.25	0.00	6.25	7.25	7.50	0.00	80.75
SIAF_Peel	7.75	7.75	7.75	8.00	10.00	10.00	8.00	7.75	0.00	6.75	7.75	8.00	0.00	82.75

APPENDIX D

DETERMINATION QUALITY OF GREEN TEA FERMENTATION WITH FUNGI

8. The caffeine, catechin, and gallic acid by HPLC in tea brick after fermented with *A. cristatus* at different fermentation stages in triplicate.

Table D1 The standard of phenolic compounds

Concentration (ppm)	Caffeine (ppm)	Catechin (ppm)	Galic acid (ppm)
3.125	19803.44	9566.39	28190.30
6.250	50749.15	20516.82	52715.89
12.500	97127.46	45627.99	104557.66
25.000	189324.86	90933.26	203910.81
50.000	386817.67	185496.77	407318.56
100.000	715261.84	391017.02	771130.36

Table D2 The caffeine, catechin, and gallic acid in tea brick after fermented

Fermentation stage	Replication	Caffeine (ppm)	Catechin (ppm)	Gallic acid (ppm)
Control	1	5828.0	3922.4	11385.2
High density	2	5379.6	3670.2	11639.0
	3	5577.6	3883.5	11711.5
Control	1	5676.2	3537.9	17092.4
Low density	2	5125.3	3119.3	13926.3
	3	5922.3	3804.7	15714.1
BT01	1	8876.5	3256.7	11610.8
High density	2	7656.7	2700.1	9992.1
	3	8392.3	3113.5	11035.4
BT01	1	8550.1	2387.2	10598.5
Low density	2	8322.9	2530.6	10794.5
	3	7780.1	2416.6	10924.4

9. The moisture content in brick tea (control, BT01) after fermented with *A. cristatus* at different fermentation stages in triplicate.

Table D3 The moisture content in brick tea after fermented

Fermentation stage	Replication	Weight of can (g)	Weight of can + wet samples (g)	Weight of can + dried samples after 20 h (g)	Moisture (%w/w)
Control	1	12.3222	13.3334	13.2669	6.58
High density	2	12.4311	13.4262	13.3691	5.74
	3	12.0160	13.0227	12.9542	6.80
BT01	1	12.1364	13.1415	13.0553	8.58
High density	2	12.4343	13.4274	13.3486	7.93
	3	12.8988	13.9049	13.8176	8.68

10. The ash content in brick tea (control, BT01) after fermented with *A. cristatus* at different fermentation stages in triplicate.

Table D4 The ash content in brick tea after fermented

Fermentation stage	Replication	Weight of crucible (g)	Weight of crucible + wet samples (g)	Weight of crucible + ash samples (g)	%Ash (wwb)
Control	1	38.0803	40.0889	38.1871	5.32
High density	2	35.4410	37.4461	35.5463	5.25
	3	36.0891	38.0900	36.1981	5.45
BT01	1	37.7172	39.7230	37.8231	5.28
High density	2	35.4605	37.4631	35.5668	5.31
	3	35.8466	37.8525	35.9517	5.24

11. The water activity in brick tea (control, BT01) after fermented with *A. cristatus* at different fermentation stages in triplicate.

Table D5 The water activity in brick tea after fermented

Fermentation stage	Replication	Water activity (aw)	Time (min)	Temperature (°C)	%TSS
Control	1	0.5235	3.51	25	0.4
High density	2	0.5260	6.56	25	0.4
	3	0.5216	12.07	25	0.6
BT01	1	0.5521	9.35	25	0.5
High density	2	0.5597	6.31	25	0.5
	3	0.5608	5.27	25	0.4

12. The antioxidant content in brick tea (control, BT01) after fermented with *A. cristatus* at different fermentation stages in triplicate.

Table D6 The antioxidant content in brick tea after fermented

Fermentation stage	Replication	Abs765	(Acontrol-Asample)/Acontrol	%scavenging activity
Control (1)	1	0.061	0.92	92.37
	2	0.070	0.91	91.24
	3	0.065	0.92	91.86
Control (2)	1	0.060	0.92	92.49
	2	0.056	0.93	92.99
	3	0.059	0.93	92.62
Control (3)	1	0.147	0.82	81.60
	2	0.140	0.82	82.48
	3	0.145	0.82	81.85
BT01 (1)	1	0.056	0.93	92.99
	2	0.061	0.92	92.37
	3	0.062	0.92	92.24
BT01 (2)	1	0.053	0.93	93.37
	2	0.053	0.93	93.37
	3	0.055	0.93	93.12
BT01 (3)	1	0.078	0.90	90.24
	2	0.074	0.91	90.74
	3	0.070	0.91	91.24

13. The color content in brick tea (control, BT01) after fermented with *A. cristatus* at different fermentation stages in triplicate.

Table D7 The color content in brick tea after fermented

Fermentation stage	Replication	*L**	*a**	*b**
Control (1)	1	35.56	2.01	5.56
	2	35.56	2.02	5.45
	3	35.56	2.07	5.46
Control (2)	1	36.74	1.16	5.74
	2	36.72	1.14	5.77
	3	36.72	1.15	5.74
Control (3)	1	37.04	0.83	5.60
	2	37.01	0.88	5.54
	3	37.01	0.81	5.60
BT01 (1)	1	34.10	3.06	3.98
	2	34.10	3.07	3.98
	3	34.12	3.04	3.92
BT01 (2)	1	34.81	2.47	4.65
	2	34.78	2.55	4.60
	3	34.78	2.55	4.58
BT01 (3)	1	34.25	2.76	4.29
	2	34.25	2.78	4.27
	3	34.25	0.91	91.24

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