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MICROBIOLOGICAL AND CHEMICAL PROFILING OF LACTO-FERMENTED SHIMEJI MUSHROOM (*Hypsizygus* sp.) PICKLE JUICE USING *Lactobacillus bulgaricus* AS A STARTER CULTURE

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ABSTRACT

Fermentation is a way to preserve food, extend its shelf life and improve its nutritional and sensory qualities. However, the use of probiotic strains in mushroom bio-preservation has been rarely investigated. A probiotic such as *Lactobacillus bulgaricus* is commonly used as a starter for fermented food and dairy products. This study evaluated the microbiological, chemical and sensory quality of the pickled juice of shimeji mushroom (*Hypsizygus* sp.) fermented using *L. bulgaricus* FNCC 0041 for 18 days and identified the volatile compounds. The microbiological profiles of lactic acid bacteria (LABs), yeast, molds and Enterobacteriaceae in the pickle juice were determined through plate counting, and the chemical profile was determined by analysing total titratable acidity, pH, nitrite content, and volatile compounds. Moreover, the sensory characteristics of the final products were evaluated according to colour, texture, aroma and overall appearance. Results showed that LABs were dominant in the mushroom pickle juice, whereas yeast and Enterobacteriaceae decreased but exceeded the microbiological quality limit. The pH of the fermented shimeji mushroom was 3.72, and the total lactic acid content increased from 0.18% to 0.32%. The nitrite concentrations of the final products decreased and were lower than the maximum limit for nitrite content in fermented food (0.42 mg/kg) on the last day of fermentation. Aromatic hydrocarbons, alkanes, alkenes, alcohols, organic acids, ketones, aldehydes, and fatty acids, such as *o*-xylene (27.880%), *p*-xylene (5.890%), styrene (0.56%), and mesitylene (0.23%), were found as major volatile compounds. The sensory characteristics of fermented shimeji were acceptable. The study suggests that fermentation using *L. bulgaricus* can be further developed into a bio-preservation method for preserving shimeji mushrooms.

Keywords: Fermentation, lactic acid bacteria *Lactobacillus bulgaricus,* mushroom, shimeji

INTRODUCTION

Food fermentation is a traditional method for preserving food and involves the use of desirable microorganisms and enzymatic processes. The process can occur spontaneously or can be induced with a starter culture, offering several advantages such as increased shelf life, enhanced nutrition, improved sensory qualities and health benefits (**Mudoor Sooresh *et al.*, 2023; Zapaśnik *et al.*, 2022**). Lactic acid fermentation is a common preservation method in Asia, Eastern Europe and Africa and usually used in preserving raw materials, such as fruits, vegetables, fish, dairy and even mushrooms, and producing pickles, kimchi, sauerkraut or fermented olive (**Manowan *et al.*, 2020**). However, spontaneous fermentation is time-consuming, carries a high risk of failure, is inefficient and yields products of variable quality (**Jabłońska-Ryś *et al.*, 2022; Zapaśnik *et al.*, 2022**).
The application of lactic acid bacteria (LABs) as staters for non-spontaneous lactic acid fermentation can yield more consistent product quality and safety than spontaneous fermentation (**Ayivi *et al.*, 2020; Manowan *et al*,. 2020**). Using LABs as starters have several benefits, including enhanced food nutrition, shortened fermentation period and improved organoleptic quality consistency and microbial safety (**Zheng *et al.*, 2018**). LAB species ensure the safety of food by preventing the spread of microbes that cause unwanted spoiling or harmful effects **(Aziz *et al.*, 2023[a])**. Moreover, *Lactiplantibacillus planctarum* K25, which is used to ferment food products, such as kimchi and sauerkraut, has been linked to the treatment of inflammatory bowel disease, prevention of cancer, maintenance of gut health, immune system strengthening, lowered cholesterol level and angiotensin I-converting enzyme production (**Aziz *et al.*, 2023[b]**; **Aziz *et al.*, 2024**).
LABs can produce antimicrobial compounds, sugar polymers, sweeteners and vitamins and act as probiotics (**Speranza *et al.*, 2017**), converting polyunsaturated fatty acids, including linoleic acid (LA), into bioactive compounds and other fatty acid metabolites that are not toxic to the bacteria themselves. Some strains of LABs, such as *Lactiplantibacillus plantarum* YW11, *L. plantarum* K25, *L. plantarum* 12–3 and *L. plantarum* 13–3, are important because of their role as probiotics that convert LA into conjugated linoleic acid (CLA) (**Aziz *et al.*, 2022[a]**). In addition, food safety bacteriocins were found in the *L. plantarum* 13-3 genome, including cyclic lactone autoinducers, terpenes, T3PKS and RiPP-like compounds (**Aziz *et al.*, 2022[b]**). Streptin and ruminococcin-A were found in *L. plantarum* YW11 as antimicrobial agents (**Aziz *et al.*, 2023[c]**). Eight fatty acid metabolites of *L. plantarum* YW11 were identified, ncluding ethyl oleate, (Z)-ethyl heptadec-9-enoate, butyl 9,12-octadecadienoate, *trans*/*trans*-9,12-octadecadienoic acid propyl ester, octadec-9-enoic acid, (Z)-18-octadec-9- enolide, *cis*-11,14-eicosadienoic acid methyl ester and (Z-Z)-9,12-octadecadien-1-ol, which also effectively convert LA into CLA (rumenic acid) and other metabolites in a dose-dependent manner (**Aziz *et al.*, 2021**).
Edible mushrooms have been used as food and medicines, offering unique sensory characteristics, such as flavour, fragrance, texture and high nutritional content and containing numerous bioactive compounds. Thus, they are commonly utilised as functional food, dietary supplements and traditional medicines (**Ogidi & Agbaje, 2021**). However, fresh mushrooms are highly perishable because they have high water content, respiration rate and enzyme activity and contain microorganisms. Additionally, the quality of mushrooms deteriorates over time because of their short shelf lives after harvest (**Liu *et al.*, 2016; Boylu *et al.*, 2023**).
Canning and salting are the common methods for mushroom preservation but can alter chemical and physical properties, such as colour, flavour and nutritional content (**Liu *et al.*, 2016**). Moreover, blanching or rapid heating, which should be applied to raw materials, impedes the spontaneous fermentation of mushrooms. Thus, starter microorganisms are required to induce fermentation, which can be obtained from various sources, such as local microflora, or can be isolated from raw materials (**Jabłońska-Ryś *et al.*, 2022**).
In several European and Asian countries, fermentation is an economical and practical way to preserve mushrooms. Many species of mushrooms, including *Termitomyces robustus*, *Agaricus bisporus*, *Boletus edulis*, *Auricularia auricularia*, *Cantharellus cibarus*, *Russula* spp., *Leccinum* spp. and *Pleurotus* spp., have been fermented (**Jabłońska-Ryś *et al.*, 2019**). LABs can can increase the flavour and nutrient content of shiitake mushrooms (*Lentinus edodes*; **Nie *et al.*, 2023**). *Lactobacillus* is one of the commonly used LABs as a starter for fermenting vegetables, cheese and meat. *Lactobacillus bulgaricus* is widely used as fermentation starter for the production of dairy products, such as yogurt (**Ayivi *et al.*, 2020**). However, study on the use of *Lactobacillus* as a starter in mushroom fermentation is limited (**Liu *et al.*, 2016**).
Liu *et al.* (2016) found that *Lactobacillus pentosus* as a fermentation starter increased the abundances of *Pleurotus cornucopiae, Pleurotus ostreatus* and *Pleurotus sajor-caju*, reduced the abundance of Enterobacteriaceae in the final fermentation process, increased lactic acid content and decreased nitrite concentration; the final product showed enhanced sensory characteristics, although the mushroom colour became brown after 18 days of fermentation. **Sun *et al.***

(**2022**) demonstrated that the *L. plantarum* starter is more effective in enhancing the nutritional quality and organoleptic properties of fermented *A. auricularia* than *L. rhamnosus* and *Leuconostoc mesenteroides* and improves antioxidant activity.
Shimeji mushrooms (*Hypsizygus* sp.) are widely consumed in North America, China and Japan but are rarely fermented. These mushrooms are known for their distinct flavour and bioactive components, including proteins, phenols, flavonoids and polysaccharides, which are highly beneficial for health and disease prevention (**Zhao *et al*., 2023**, **Chauhan *et al*., 2017**). In the present study, we investigated the use of *L. bulgaricus* in lactic fermentation for preserving shimeji mushrooms and evaluated the process by analysing the microbiological and chemical properties, sensory quality and volatile compounds of the produced pickle juice as the final fermentation product.

MATERIAL AND METHODS

Materials and microorganisms

L. bulgaricus FNCC 0041 were obtained from Food and Nutrition Culture Collection, Universitas Gadjah Mada, Indonesia. Shimeji mushroom (*Hypsizygus* sp.), chilli powder (Koepoe-Koepoe) and garlic powder (Jays Kitchen) were purchased from a local market in Yogyakarta, Indonesia. De Man, Rogosa and Sharpe Agar (MRSA), violet red bile glucose (VRBGA) and potato dextrose agar (PDA) were obtained from Himedia, India. NaCl, sucrose, phenolphthalein, 1% indicator, 0.1 N NaOH, 1 N HCl , 99.8% sulphanilamide and *N*-1-naphtyethylene diamine dihydro chloride (NED) were obtained from Sigma Aldrich, USA.

LAB starter culture preparation

The starter was prepared according to the methods of **Liu *et al*.** (**2016**) with modifications. A loop of *L. bulgaricus* was inoculated into an MRS broth and incubated at 37 °C for 24–48 h. Bacterial cells were then harvested at 5000 rpm for 10 min. Bacterial pellets were washed twice with 0.9% NaCl solution, suspended in 0.9% NaCl solution and diluted with distilled water.

Mushroom fermentation

The methods for fermentating shimeji mushrooms were based on the methods described by **Zheng *et al*.** (**2018**) but were modified. Approximately 150 g of fresh shimeji mushrooms were sliced, blanched for 2 min and placed in 300 ml sterilised glass jars. After cooling, 3% (wt/wt) red chilli powder, 2% garlic powder (wt/wt), 2% NaCl (wt/wt) and 1% sucrose (wt/wt) in 120 ml of sterile water were added to the jars. Fermentation was induced by inoculating 1.5×10^8 CFU/ml *L. bulgaricus* starter culture into each sealed glass jar and conducted for 18 days at 25 ± 2 °C.

Microbial enumeration

Pickle juice was collected after 0, 3, 6, 9, 12, 15 and 18 days of fermentation and then analysed for the viability of LABs, Enterobacteriaceae and yeast with spread-plate methods. MRSA, PDA and VRBGA were used for LAB enumeration, yeast enumeration and Enterobacteriaceae cultivation, respectively, and the cultures were incubated at 37 °C for 48 h, 25 °C for 48 h and 37 °C for 24 h (**Liu *et al.* 2016**).

pH and lactic acid measurement

Approximately 10 ml of pickle juice samples was collected, and pH was measured using a digital pH meter (Eutech Instruments, Singapore). Lactic acid content was measured on the basis of total titratable acidity, and pickle juice was diluted with water and mixed with 1% phenolphthalein indicator. The mixture was shaken and titrated with a 0.1 N NaOH solution until it turned pink. Lactic acid content was calculated using the following formula:

$$\text{Total titratable acidity (\%)} = \frac{\text{N x V1 x 0.09 x 100}}{\text{V2}},$$

where N, V_1, and V_2 represent NaOH normality, NaOH volume (ml) and sample volume (ml), respectively. A value of 0.09 is equivalent to 1 ml of lactic acid **(Matela *et al*., 2019**).

Analysis of nitrite content

The nitrite content of the fermented mushrooms was determined with the modified methods of **Liu *et al*.** (**2016**). Approximately 0.5 ml of mushroom pickled juice was filtered and mixed with 5.5 ml of sterile water. The mixture was then homogenised, and 4.5 ml of the mixture was mixed with 0.25 ml of sulphanilamide. The mixture was then homogenised and left for 5 min, mixed with 0.25 ml of NED and incubated again for 5 min, and the absorbance was measured at a wavelength of 538 nm with a UV-Vis spectrophotometer (Thermo Scientific Genesys 150). Sodium nitrite was used in obtaining the standard curve of nitrite content

Evaluation of sensory properties

Shimeji mushrooms fermented for 18 days were evaluated by seven untrained panellists. The sensory parameters of fermented shimeji mushroom were rated for colour, aroma, texture and overall appearance on a 1–5 scale (1 = extremely dislike, 2 = dislike, 3 = neither like nor dislike, 4 = like, 5 = extremely like).

Determination of volatile compounds

The volatile compound profile was determined according to the methods of **Balakrishnan and Agrawal** (**2014**). The volatile compounds contained in fermented shimeji mushrooms were determined using GC-MS. The fermentation solution (5 ml) was mixed with methanolic KOH (2N; 0.1 ml) and then heated at 45 °C for 1 h. After the reaction, the sample solution was cooled, 1 ml of hexane was added and the mixture was homogenised. GC-MS analysis was performed using an HP5MS-09012024 instrument (Agilent, USA) with an HP-5MS UI column (30 m × 250 μm × 0.25 μm). The time was 61 min, injector temperature was 250 °C, detector temperature was 280 °C and column temperature was 60 °C. The temperature was increased at an average rate of 5 °C/min to 280 °C. Helium gas was used as the carrier gas, and its constant flow rate was 1 ml/min. GC-MS procedures were used in detecting bioactive chemicals, mass spectra and chromatogram peak data were analysed and the spectrum was compared with the National Institute of Standard and Technology standards for identification.

Statistical analysis

The experiments were carried out three times, and differences among the samples were evaluated using means and standard deviation. The results were analysed using SPSS statistical analysis software version 25.0 (IBM, NY, USA) with one-way analysis of variance, and the difference in sample means was further analysed using the Duncan's multiple-range test. A *p value of* <0.05 indicated significant difference.

RESULTS AND DISCUSSION

Appearance of shimeji mushroom fermentation

Mushroom fermentation is still relatively uncommon compared with vegetable fermentation. Several fermented mushrooms have been studied, including *Auricularia auricularia, Pleurotus eryngii, Pleurotus ostreatus, Agaricus bisporus, Cantharellus cibarus* and *Termotimyces robustus* (**Jabłońska-Ryś *et al*. 2019**). Although shimeji mushroom (*Hypsizygus* sp.) is highly nutritious and widely consumed, the fermentation of shimeji mushroom has never been investigated. Thus, this study evaluated the effect of *L. bulgaricus* inoculation as a starter for shimeji fermentation (18 days).
On the first day of shimeji fermentation, the pickle juice was clear. On the 18th day, the liquid became cloudy. In this study, pickle juice samples were tested every 3 days for microbiological quality, pH and chemical quality for the analysis of microbial growth and fermentation process (**Figure 1**). The time of fermentation may affect the microbial population and biochemical composition. According to **Park *et al*.** (**2022**), the number of microorganisms increases at the early stage of fermentation and then decreases after peaking. These changes are accompanied by biochemical changes, which enhanc flavour and aroma. If lactic acid fermentation time is extremely short, LABs will not grow optimally. Conversely, extremely long lactic acid fermentation time might result in an extremely sour taste and considerable decrease in LAB population. The role of salt in controlling fermentation processes were analysed in this study. Salt can reduce water activity (Aw) from a substrate, and this effect is essential for microorganism growth. In addition, it can inhibit the proliferation of unwanted microorganisms through osmotic shock and prevent substrate softening (**Yang *et al*., 2020**). The addition of NaCl as salt enhances LAB growth by dissociating NaCl into Na^+, which is crucial for growth, and Cl^- ions, which decrease water availability, affecting LAB growth. Microbial growth is inhibited when the Aw of food is below the minimum levels for microbial growth. In addition, osmotic pressure causes plasmolysis, dehydration and microorganism death (**Barcenilla *et al*., 2022; Koesoemawardani *et al*., 2021**). The optimal salt concentration for fermented vegetables and fruits is 2%–3% and may promote LAB growth while inhibiting pathogenic bacteria (**Lee *et al*., 2022**). *L. bulgaricus* has a high tolerance to 3% salt concentration (**Samuel *et al*., 2019**).

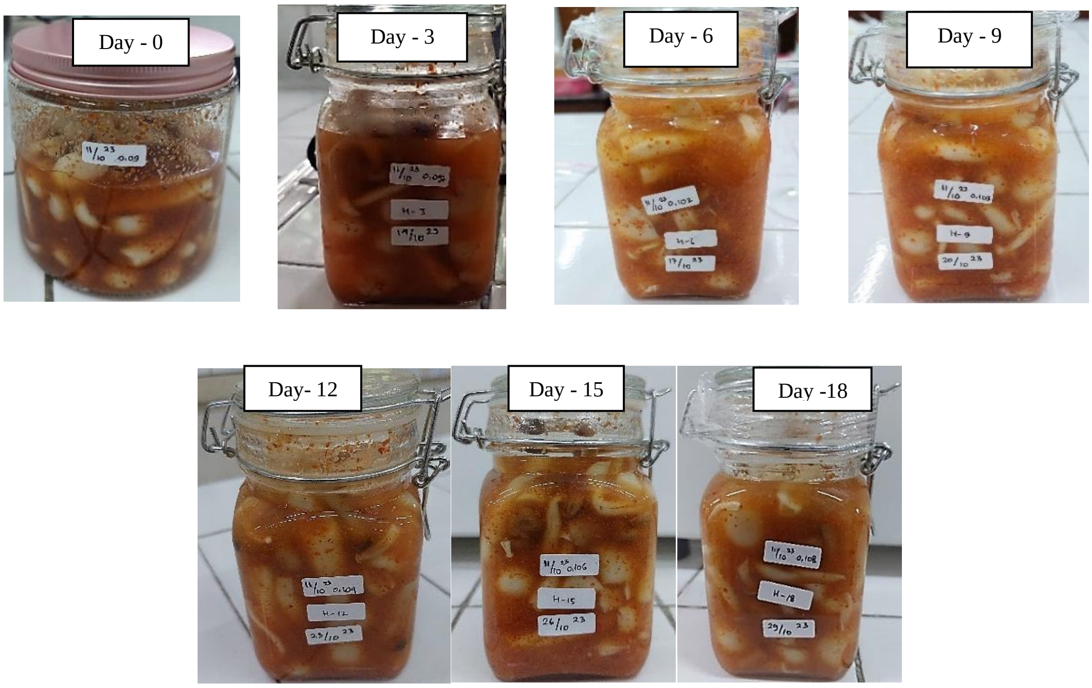

Figure 1 Fermentation of shimeji mushrooms by *L. bulgaricus* for 18 days.

The study focuses on the fermentation of shimeji mushrooms. Sucrose at 1% concentration was added to provide energy and carbon for LAB growth. According to **Kong *et al*. (2018)**, sucrose is a disaccharide sugar that is easily metabolised by LABs and can be applied to produce lactic acid. The fermentation process was modified by adding 2% red chilli powder and 3% garlic powder, which enhanced the sensory qualities of the final product. Previous research used black pepper, bay leaves and 5% onions in the fermentation of *A. bisporus and Pleurotus eryingii* and achieved products with good sensory quality (**Jabłońska-Ryś *et al*., 2019**).
Spices, such as onions and chilli peppers, contain antimicrobial compounds that may extend the shelf life of food prevent spoilage. Onions contain an allicin compound that inhibits the proliferation of pathogenic microorganisms by inhibiting lipid synthesis and RNA production in bacterial cells, inhibiting protein synthesis and potentially causing their death. Allicin affects the production of lipids in the bacterial cell membrane. Garlic powder can increase food shelf life by inhibiting the proliferation of pathogenic and spoilage microorganisms (**Geremew *et al*., 2015**). Garlic and chilli are commonly used in food fermentation as preservatives to improve product shelf life. Capsaicin, the primary capsaicinoid found in chilli peppers, inhibits the growth of pathogenic microorganisms by preventing cell membrane synthesis (**Behbehani, 2023**).

Population of lactic acid bacteria (LABs) in lacto-fermented shimeji pickle juice

The LAB population in the lacto-fermented shimeji pickle juice during fermentation is shown in **Figure 2**. The LAB population in shimeji mushrooms increased and decreased during 18 days of fermentation. On day 0 of fermentation, the LAB population was 6.95 log cfu/ml and probably in the lag phase, and the fermentation process was not optimal. During the lag phase, the bacteria began to adapt to the new fermentation environment, and thus the cells most likely grew in size rather than in quantity and reproduction was minimal (**Ughy *et al*., 2023**). The LAB population then increased rapidly to 8.05 log cfu/ml on the third day of fermentation. At this stage, LABs were in the log or exponential phase and showed rapid growth. The gradual formation of anaerobic conditions in the fermentation jar can promote LAB growth, leading to the rapid increase in LAB populations **(Liu *et al*., 2016).** As fermentation progresses and microorganisms reproduce, sugar is utilised by microorganisms or dissolved into pickle juice through osmosis and soaking **(Lin *et al.*, 2023).** The LAB population decreased by 7.11 log cfu/ml from the 3rd to 18th day of fermentation. This decrease was attributed to the availability of nutrients during fermentation because a large amount of carbohydrates were used for LAB metabolism (**Jabłońska-Ryś *et al.*, 2022**). The LABs continuously utilised nutrients from the mushroom fermentation solution, although the gradual increase in acidity and high concentration of organic acids may have contributed to the decrease **(Sun *et al.*, 2022).** These trends were in line with the findings of **Liu *et al.* (2016)**, who discovered that the LAB population in fermented oyster mushrooms (*Pleurotus* spp.) increased in the first three days of fermentation before declining. The predominant LAB population during the lactic acid fermentation of mushrooms can control pathogenic and food spoilage microorganisms (**Gao *et al.*, 2014**).

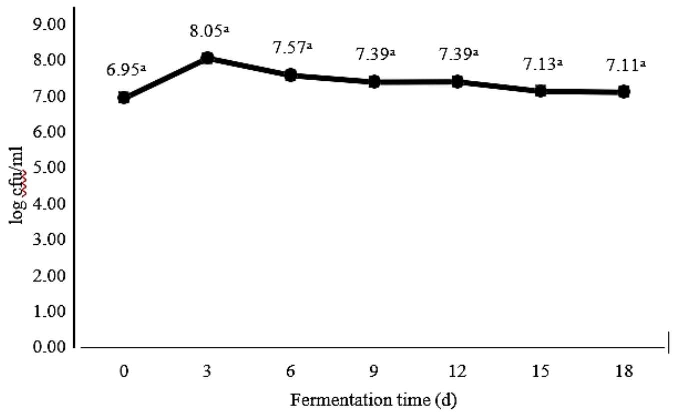

Figure 2 LAB population in lacto-fermented shimeji pickle juice
*Value with a different letter (superscript) indicates significant difference ($p < 0.05$).

Trends of yeast population in lacto-fermented shimeji pickle juice

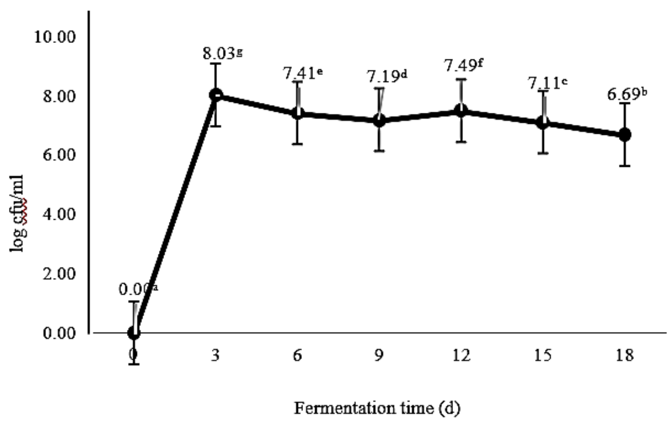

Figure 3 Trends of yeast population in lacto-fermented shimeji pickle juice
*Value with a different letter (superscript) indicates significant difference ($p < 0.05$).

The yeast population during fermentation of shimeji is illustrated in **Figure 3**. The yeast population initially increased and decreased in the final fermentation process. On the first day, no yeast was detected, indicating that the fermentation environment had not yet begun. This result suggested that yeast had not adapted to the new conditions, as it can grow optimally in acidic and anaerobic conditions **(Riesute *et al*., 2021**). On the third day, the population of LABs in the fermented shimeji mushroom increased rapidly, reaching 8.03 log cfu/ml. However, the yeast population decreased to 6.69 log cfu/ml on the last day. The yeast population was dominant during fermentation, LABs were dominant and the yeast population was lower than the LAB population after 6 days. In general, yeast and LABs co-existed throughout the fermentation process (**Liu *et al*., 2016**).

Yeast population increased on day 3 because the yeast adapted to the fermentation environment. On day 3, the pH of the fermentation products rapidly decreased below 4, which is suitable for yeast growth. Acidic fermentation conditions can promote yeast growth (**Akphogelie *et al*., 2024**), and the high availability of nutrients and carbohydrates during early fermentation can promote yeast growth (**Riesute *et al*., 2021**). The final yeast population in shimeji mushroom fermentation decreased to 6.69 log cfu/ml. Similar results were obtained by **Liu *et al*.** (**2016**), but the populations of yeast in other mushrooms, such as *A. bisporus* and *P. eryngii*, were 4–5 log cfu/ml below that of the LABs (**Bartkiene *et al*., 2023; Zheng *et al*., 2018**). The decrease in yeast population may be due to extremely acidic fermentation conditions and accumulation of organic acids with antifungal activity (**Riesute *et al*., 2021**). LABs, such as *Lactobacillus, Leuconostoc* and Propionibacterium, can inhibit yeast growth by producing bacteriocins (**Zapaśnik *et al*., 2022**). Therefore, more study is needed to control yeast growth during shimeji mushroom fermentation.

Trends of Enterobacteriaceae population in lacto-fermented shimeji pickle juice

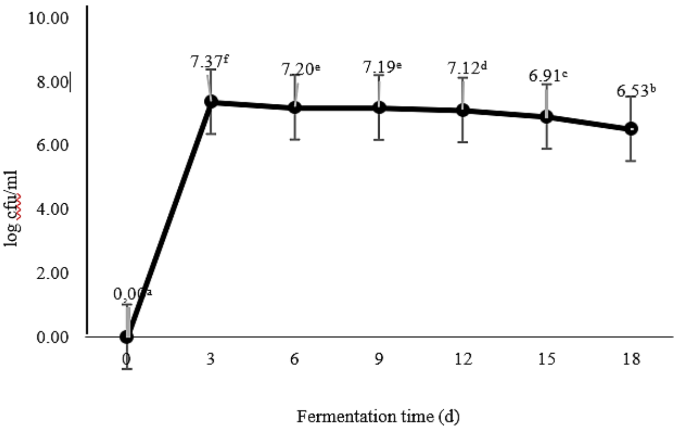

Figure 4 Trends of Enterobacteriaceae population in lacto-fermented shimeji pickle juice
*Value with a different letter (superscript) indicates significant difference ($p < 0.05$).

The population of Enterobacteriaceae during shimeji mushroom fermentation is shown in **Figure 4**. Initially, the population of Enterobacteriaceae on day 0 indicated no growth because of sterile fermentation conditions. However, the population increased during the first 3 days of fermentation becasue carbohydrates, such as sucrose, maltose and fructose, were used as nutrient sources by Enterobacteriaceae (**Mudoor Sooresh *et al*., 2023**). After 3 days, the population of Enterobacteriaceae decreased gradually to 6.53 log cfu/ml until the 18th day. This decrease was due to the rapid decrease in pH and the production of organic acids and antimicrobial compounds, including bacteriocins **(Esmaeilzadeh *et al*., 2013**). Lactic acid, a primary factor in fermentation, inhibits the proliferation of undesirable bacteria in food, such as Enterobacteriaceae, because they cannot survive under acidic conditions **(Zapaśnik *et al*., 2022; Zheng *et al*., 2018**). Similar results were reported by **Zheng *et al*.** (**2018**), who showed the Enterobacteriaceae population in sauerkraut and kimchi prepared using king oyster ws detected after 30 days of fermentation. Lactic acid at concentrations of 1%–2% is a key factor in fermentation and effectively kills bacteria, such as Enterobacteriaceae, preventing spoilage in food. At 0.5% con, it could kill *Escherichia coli, Listeria monocytogenes, Salmonela* spp.(**Zapaśnik *et al*., *2022*; Bangar *et al*., 2022**). In this study, lactic acid produced in shimeji mushroom fermentation was less effective in inhibiting Enterobacteriaceae proliferation because its concentration was lower than 0.5%.

pH and lactic acid content in lacto-fermented shimeji

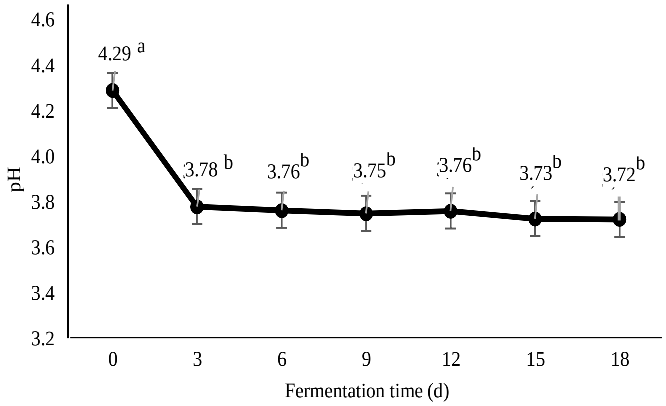

Figure 5 pH value during lacto-fermented shimeji
*Value with different latter (superscript) indicate are significantly different ($p < 0.05$)

The pH value of fermentation products is crucial for product storage durability and microbial safety (**Boylu *et al*., 2023**). The pH of shimeji mushrooms considerably decreased during fermentation, reaching 3.78 on the first 3 days of fermentation (**Figure 5**). This decrease was due to the presence of organic acids, which are the end product of fermentation. The increase in LAB population on day 3 promoted sugar metabolism, increasing acidity (**Sun *et al*., 2022**). On the 18th day, the pH value was 3.71 because of the accumulation of lactic acid from LAB metabolism. LABs can ferment carbohydrates in mushrooms, such as monosaccharides, disaccharides, polysaccharides and sugar alcohol, and produce organic acids (**Bartkiene *et al*., 2023**). A long fermentation time results in weak organic acids, such as acetic acid and propionic acid, and many more from lactic acids hydrolysis process (**Bangar *et al*., 2022**). Fermented mushrooms have a pH of 3.3–4.6 because of temperature, carbohydrate content and chemical processes **(Boylu *et al*., 2023**). Similar results were reported by **Jabłońska-Ryś *et al*.** (**2022**) and **Zheng *et al*.** (**2018**) for fermented button mushroom (pH 3.68) and king oyster mushroom (pH 3.3).

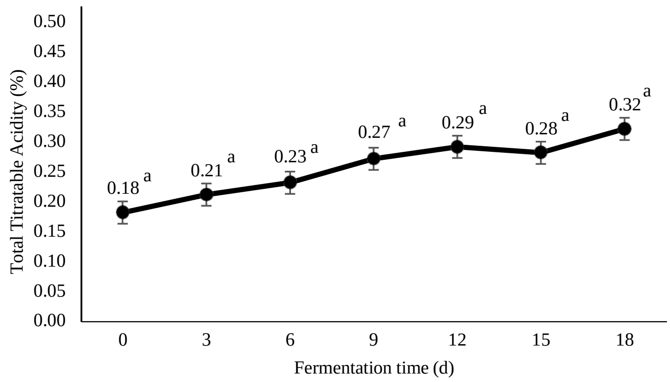

Figure 6 Lactic acid content during fermented shimeji mushroom
*Values with the same letter (superscript) are not significantly different ($p > 0.05$).

The total lactic acid content during mushroom fermentation was calculated in percentages of lactic acid equivalent. The lactic acid content increased gradually during fermentation, and no significant difference was observed. In shimeji mushroom fermentation, the lactic acid content increased from 0.18% to 0.32% on the last day **(Figure 6)**. However, the total lactic acid content showed no significant difference ($p < 0.05$). Some studies suggest adding sucrose to provide simple sugars, but LABs prefer reducing sugars, including glucose and fructose, which are low in mushroom fruiting bodies and insufficient for LAB growth (**Jabłońska-Ryś *et al*., 2022**). LABs continuously produce lactic acid, for example, *L. bulgaricus*, which converts glucose into lactic acid through the Embden–Meyerhof–Parnas pathway using lactate dehydrogenases (**Ojo & de Smidt, 2023**). *Lactobacillus* produces organic acids essential for flavour in pickles by fermenting carbohydrates. These acids are catabolised by microorganisms and are influenced by the microbial community. Common types include lactic, acetic, malic, succinic, oxalic, tartaric and citric acids **(Lin *et al*., 2023).** The lactic acid content increases until the 18th day of fermentation to 0.32% because LABs continuously use nutrients in the fermentation solution. However, organic acid accumulation can inhibit LAB growth **(Sun *et al*., 2022**). The preservation of king oyster mushroom with pickle, kimchi and sauerkraut methods produces 0.45%–1.25% lactic acid (**Zheng *et al*., 2018**). The difference of the lactic acid concentration was influenced by temperature, pH, nutrients, substrate type and final product concentration (**Ojo & de Smidt, 2023**).

Nitrite content during lacto-fermented shimeji

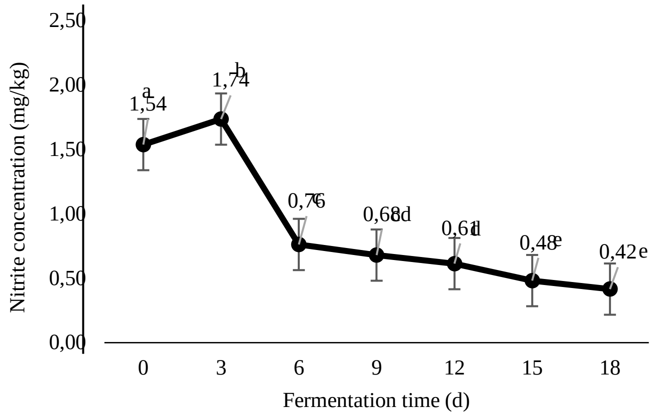

Figure 7 Nitrite concentration during shimeji mushroom fermentation
*Value with different latter (superscript) indicate are significantly different ($p < 0.05$)

The accumulation of nitrite is an important problem in fermented food production. Excessive nitrite in food can be harmful to health (**Hou et al., 2013**). In shimeji mushroom fermentation, nitrite content initially increased and then decreased gradually **(Figure 7)**. It reached 1.74 mg/kg during the first 3 days and then decreased to 0.42 mg/kg on the last day. The amount of Enterobacteriaceae present during fermentation may be related to the increase in nitrite. At the beginning of the fermentation, some Enterobacteriaceae or other gram-negative bacteria are nitrate-reducing bacteria (**Hou *et al*., 2013**). **Hou ai *et al*. (2013)** suggested that during fermentation, nitrate in a substrate is converted into nitrite by microorganisms, and the process increases nitrite concentration. Thus, the decrease in nitrate is attributed to the growth of nitrate-reducing bacteria (**Huang *et al*,. 2021**).
The decrease continued until 18 days, reaching 0.42 mg/kg. LABs, such as *Lactobacillus brevis, L. plantarum* and *Leuconostoc mesenteroides*, can metabolise nitrite during fermentation. The addition of ingredients, including sugars, salt, onion and ginger, can influence nitrite concentration (**Ding *et al*., 2018**). LABs, especially *Lactobacillus* sp., effectively reduce nitrite content (**Xia *et al*., 2022**). The nitrite concentration in fermented shimeji mushroom was 0.42 mg/kg, which was lower than the maximum permissible level (20 mg/kg; **Huang et al. 2021**). Similar results were reported by **Ding *et al*. (2018)**, who reported that nitrite content in fermented pickled cucumber and sauerkraut in south China is 0.4–0.6 mg/100 g. **Zheng *et al*. (2018)** reported that on king oyster mushroom preserved in sauerkraut, pickles and kimchi showed a decrease in nitrite content to below 0.50 mg/kg.

Evaluation of sensory properties lacto-fermented shimeji

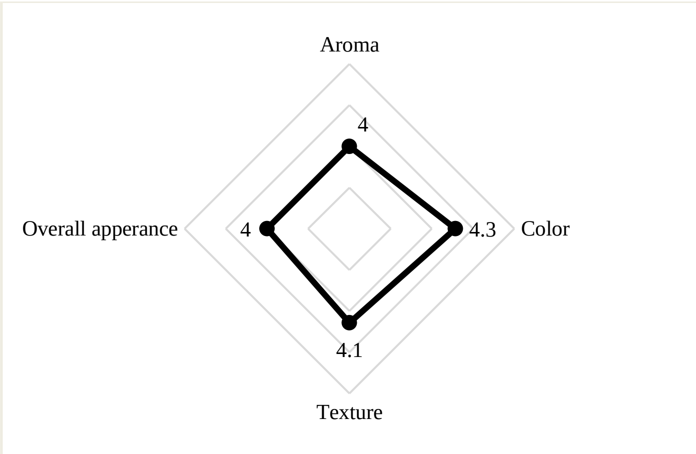

Figure 8 Sensory characteristic analysis of lacto-fermented shimeji

Table 1 Volatile compound by GC-MS

Compound Name[(a)]	**Formula**	**RT**[(b)]	**Peak Area (%)**[(c)]
Aromatic hydrocarbon			
o-Xylene	C_8H_{10}	5.295	27.880
p-Xylene	C_8H_{10}	5.829	5.890
Styrene	C_8H_8	5.776	0.560
Mesitylene	C_9H_{12}	8.387	0.230
Fatty acid amides			
9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	45.110	1.680
Alkene			
2,4-Dimethyl-1-heptene	C_9H_{18}	4.678	0.920
Cyclopropane, 1-methyl-2-(3-methylpentyl)-	$C_{10}H_{20}$	10.910	0.650
Heptane, 3-ethyl-5-methylene-	$C_{10}H_{20}$	3.414	0.650
Cyclopentane, 1,2,3-trimethyl-, (1.alpha., 2.alpha., 3.alpha.)-	C_8H_{16}	3.182	0.400
1-Undecene, 7-methyl-	$C_{12}H_{24}$	19.605	0.390
Cyclopentane, 1,1,3,4-tetramethyl-, cis-	C_9H_{18}	19.041	0.380
1-Nonene, 4,6,8-trimethyl-	$C_{12}H_{24}$	33.144	0.350
Cyclohexane, 1,2-dimethyl- (cis/trans)	C_8H_{16}	3.609	0.260
Alkane			
Hexane, 2,3,4-trimethyl-	C_9H_{20}	8.791	0.330
Hexane, 2,3,5-trimethyl-	C_9H_{20}	4.298	0.190
Pentane, 2,2,3,4-tetramethyl-	C_9H_{20}	8.530	0.150
Alcohol			
o-Menthan-8-ol	$C_{10}H_{20}O$	45.305	0.710
(**Secondary alcohol)**			
1-Hexanol, 3,5,5-trimethyl-	$C_9H_{20}O$	37.637	0.330
(aliphatic alcohol)			
1-Hexanol, 5-methyl-2-(1-methylethyl)-	$C_{10}H_{22}O$	42.183	0.350
(**Fatty alcohol)**			
Organic acid			
Acetic acid, trifluoro-, 3,7-dimethyloctyl ester	$C_{12}H_{21}F_3O_2$	19.326	0.550
Pyruvic acid, butyl ester	$C_7H_{12}O_3$	3.799	0.190
Aldehyde			
Acetaldehyde	C_2H_4O	41.952	0.290
Ketone			
3-Hexanone	$C_6H_{12}O$	3.722	0.120

a Volatile organic compounds detected in mushroom fermentation
b Retention time of gas chromatography
c Peak area

The sensory characteristics of fermented shimeji mushrooms were evaluated by seven panellists and scored from 1 to 5 (dislike to like) in terms of colour, aroma, texture and overall appearance. In general, shimeji mushrooms fermented with *L. bulgaricus* culture had good sensory and acceptable characteristics according to the panellists. The sensory evaluation of shimeji mushrooms fermented with *L. bulgaricus* showed the highest sensory value in terms of colour (4.3), texture (4.1), aroma (4) and overall appearance (4). On the other study **Jablonska-Rys *et al.*** (**2016**), the sensory evaluation of button mushroom fermented with *L. plantarum* was also similar, with a colour value of 4.3, texture of 5 and aroma of 4.

Volatile compounds analysis by GC-MS

Volatile compound profile of the fermented shimeji mushrooms are shown in Table 1. Volatile compounds produced by shimeji mushroom fermentation included aromatic hydrocarbons, alkanes, alkenes, organic acids, alcohols, ketones, aldehydes and fatty acids. Volatile compounds are complex and easily change, primarily facilitating the oxidation of unsaturated fatty acids by chemicals or enzymes, interacting with proteins, peptides or free amino acids and undergoing the Maillard process **(Chen *et al.*, 2017**). The distinctive smell of mushrooms are due to a variety of chemical groups, including alcohols, aldehydes, ketones, acid, hydrocarbons, esters, aromatic and heterocyclic compounds. Over 110 volatile compounds have been found in mushroom, and eight-carbon (C8) compounds (44%–97%) constitute the volatile fraction (**Tagkouli *et al.*, 2021**). Volatile compounds that produce aroma and prevalent in fermented shimeji mushroom products are *o*-xylene, *p*-xylene, styrene and mesitylene, which have percent peak areas of 27.88%, 5.89%, 0.56% and 0.23%, respectively. These volatile compounds play a significant role in the unique aroma and flavour of mushrooms. Mesitylene is a sweet-tasting substance from benzene-derived volatile compounds structurally related to toluene. *p*-Xylene, a sweet substance, may be produced through β-carotene degradation. Styrene, a woody balsamic compound, is produced by the degradation of cinnamic acid and is a product of microbial metabolism (**Clarke *et al.*, 2020**).

Fermented products have a characteristic aroma because LABs can produce a variety of volatile compounds during fermentation. Aldehydes provide a fresh, floral, grassy and fatty aroma in the final products. Mushroom alcohol derived from LA is responsible for the earthy, green and oily odour because of its key odour compound. Three alkanes were detected in fermented shimeji. Alkanes provide mushroom waxy, terpene and woody aroma (**Tagkouli *et al.*, 2021**). **Sun *et al.*** (**2022**) reported that the fermentation of *A. auricularia* with *Lactiplantibacillus plantrarum* produced volatile compounds, such as alcohols, ketones, acids, hydrocarbons, aldehydes and esters. **Bartkiene *et al.*** (**2023**) showed that the volatile compound produced by fermentation with *A. bisporus* w *Lc. casei*, *L. plantarum*, *Lc. paracasei* and *P. acidilactici* are alcohols, aldehydes, ketones, esters and aromatic hydrocarbons.

CONCLUSIONS

The fermentation of shimeji (*Hypsizygus* sp.) mushrooms using *L. bulgaricus* as starter showed that LABs were prevalent over the other microorganisms, with a decrease in yeast and Enterobacteriaceae population. The fermentation increased total lactic acid content and decrease in nitrite concentration.The sensory characteristics were good and acceptable. The fermented shimeji mushrooms produced volatile compounds, such as aromatic hydrocarbons, alkanes, alkenes, alcohols, organic acids, ketones, aldehydes and fatty acids, especially *o*-xylene, *p*-xylene, styrene and mesitylene. Fermentation using *L.bulgaricus* should be further studied and developed as a bio-preservation method for preserving shimeji mushrooms, extending their shelf lives and enhancing sensory qualities.

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