# Effects of Mixed Yeast Fermentation on Volatile Compounds Composition of Arabica Coffee Beans

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**ABSTRACT**

Coffee is part of the most highly valued agricultural commodities, and fermentation is an alternative method to enhance the quality of coffee beans. Therefore, this study aimed to assess the effects of *Wickerhamomyces anomalus* and *Kluyveromyces lactis* on the fermentation of Arabica coffee, particularly the contributions to volatile compounds formed in roasted beans. The fermentation process was further carried out by incorporating *W. anomalus* and *K. lactis* for 48 hours at room temperature. The results showed that fermenting for 12 hours with mixed yeast inoculation significantly increased the total yeast count and volatile compounds. Additionally, the fermentation of Arabica coffee with mixed yeast inoculation at a 1:1 ratio produced the highest total titratable acidity and yeast count. The release of volatile compounds varied based on the activity of the microorganisms with the highest concentrations of naphthalene, α-himachalene, toluene 2, 4-diamine, and 3-pentanol detected in the samples. These results suggested that fermenting Arabica coffee with *W. anomalus* and *K. lactis* not only enhanced bean quality but also held promise for industrial application.

**Keywords**: Arabica coffee; fermentation; *Kluyveromyces lactis*; *Wickerhamomyces anomalus*

## INTRODUCTION

Coffee is part of the most highly valued agricultural commodities worldwide enjoying high demand and consumption in the global market. Coffee beans contain numerous chemical compounds including organic acids, sugars, heterocyclic compounds, and polyphenols (Bressani et al., 2020). The quality is further dependent on various factors such as environmental conditions, harvesting methods, processing methods, storage, and the degree of roasting (Elhalis et al., 2020). Among these processing methods, fermentation plays a crucial role in influencing coffee flavors by removing the pectin-containing mucilage from the parchment, thereby influencing the final quality of coffee. Typically, coffee fermentation occurs spontaneously due to the naturally occurring microorganisms on coffee beans, including yeast and lactic acid bacteria (LAB). However, external microbes have been added in earlier studies and in-farm conditions to generate coffee beans with unique flavor compounds as well as higher quality. Microorganisms, specifically yeast and bacteria, are present in coffee beans and produce enzymes (pectinolytic), acids, and alcohol to degrade mucilage (Haile and Kang, 2019). Yeast and LAB are also known to generate secondary metabolites such as higher aldehydes, ketones,

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esters, and alcohols which can permeate beans and improve the flavor of coffee. During fermentation, microorganisms generate secondary metabolites-such as higher aldehydes, ketones, esters, and alcohols—that permeate beans and enhance coffee's flavor (Elhalis et al., 2021b).

Several microorganisms have been identified during the fermentation process including *Saccharomyces cerevisiae*, *Pichia*, *Candida*, *Torulaspora, Wickerhamomyces anomalus,* and *Kluyveromyces lactis*. These microbes influence the chemical composition of beans by producing volatile and organic compounds that contribute to the flavor and aroma of the final product (Elhalis et al., 2020). For example, *W. anomalus* produces organic acids and metabolites by fermenting mucilage, using the product as a source of carbon and nitrogen. This process leads to a decrease in pH and the creation of distinctive flavors and aromas, enhancing the overall quality of coffee beverage (Mahingsapun et al., 2022; Krajangsang et al., 2022). The ethyl acetate produced by *W. anomalus* can also improve product quality including making Chinese baiju and generating antioxidants during the fermentation (Fan et al., 2019). Furthermore, *K. lactis* produces various volatile compounds to enhance flavor in fermentation and potential attractants in monitoring and control systems against insects in the production of Chinese daqu. The addition of *K. lactis* can inhibit the growth of fungi during coffee bean fermentation (Yang et al., 2017). Although *K. lactis* is primarily used in the dairy industry, for example, in infant formula to mimic the molecular sizes and prebiotic functions of human milk oligosaccharides (Yin et al., 2017) and in blue cheese production through its ester and alcohol production (Price et al., 2014)—its application in coffee fermentation is promising.

Both *W. anomalus* and *K. lactis,* also identified as *Candida sphaerica* and *Candida pelliculosa* have exhibited pectinolytic activity and show great potential as control agents against ochratoxigenic fungi. These fungi are significant contaminants during coffee fermentation and represent a significant market barrier in tropical countries (Celestine, 2017). Although indigenous yeast has shown potential in controlling these fungi, no study has investigated the combined use of *W. anomalus* and *K. lactis* in fermenting Arabica coffee. Fermentations using mixed microbial cultures can significantly influence the physical and chemical characteristics, flavor, aroma, as well as the overall sensory quality of coffee while pure cultures typically produce specific compounds (Darwin et al., 2021). Therefore, this study aimed to determine the microbiological profile, pH, total, and chlorogenic acid content, as well as volatile compound composition during coffee fermentation with the addition of indigenous yeasts *K. lactis* and *W. anomalus.*

## METHODS

### Materials

The media used in this study included peptone glucose yeast (PGY, Merck), Malt Extract Agar (MEA), de Man media, as well as Rogosa and Shape Agar (MRSA). The chemicals namely Plate Count Agar (PCA), phenolphthalein (PP) indicator, and NaOH were obtained from the Laboratory of Biotechnology of the Faculty of Agricultural Technology at Universitas Gadjah Mada. The sample Arabica coffee beans (*Coffea arabica*) were obtained from Temanggung Regency, Central Java. Coffee beans were de-pulped and preserved in hermetic packaging made of PVC plastic and sacks to generate a low-oxygen condition during transportation to the Laboratory of Biotechnology for the fermentation process. Following to Martinez et al. (2019) protocol, the yeast cultures used in this study were obtained from the preserved *W. anomalus and K. lactis* in the Laboratory of Biotechnology. The inoculum was then rejuvenated on an agar slant of peptone glucose yeast extract (PGY, Merck) and incubated at 27°C for 24 h. Subsequently, yeast was cultivated in PGY broth at 28°C for 48 h until reaching a concentration of $10^9$ CFU/mL. The equipment used in this study included a cabinet dryer (Model UN55, Memmert, Germany), a Roaster (Model Probatino, Probat, Germany), a 0.22 m Nylon syringe filter, an HPLC system, and a GC Agilent 7890A model equipped with an Agilent 5975C XL EI/CI mass spectrometry (MS) and a silica capillary DB-Wax column (30m x 0.25 mm x 0.25 mm).

### Coffee Fermentation

According to Kwak et al. (2018), wet fermentation was used in this study with several modifications. A portion of 100 g de-pulped coffee beans was mixed with 200 mL water (a 1:2 ratio). De-pulped coffee beans were divided into several groups, namely a control group without the addition of yeast, groups with the addition of a single culture of *W. anomalus* or *K. lactis*, and groups with the addition of mixture of *W. anomalus* and *K. Lactis* at ratio of 1:1, 1:2, and 1:3. The inoculum was added at the concentration of $3x10^7$ CFU/mL with the fermentation conducted for 48 h. Samples were taken every 12 h (from 0 to 48 h) for microbial evaluation, pH measurement, and titratable acid analysis.

### Coffee Drying and Roasting

The green coffee beans were separated from the medium and spread on a tray, which was placed in a cabinet dryer at 60°C for 16 h. Subsequently, the green coffee beans were transferred to a roaster and roasted

for 10–15 min at 200°C until a medium roast level was achieved. The dried and roasted samples were further subjected to chlorogenic acid determination.

### Total Microbial Evaluation

Microbiological analysis was performed using the pour plate method according to Avallone et al., (2001) and Nasanit & Satayawut (2015) in duplicate. Three types of media were used namely (1) Malt Extract Agar (MEA) to enumerate yeast, (2) de Man media and Rogosa & Shape Agar (MRSA) to enumerate and analyze the total growth of LAB, and (3) Plate Count Agar (PCA) to enumerate the total aerobic microorganisms during fermentation. The plates were inverted and incubated at room temperature of 25-27 °C for 48 h. For the analysis of LAB, colonies forming a clear zone on the media were counted. Microbial activity was assessed every 12 h, starting at 0 h and continuing until 48 h with evaluations conducted at 0, 12, 24, and 48 h.

### pH and Total Titratable Acidity Analysis

Analysis of pH method was determined according to AOAC (1995). The total titratable acidity analysis was determined based on Sinaga et al. (2021) with several modifications. A total of 0.5 g ground fermented coffee beans was mixed with 50 mL distilled water and stirred to obtain a 1% (w/v) coffee solution. The solution was filtered using filter paper, and 5 mL filtrate was mixed with 3 drops of a 1% phenolphthalein (PP) indicator. The samples were titrated with 0.1 N NaOH solution until a pink color appeared.

### Chlorogenic Acid Content

The samples in this study included dried green coffee beans and roasted coffee beans that had been fermented for different durations. Dried and roasted coffee beans were ground using a blender. A total of 5 g ground coffee beans was mixed with 50 mL heated distilled water to obtain a 1% coffee solution. The solution was stirred for 5 min to achieve homogeneity. A 2 mL aliquot of the homogeneous solution was extracted using a syringe, and 1.5 mL was injected into a coded vial through a 0.22 µm Nylon syringe filter. The vial was then injected into High Performance Liquid Chromatography (HPLC) system, and chlorogenic acid content was determined from the resulting chromatogram.

### Volatile Compounds

The analysis of volatile compounds in coffee beans was conducted using Gas Chromatography-Mass Spectrometry (GC-MS) method, following the procedure outlined by Bressani et al., (2018). The compounds were examined using a GC Agilent 7890A system equipped with an Agilent 5975C XL EI/CI mass spectrometer and a DB-Wax silica capillary column (30 m x 0.25 mm x 0.25 mm). Samples for volatile compound analysis included the control group, coffee beans with the addition of a single culture of *W. anomalus* or *K. lactis*, and coffee beans with the addition of mixture of *W. anomalus* and *K. lactis* at a ratio of 1:1. The samples were injected in spitless mode at 250°C and analyzed. Helium served as carrier gas with the temperature maintained at 50°C for 5 min before being increased. Volatile compounds were identified by comparing their mass spectra with the NIST14 library. Additionally, an alkane series ($C_{10}$–$C_{40}$) was used to calculate the retention index (RI) for each compound, which was further compared with RI values reported in the literature.

### Statistical Analysis

The pH, total titrated acid, and chlorogenic acid content in the fermented coffee beans were analyzed using SPSS (IBM Statistic ver. 25) with a one-way analysis of variance (ANOVA), considering the statistically significant differences ($p$<0,05). Duncan's test and paired samples t-test were used to determine significant differences ($p$<0,05) between treatments. The results were expressed as means ± standard deviation (SD) of duplicate analyses unless otherwise stated.

## RESULTS AND DISCUSSION

### Total Microbial Evaluation

The effect of fermenting coffee using *W. anomalus*, *K. lactis*, and mixed yeasts at different ratios on total microbial counts was shown in Figure 1. The growth dynamics, metabolic activities, and interactions among microorganisms in food affected the quality and safety of the final product. During fermentation, LAB converted sugars into lactic acid which led to a decrease in pH. In addition to producing organic acids, LAB generated bioactive substances that contributed to the nutritional value and organoleptic properties of coffee (Šipošová et al., 2021). LAB activity on *W. anomalus* showed during the first hour of fermentation was 6.87 log CFU/mL which further grew to 9. 18 log CFU/mL in 48 h of fermentation. LAB on *K. lactis* sample was also 6.50 log CFU/mL at initial observation and increased to a maximum population of 8.61 log CFU/mL in 48 h of fermentation.

Meanwhile, in the sample with mixed yeast ratio of 1:1, LAB count was 6.13 log CFU/mL in the first hour and subsequently increased to 8.50 log CFU/mL after 48 h. In mixed yeast sample with a ratio of 1:2, the count was 6.80 log CFU/mL initially and increased to

8.15 log CFU/mL by the end of fermentation. For mixed yeast sample with a ratio of 1:3, the initial count was 6.96 log CFU/mL, and it increased to 8.63 log CFU/mL in 48 h. The influence of different yeast mixture ratios on the growth of LAB varied depending on the specific interactions between yeast strains and bacteria. In some cases, higher yeast ratios supported greater LAB growth because yeast produced compounds such as organic acids or specific nutrients. Conversely, yeast could also compete with LAB for resources or even produce antimicrobial compounds that inhibited bacterial growth (De Vuyst & Leroy, 2020).

Figure 1(B) showed that the growth rate of LAB fluctuated over time. These fluctuations might have been caused by regrowth after a decline in the bacterial population. According to Azizah et al. (2019), bacteria might have regenerated due to nutrients released from dead cells. The released compounds, including

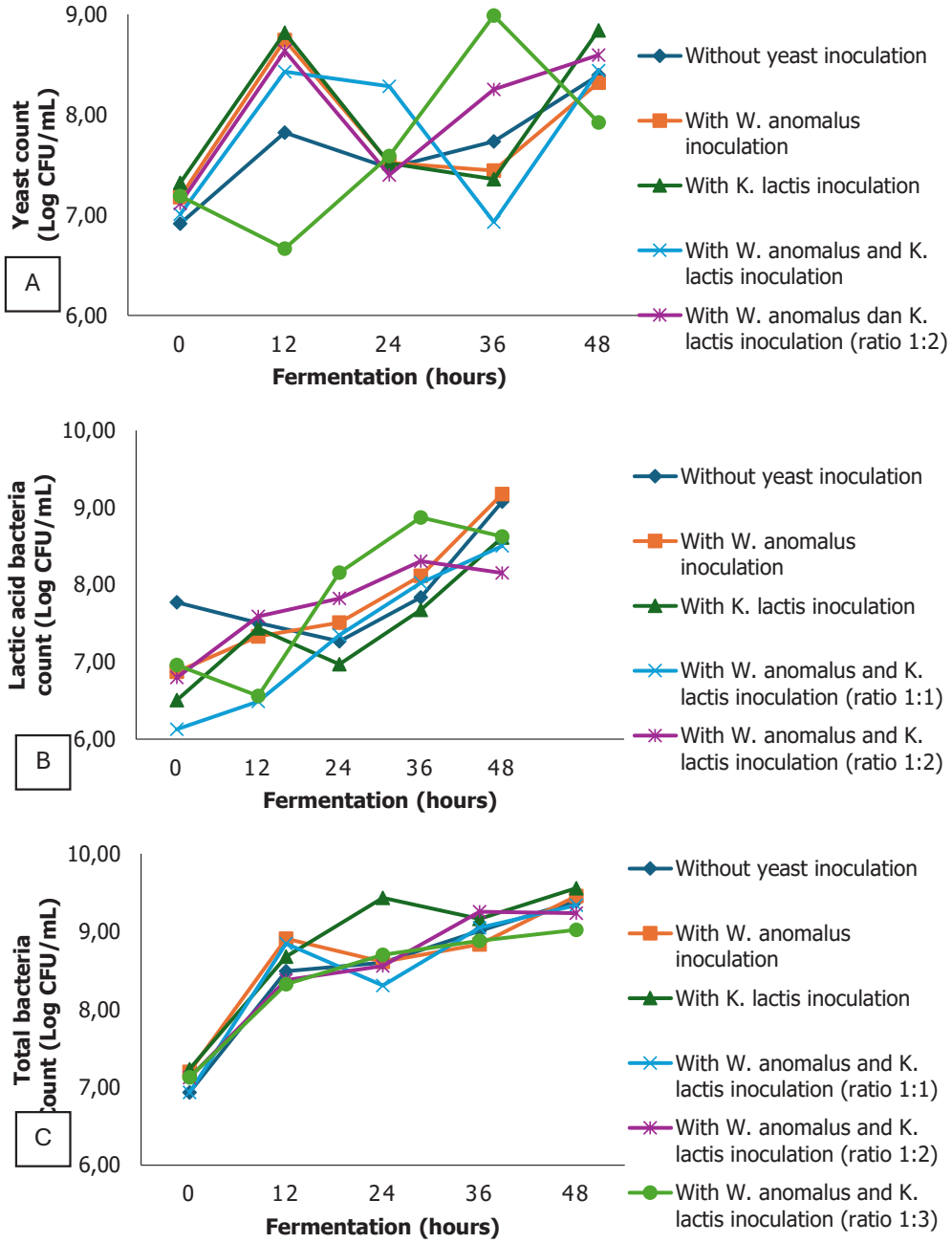


Figure 1. Microbial evaluation on total yeast count (A), LAB (b), and total plate count (c) on coffee beans after 48 h fermentation: without addition of culture (♦), with addition of *W. anomalus* (■), *K. lactis* (▲), and mixed yeast at different ratios

proteins and vesicles, could have contributed to biofilm formation and served as nutrients for microbial growth (Smakman & Hall, 2022). Lactic acid was produced by the naturally occurring population of LAB in coffee beans. Fermentation under low oxygen conditions promoted the growth of both yeast and LAB, which in turn increased lactic acid production and coffee acidity (Bressani et al., 2020). In a previous study by Elhalis et al. (2020), the population of *Leuconostoc mesentroides* and *Lactococcus* reached 5 and 4.7 log CFU/g respectively after 36 h of fermentation.

The fermentation process using yeast was also evaluated by measuring the total yeast population. Figure 1(A) showed that most samples reached their highest yeast populations after 12 h of fermentation. According to Elhalis et al. (2021a), yeast consumed a significant amount of sugar from the mucilage, which increased their populations. The consumption of sugar in the endosperm during wet fermentation allowed yeast to thrive and form numerous colonies. In a previous study by Elhalis et al. (2020), yeast population during the wet fermentation of coffee beans consistently increased with *Hanseniaspora uvarum* evolving as the dominant species. *Hanseniaspora uvarum* and *W. anomalus* reached 5.2 and 2 log CFU/g, respectively, after 36 h of fermentation.

Total plate count (TPC) was performed to determine the number of bacteria present in fermentation samples. Figure 1(C) showed that the total bacterial count increased in all samples over the 48-h fermentation period. Microorganisms such as bacteria, LAB, and yeast played a dominant role in fermentation process by producing organic acids, proteolytic enzymes, and pectinolytic enzymes. The production of lactic acid was associated with the decrease in pH observed during fermentation (Elhalis et al., 2020).

## pH and Total Titratable Acidity

The effects of fermenting coffee with *W. anomalus*, *K. lactis*, and mixed yeasts at different ratios of pH and total titratable acidity were shown in Figure 2. pH value was measured every 12 h over the 48 h fermentation period. Figure 2 showed that pH of coffee steadily decreased over time. The variation in the final pH for each treatment was attributed to the differing abilities of the inoculants during fermentation. This process converted glucose and fructose into lactic acid, an indicator of fermentation success (Wang et

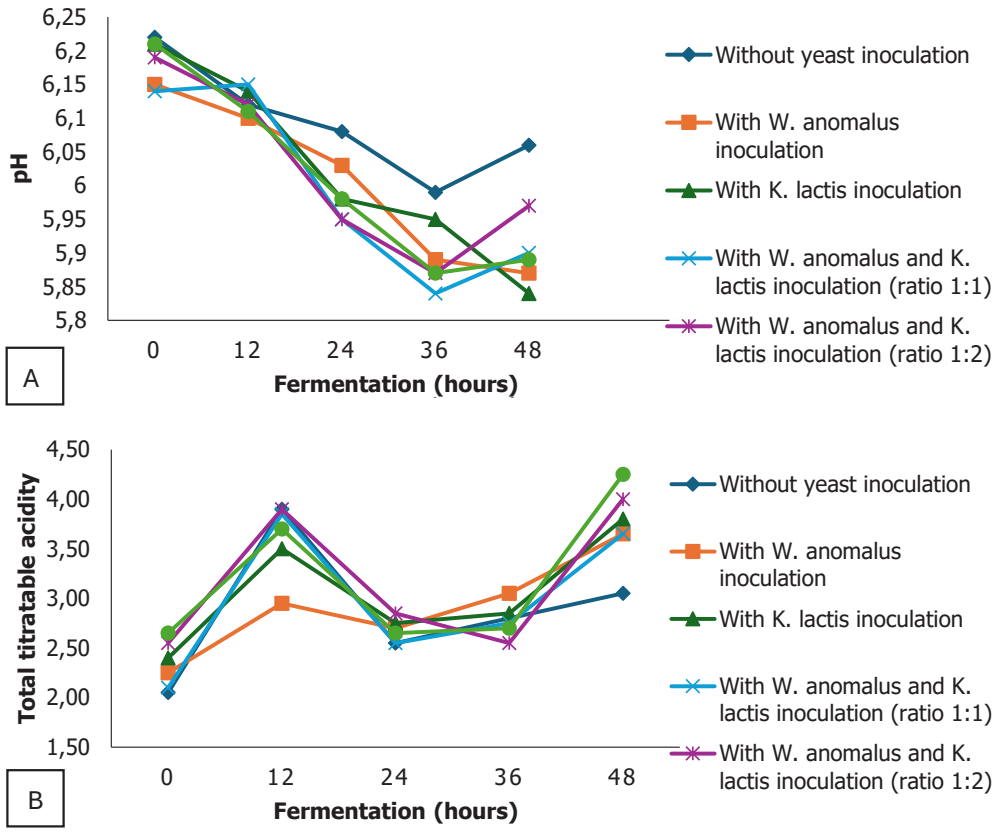


Figure 2. The effect of fermentation coffee using *W. anomalus* (■), *K. lactis* (▲), and mixed yeast at different ratios of pH (A) and total titratable acidity (B) over 48 h of fermentation

al., 2020a). Although fermentation with pure cultures typically produced a specific compound, fermentations using mixed microbial cultures considerably influenced coffee quality, including its physical and chemical characteristics, flavors, aroma, and overall sensory quality (Darwin et al., 2021). Changes in pH during fermentation were closely associated with the production of organic acids, as well as with the absorption of amino acids, which significantly affected pH levels (Haile and Kang, 2019). pH data obtained in this study were similar to those reported by Galarza and Figueroa (2022) who obtained pH values of 5.7± 0.1 and pH 5.6 by Wang et al. (2020).

A negative correlation was observed between total titratable acidity and pH which was shown in the increase in titratable acidity and the decrease of pH. The total titratable acidity was measured to quantify the amount of acetic acid generated by yeast during fermentation. Figure 2 showed that most samples exhibited the highest acid levels after 12 h of fermentation. The starch present in coffee beans was broken down into glucose during fermentation, which was then converted into lactic acid. Fermentation of the mucilage by bacteria and yeast produced ethanol and organic acids such as succinic, malic, citric, and acetic acids, leading to a decrease in pH (Azizah et al., 2019). The total titratable acidity data obtained in this study were like those reported by Elhalis et al. (2020) who found that the total acidity of wet fermented coffee was 1.70 (mg/g).

## Chlorogenic Acid Content

The effect of fermenting coffee using *W. anomalus*, *K. lactis*, and mixed yeasts at different ratios on chlorogenic acid content was shown in Figure 3. Chlorogenic acid is an essential component in coffee due to the antioxidant properties, as it belongs to the phenol group (Sinaga et al., 2021). During fermentation, chlorogenic acid was hydrolyzed into quinic acid and phenolic acids, such as caffeic acid. These phenolic acids further degraded into volatile phenolic compounds (Wang et al., 2020; Cassimiro et al., 2022). Figure 3 showed that green beans had a higher chlorogenic acid content than roasted beans. Specifically, after 48 h of fermentation using mixed yeast ratio of 1:1, chlorogenic

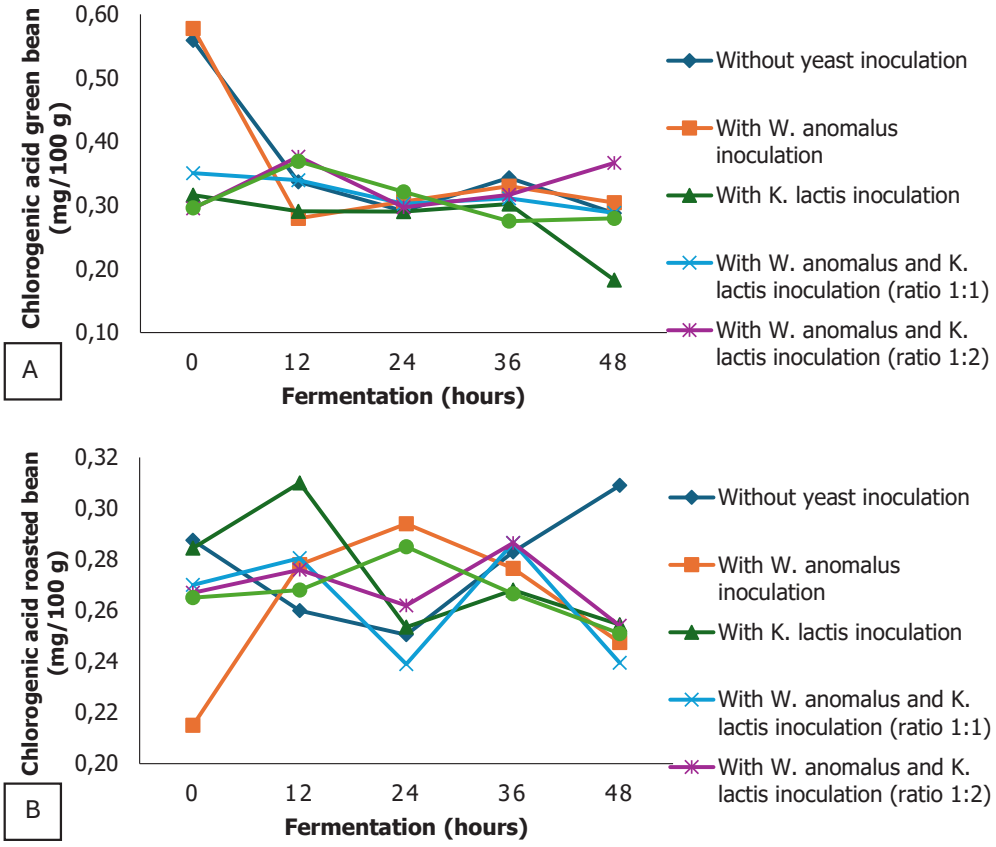


Figure 3. Chlorogenic acid content in green bean (A) and roasted bean (B) after yeast inoculation over 48 h of fermentation using *W. anomalus* (■), *K. lactis* (▲), and mixed yeast with different ratios

acid content in green beans was 0.29 mg/100 g, which was higher than roasted beans at 0.24 mg/100 g. Similarly, after 48 h of fermentation using mixed yeast ratios of 1:2 and 1:3, chlorogenic acid content in green beans was 0.37 mg/100 g and 0.28 mg/100 g, respectively. Chlorogenic acid content in roasted beans for the 1:2 and 1:3 yeast mixtures were 0.25 mg/100 g. The variation in chlorogenic acid levels was due to the influence of yeast mixture ratios, which could either increase or decrease chlorogenic acid content depending on several factors (Liang & Kitts, 2015). According to Pereira et al. (2014), the effect of different blending ratios on chlorogenic acid content in Arabica coffee varied depending on factors such as the type of yeast used, fermentation temperature, fermentation duration, and other environmental conditions. However, increasing the ratio of yeast mixtures used in fermentation could enhance chlorogenic acid content in coffee beans. This increase was attributed to the interaction between yeast and coffee beans during fermentation which influenced the production and degradation of various chemical compounds including chlorogenic acid.

The higher chlorogenic acid content in green beans compared to roasted beans was due to the thermal degradation of chlorogenic acid during roasting. Roasted beans underwent roasting at 200°C, which altered the chemical composition of coffee due to the Maillard reaction. The roasting process led to medium-roasted beans, which balanced the bitterness and acidity of coffee flavor (Preedy, 2015). Chlorogenic acid, an ester of trans-cinnamic acid and quinic acid, was degraded by microflora into various aromatic acids, such as coumaric acid, benzoic acid derivatives, and phenylpropionic acid. Chlorogenic acid content observed in this study was similar to the findings of Santosa et al. (2021), showing that chlorogenic acid levels in green beans was 12.70 ± 0.04 mg/g, higher than the roasted beans (10.60 mg/g). Additionally, a study by Cassimiro et al. (2022) also reported that chlorogenic acid content reached 12.79 ± 0.01 g/kg after 72 h of fermentation using *L. plantarum* and *S. cerevisiae*.

**Volatile Compounds**

Fermentation process possessed the most influence on the formation of coffee flavors among the processing methods (Santosa et al., 2021). The different concentrations of volatile compounds in fermented coffee using *W. anomalus*, *K. lactis*, and mixed yeast with a ratio of 1:1 was shown in Table 1. Mixed yeast ratio of 1:1 was selected because it had the highest total titratable acidity among the other ratios. A high-quality coffee flavor was characterized by a pleasant balance of flavor, body, and aroma, without defects such as over-fermentation, earthiness, or greasiness. The main coffee flavors included acidity, sweetness, astringency, and aroma (Santosa et al., 2021). These characteristics were analyzed through volatile compounds produced during coffee fermentation, such as sulfur compounds, aldehydes, organic acids, esters, and alcohols (Galarza & Figueroa, 2022).

During fermentation, yeast and LAB generated secondary metabolites, including higher alcohols, esters, ketones, and aldehydes, which diffused into coffee beans and enhanced their flavor. Simultaneously, endogenous plant enzymes degraded the macromolecules present in the mucilage attached to beans, increasing the release of compounds into fermentation water and significantly altering the chemical composition, thereby affecting coffee's flavor. The duration of fermentation played a crucial role in determining the composition of green coffee beans and the overall quality of the brewed coffee (Várady et al., 2022).

Furan was a highly volatile heterocyclic compound formed during coffee roasting process. Several precursors, including sucrose, glucose, linoleic acid, and linolenic acid, played a crucial role in the formation of furan in roasted coffee (Cassimiro et al., 2022). The formation of furan in roasted coffee originated from the caramelization of sugar in the Maillard reaction. Glucose and sugar polymers broke down into furanic compounds as primary decomposition products (Dippong et al., 2022). The most prevalent furan compound responsible for caramel flavors in coffee was 2-furanmethanol. The control samples contained the highest amount of 2-furanmethanol (11.73%), followed by *K. lactis* (10.94%). Volatile compound in furan was identified as 2-furanmethanol which possessed antioxidant properties and contributed caramel, sweet, and astringent notes to the flavors during spontaneous fermentation (Cassimiro et al., 2022). Furthermore, furfural (6.05−5.50%) was identified in high concentrations in all samples and was responsible for the sweet and woody flavors in coffee.

Pyrazine derivatives are ranked as the second most significant volatile compounds responsible for the distinctive aroma of coffee (18−24%). Schiff bases were formed through the condensation of carbonyl and amine groups in pyrazine derivatives. After roasting, the fermentation process reduced the alcohol (Cassimiro et al., 2022). The most abundant pyrazine compounds were methylpyrazine and 2,6-dimethylpyrazine, which contributed to the nutty flavor. Pyrazines provided nutty, roasted, corn, hazelnut, potato, or earthy aromas. Pyridine had bitter, fishy, or burnt odors depending on the pyrazine ring substituents (Dippong et al., 2022). The methylpyrazine content in *W. anomalus* samples

was 3.74%, in *K. lactis* samples was 4.13%, and in mixed yeast with a ratio of 1:1 was 4.36%. Meanwhile, the 2,6-dimethylpyrazine content in *W. anomalus* samples was 2.73%, in *K. lactis* samples was 3%, and in mixed yeast with a ratio of 1:1 was 2.75%. *K. lactis* sample had the highest levels of methylpyrazine and 2,6-dimethylpyrazine, which might have been related to pH changes, as *K. lactis* sample had the lowest pH among the samples. According to Elhalis et al. (2021b), during coffee roasting, pH decreased to facilitate the formation of pyrazines.

The highest concentration of acidic compound was detected in acetic acid in the control samples reaching 7.42%, followed by isovaleric acid which was predominantly detected in *W. anomalus* sample at 4.48%. Acetic acid contributed to the pungent and sour aroma of coffee while isovaleric acid contributed to the dairy and sour aroma. Previous studies found that acetic acid in roasted coffee was present at high concentration (7.3%). The temperature and duration of roasting influenced the development of coffee aroma (Dippong et al., 2022). Furthermore, the results also showed that the control sample had the highest percentage of acetic acid compared to the fermentation samples. This indicated that the microbial in the control sample consumed more sugar and produced more organic acids than in other samples during coffee fermentation.

Aldehydes and ketones were discovered to represent approximately 10 – 13% of volatile organic compounds in various fermentation treatment samples. Aldehydes produced during the breakdown of mucilage were essential precursors for the creation of aromatic compounds including alcohols and higher esters, through the action of alcohol dehydrogenase. Additionally, during apoptosis and diffusion events in coffee beans, several aldehydes with a vibrant aroma were released from yeast cells, contributing to fruity and floral notes in coffee beverages (Bressani et al., 2020). The main mechanism of ketones production in green beans was fatty acid oxidation. Ketones formed as a result of the Maillard reaction and caramelization during roasting. The self-oxidation of alcohol and the autoxidation of unsaturated fatty acids, through the decomposition of hydroperoxide intermediates, were also associated with the production of volatile ketones and aldehydes (Zakidou et al., 2021). The most abundant volatile compounds found were isovaleraldehyde and acetaldehyde-hydroxy, which were responsible for the fruity flavors of coffee. The highest concentrations of isovaleraldehyde (1.88%) and acetaldehyde-hydroxy (2.43%) were found in *K. lactis* samples.

The percentage of 3-pentanol in control samples was the lowest compared to other samples during fermentation, possibly due to the fermentation and demucilaging process caused by microbial activity. The 3-pentanol in the control sample was not detected, while the 3-pentanol levels in *W. anomalus*, *K. lactis*, and mixed yeast with a ratio of 1:1 was 0.16%, 0.17%, and 0.17%, respectively. Ethanol was found in high amounts in all samples, specifically in the control sample, and contributed to the alcoholic flavor of coffee. Benzyl alcohol was also found in fermentation and was produced by microorganisms. It participated in esterification reactions between alcohols and fatty acids, contributing to the formation of coffee's ester-related aroma. Additionally, LAB produced it through alternative pathways of pyruvate degradation, adding sweet and floral notes to the fermentation process (Cassimiro et al., 2022).

Ester compounds which are prevalent in coffee, were produced through esterification, fermentation, acidosis, and transesterification. The production of ester compounds was frequently linked to microbial development during the early stages of fermentation (Cassimiro et al., 2022). The 2-furfuryl-acetate was the most prevalent ester compound detected in the fermentation process. Mixed yeast (1:1) sample had the highest 2-furfuryl-acetate with a percentage of 1.77% and was responsible for fruity and banana flavor in coffee. Ester compounds played a role in creating floral and fruity scents in alcoholic beverages (de Melo Pereira et al., 2019).

Pyrroles compounds in coffee contributed to its aroma and taste as it was created by the interaction of aldoses with alkylamines, as well as by the condensation of glucose with alanine, proline, or hydroxyproline (Dippong et al., 2022). The most prevalent pyrrole compound detected was 2-formyl-1-methylpyrrole, which contributed to roasted and nutty flavors. Mixed inoculant sample had the highest concentration of 2-formyl-1-methylpyrrole, at 0.55%. Terpene compounds in coffee fermented using *W. anomalus* and *K. lactis* contributed to its aroma and flavor. The terpenes produced during coffee mucilage removal process originated from glycoside precursors through yeast *β-glucosidase* enzymes. Additionally, certain yeast species such as *Saccharomyces cerevisiae* generated terpene derivatives through the mevalonic acid pathway (de Melo Pereira et al., 2019).

Pyridine compounds in coffee were decomposition products of trigonelline, an alkaloid compound commonly found in green beans (Caporaso et al., 2018). Green coffee contained high concentrations of nitrogen-containing compounds such as alkaloids, proteins, and volatile acids. During the roasting process, these chemicals degraded and produced flavor-active metabolites such

Table 1. Main volatile compounds in yeast fermented coffee analyzed with GC-MS

| No | Compound | K (%) | *WA* (%) | *KL* (%) | WA: KL (1:1) (%) | Aroma description |
|---|---|---|---|---|---|---|
| 1 | Acetic acid | 7.42±0.25 | 6.28±1.32 | 6.20±0.86 | 5.59±0.38 | Vinegar, sour |
| 2 | Isovaleric acid | 3.87±0,1 | 4.48±0.31 | 4.26±0.15 | 4.16±0.50 | Dairy, sour |
| 3 | Methylpyrazine | 3.85±0.03 | 3.74±0.52 | 4.13±0.33 | 4.36±0.49 | Nutty, roasty |
| 4 | 2,6-Dimethylpyrazine | 2.52±0.22 | 2.73±0.63 | 3.00±0.5 | 2.75±0.27 | Nutty, earthy |
| 5 | 2-Furanmethanol | 11.73±0.78 | 9.41±1.70 | 10.94±1.40 | 9.95±0.36 | Caramel |
| 6 | Furfural | 6.05±0.51 | 5.50±0.22 | 5.80±0.30 | 5.59±0.03 | Sweet, woody |
| 7 | Isovaleraldehyde | 1.78±0.06 | 1.82±0.04 | 1.88±0.02 | 1.86±0.02 | Fruity, cherry |
| 8 | Hydroxy-acetaldehyde | 2.09±0.24 | 1.59±0.44 | 2.43±0.28 | 2.23±0.07 | Corn, nutty |
| 9 | Ethanol | 0.83±0.26 | 0.48±0.19 | 0.82±0.15 | 0.72±0.08 | Alcoholic |
| 10 | 3-Pentanol | nd[a] | 0.16±0.01[b] | 0.17±0.01[b] | 0.17±0.02[b] | Fruity |
| 11 | Benzyl alcohol | 0.38±0.06 | 0.43±0.06 | 0.58±0.06 | 0.54±0.11 | Sweet, flowery |
| 12 | 2-Furfuryl-acetate | 1.61±0.13 | 1.70±0.32 | 1.63±0.20 | 1.77±0.02 | Fruity, banana |
| 13 | 2-Formyl-1-methylpyrrole | 0.47±0.07 | 0.51±0.06 | 0.49±0.01 | 0.50±0.0 | Roasted, nutty |
| 14 | 2-Acetonitrile, 1- methylpyrrole | 0.39±0.04 | 0.46±0.05 | 0.42±0.01 | 0.41±0.03 | Smoky, woody |
| 15 | Toluene-2,4-diamine | 1.06±1.31 | 2.13±0.35 | 2.08±0.11 | 2.16±0.06 | Floral, woody |
| 16 | α-Himachalene | 0.11±0.02[a] | 1.99±2.45[ab] | 3.69±0.12b | 3.77±0.05[b] | Sweet |
| 17 | Naphthalene | 1.60±1.09[a] | 1.77±0.10[ab] | 1.91±0.15b | 2.02±0.01[b] | Earthy |

*K: control, WA: Wickerhamomyces anomalus, KL: Kluyveromyces lactis, WA:KL:Mixed W. anomalus* and *K. Lactis* with a ratio of 1:1
Notes: Different letters in the same row are significantly different ($p$<0.05)

as pyridines and pyrroles (de Melo Pereira et al., 2019). The main amine detected in the fermentation was toluene-2,4-diamine pyrazinamide, which was found in mixed inoculant sample at 2.24% and contributed to the sweet aroma of coffee. Additionally, fermentation with microorganisms increased organic compound production. In control samples, *α*-himachalene and naphthalene were detected at 0% and 1.60%, respectively. Other treatments showed an increase in *α*-himachalene and naphthalene during fermentation with levels in mixed yeast samples reaching 3.77% and 2.02%, respectively. *α*-himachalene contributed to citrus and woody aromas in coffee while naphthalene was responsible for sweet and citrus flavors. The development of volatile compounds was influenced by the stability of the precursors and the position in the seed. The fermentation process played a significant role in creating favorable qualities in coffee. Fermentation decreased alcohol content due to microbial activity and its conversion into other organic compounds (Galarza & Figueroa, 2022).

During fermentation process, some volatile compounds were broken down while others were produced through the metabolism of aroma precursors such as pyrazines, thiols, furanones, and guaiacols which contributed to a distinctive coffee aroma during roasting (Haile & Kang, 2019). The concentration of volatile compounds in coffee beans changed due to factors such as bean composition, the thermal profile of the roasting process, plant genetics, post-harvest processes, environmental conditions, the presence of defective beans, and the stage of maturation. The roasting process significantly impacted volatile compounds with time and temperature profiles affecting aroma composition of coffee, the extraction efficiency of each compound, and the brewing method used (Caporaso et al., 2018). Fermentation time could increase the concentration of acidic compounds. However, extended fermentation periods led to an overall decline in volatile compound concentrations due to enzymatic activity limitations, which reduced aroma precursors such as pyrazines and furfurans (Galarza & Figueroa, 2022).

## CONCLUSION

In conclusion, mixed yeast *W. anomalus* and *K. lactis* at a ratio of 1:1 had the highest total titratable acidity and total yeast count compared to the other treatments. The addition of yeast inoculation did not have a significant effect on pH level during coffee fermentation. In this study, fermenting coffee beans for 12 h led to the optimal fermentation duration as evidenced by the highest levels of microbiological activity, pH, and total titratable acidity. Chlorogenic acid content in green beans was higher than in roasted beans. The release of volatile compounds varies based on microbial activity. The most prevalent volatile compounds found in all sample groups were 2-furanmethanol, acetic acid, and furfural. However, when comparing volatile compounds in coffee fermented with *W. anomalus* and *K. lactis* at a 1:1 ratio to other sample groups, it was observed that naphthalene, *α*-himachalene, toluene-2,4-diamine, and 3-pentanol were significantly higher in mixed yeast (1:1) group than in the other groups.

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## CONFLICT OF INTEREST

Declare whether there is conflict of interest or not, regarding the publication of the article.

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