**Article**

**Lactobacillus plantarum SK15 as a Starter Culture for Prevention of Biogenic Amine Accumulation in Fermented Beverage Containing Hericium erinaceus Mushroom**

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**Abstract:** The effectiveness of *Lactobacillus plantarum* SK15 starter culture in preventing biogenic amine production, such as putrescine (PUT) and spermidine (SPD), during the fermented mushroom beverage (FMB) process using *Hericium erinaceus* was examined. Spontaneously fermented and starter-mediated FMB was manufactured in parallel. Besides changes in pH, total acidity content, alcohol content, amino acid content, reducing sugar content, total phenolic content, antioxidant activity, and the microbial load were examined at several sampling points during the 720-h fermentation process. As a result, the *L. plantarum* SK15 starter prevented biogenic amine accumulation during FMB fermentation. Moreover, starter-mediated FMB can control pH and alcohol content at an acceptable level. In addition, FMB with *L. plantarum* SK15 had a higher level of total phenolic content (TPC) and antioxidant activity.

**Keywords:** biogenic amine; fermented mushroom beverage; *Hericium erinaceus*; lactic acid bacteria; starter culture

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**1. Introduction**

*Hericium erinaceus* is considered an edible mushroom with beneficial medicines. It is well-known for its anti-tumor, anti-microbial, anti-diabetic, anti-hypertensive, anti-aging, antioxidant activities, and protein enrichment [1–3]. *H. erinaceus* mushroom is commonly transformed into many products, in particular FMB. Consumed by people in Thailand, FMB is one of the most nutritionally functional foods in preventing sickness and maintaining good health. In the beginning, the traditional process is performed, which includes a natural fermentation without a starter culture at room temperature for 6–12 months and depends on fruit or vegetable as a material [4]. To ensure the quality and safety of consumption, FMB is commercially manufactured to improve some nutritional value and control the hazards of a toxic compound, including biogenic amine.

The biogenic amines putrescine (PUT) and spermidine (SPD) are the most abundant compounds in mushrooms and contain a high amount in *H. erinaceus* [5–7]. The amines in FMB are compounds produced by enzymes of microorganism origin from the decarboxylation of amino acids in suitable environmental conditions [8–10]. A high accumulation of biogenic amines in FMB negatively affects consumer health with rashes, flushing, nausea, vomiting, diarrhea, headaches, migraines, cardiac palpitations, and blood pressure changes [11,12]. For the manufacturing process, biogenic amines were monitored as food quality and hygiene indicators in the food process chain [13].
The microbes, such as *Bacillus*, *Clostridium*, *Pseudomonas*, *Escherichia*, *Salmonella*, *Shigella*, *Photobacterium*, and *Lactobacillus*, are generally found in the raw material or environment of the fermentation process [14]. Therefore, they can be contaminated and contribute to biogenic amine formation in products. Consequently, it is necessary to note not only the loading microbe population but also the different microorganism stains (including species) [6]. The main bacteria for fermented food are the lactic acid bacteria (LAB) as a starter culture [15–17]. The inoculated starter is one of the most productive methods in controlling biogenic amine accumulation [18,19]. Some LAB species do not produce amino acid decarboxylase enzyme, which changes amino acid to biogenic amines. Some research supports the use of a starter culture to diminish biogenic amine accumulation during a fermentation process. Lee et al. [20] studied the alteration of biogenic amines during miso fermentation with inoculation of *L. plantarum* for three months. The content of histamine (HIS), PUT, cadaverine (CAD), and tyramine (TYR) decreased by 58.0%, 12.0%, 2.4%, and 33.5%, respectively. In another study, the addition of *Lactobacillus sakei* starter culture in the sausage sample exhibited an extremely low content of biogenic amines (TYR, PUT, and CAD) [21]. Wu et al. [22] evaluated the combination of a culture system between *L. plantarum* Shanghai brewing 1.08 and *Zygosaccharomyces rouxii* CGMCC 3791 in sauerkraut (cabbage and radish pickle) fermentation. The biogenic amine (PUT, CAD, and TYR) concentration of the samples with combined starter culture was significantly lower, comparing the spontaneous sample for both pickles. Woraharn et al. reported that fermented *H. erinaceus* beverage, which used *Lactobacillus brevis* HP2 and *Lactobacillus fermentum* HP3 as a starter culture, enriched l-glutamic (GA) and γ-aminobutyric acid (GABA) production [23] and managed diabetes Mellitus along with medication [24]. The selection of proper LAB significantly enhances the functional values and health.

More knowledge about the ability of *L. plantarum* SK15 starter culture in the prevention of biogenic amines accumulation was essential to ensure food quality and safety in FMB containing *H. erinaceus*. Therefore, this study was carried out to confirm that *L. plantarum* SK15 as non-amino acid decarboxylase-producing bacteria can be used as a suitable starter culture in FMB. In addition, the change of PUT and SPD accumulation between a spontaneous sample and an inoculated sample was studied during FMB fermentation. For safety and quality, changes in the pH, total acidity, alcohol content, amino acid content, reducing sugar content, microbial load, total phenolic content (TPC), and antioxidant activity were evaluated at several sampling points during fermentation.

2. Materials and Methods

2.1. Material

*H. erinaceus* mushroom was harvested from a contact farm located in Doi Saket District, Chiang Mai, Thailand. The pretreatment method was described by a previous study for reducing biogenic amine accumulation [25]. The fresh mushroom was washed with water, excess water was removed. The sample size was shrunk using a blender (Blend-Xtract 3-in-1 Blende, BL237WG, Irving, TX, USA). After that, the blended mushroom was dried at 60 °C with a laboratory-scale hot air dryer (Drawell, DGT-G135, Shanghai, China) until final moisture content of less than 10% dry weight was reached. The dried mushroom was kept in an aluminum package at room temperature.

2.2. Starter Culture Preparation

The starter, *L. plantarum* SK15, was isolated from plant beverages, characterized by its functional properties, and provided by Innovation Center for Holistic Health, Nutraceuticals, Faculty of Pharmacy, Chiang Mai University, Thailand. The *L. plantarum* SK15 was chosen in this study because the strain is used to improve the quality of fermented *Morinda citrifolia* Linn. (Noni) fruit juice, which nullified the formation of unwanted metabolites, i.e., ethanol and methanol content [26]. *L. plantarum* SK15 was cultured using de Man Rogosa and Sharpe (MRS) broth at 37 ± 2 °C for 24 h. The bacteria were incubated in order to acquire an approximate cell concentration of $10^9$ CFU/mL for the fermentation process.
2.3. Evaluation of L. plantarum SK15 on Amino Acid Decarboxylase Production

*L. plantarum* SK15 was cultured in ornithine decarboxylase broth and arginine dihydrolase broth. A modified method has detected the ability of decarboxylation on ornithine (Himedia, Mumbai, India) and arginine (Himedia, Mumbai, India) following Falkow [27]. Briefly, 100 µL of *L. plantarum* SK15 was mixed with 900 mL of broth in a sterile microcentrifuge tube. Then, the mixture tube was incubated at 37 °C for 72 h. After incubation, the samples were centrifuged at 10,000 × g at 4 °C for 5 min, and the absorbance of the supernatant was measured at 588 nm using the multi-mode microplate reader (model SpectraMax M3, San Jose, CA, USA). *Shigella sonnei* and *Salmonella typhi* were tested as positive activity in ornithine decarboxylase broth and arginine dihydrolase broth, respectively.

2.4. Fermented Mushroom Beverage (FMB) Preparation

In this study, FMB was manufactured according to the process reported by Chaiyasut et al. [28]. FMB was prepared by mixing pretreated *H. erinaceus* mushroom, cane sugar, and water with the ratio of 3:10:1 by weight. The study process was divided into two fermentation batches in parallel—with and without *L. plantarum* SK15 starter culture. The fermentation with a starter culture, about 10% (v/v) *L. plantarum* SK15 inoculum (cell concentration of 10⁹ CFU/mL), was added to the FMB sample. FMB samples were collected in a sterile polypropylene tank at a temperature of 30 ± 2 °C for 720 h and filtered before analysis.

2.5. Determination of Biogenic Amines

The post-column derivatization high-performance liquid chromatography (HPLC) analysis was described according to Makhamrueang et al. [25]. PUT and SPD were detected with C18 reversed-phase column (4 µm, 4.6 × 150 mm, Poroshell 120 EC-C18, Agilent Technologies, Santa Clara, CA, USA) with a system controller (model SCL-10A VP, Shimadzu, Tokyo, Japan), two auto pumps (model LC-10AS, Shimadzu, Tokyo, Japan), a spectrofluorometric detector (model SCL-10A VP, Shimadzu, Tokyo, Japan), and a post-column pump (model, Waters, Milford, MA, USA). The mobile phases had phase A and phase B as a mixture of sodium acetate anhydrous (QReC, Selangor, Malaysia) and sodium octanesulfonate (Merck, Darmstadt, Germany) adjusted to pH with glacial acetic acid (RCl Labscan, Bangkok, Thailand). The flow rate of mobile phases was 1.2 mL/min in the gradient program. The post-column derivatization with ortho-phthalaldehyde (OPA) (Himedia, Mumbai, India) was freshly prepared as a reagent. The flow rate of the post-column reagent was 0.6 mL/min. The amines were detected by a fluorimetric detector after post-column derivatization at 340 nm for excitation and 445 nm for emission.

2.6. Determination of pH and Total Acidity

The alteration of pH during fermentation was measured by pH meter (model HQ40d, Hach, Loveland, CO, USA) at room temperature. The total acidity of the sample was determined by titration, and the values were represented as lactic acid equivalent [26]. The acid titrations used sodium hydroxide (RCl Labscan, Bangkok, Thailand) and phenolphthalein (QReC, Selangor, Malaysia) as an indicator. Briefly, 100 mL of DI water was mixed with 0.5 mL of phenolphthalein as an indicator, and 2.5 mL of the sample was added. The samples were titrated with 0.1 M NaOH until the formation of pink color as an endpoint. Then the total lactic acid was calculated by following Equation (1).

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\text{Total acidity} \ (\% \ \text{w/v}) = \left( M \times V \times 0.09 \times 100 \right) / V \ \text{of sample}, \quad (1)
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where M was the concentration of sodium hydroxide in M or mol/L, V was the volume of sodium hydroxide used in mL, 0.09 was the constant of lactic acid equivalent, which came from the molecular weight of lactic acid, and V of the sample is the volume of FMB in mL.
2.7. Determination of Methanol and Ethanol

The method of methanol and ethanol was analyzed using Gas chromatography (GC) [28]. The equipment consisted of a GC machine (Model GC-14B, Shimadzu, Tokyo, Japan), carbowax column (1 µm, 30 m × 0.53 mm), and flame ionization detector (FID). The carrier gas, nitrogen, was set at 40 mL/min. The temperature at the injector port and detector was set at 180 and 260 °C, respectively. The oven temperature will be controlled at an initial 38 °C for 3 min, increased to 250 °C (the rate of 50 °C per 1 min). Isobutanol (Merck, Darmstadt, Germany) 50 ppm was used as internal standards. The sample was injected at about 5 µL.

2.8. Determination of Amino Acids

The determination of the amino acid was a modified method of Kivrak et al. [29]. The sample was measured by high-performance liquid chromatography-mass spectrometry (HPLC-MS) (Model TSQ-ENDURA, ThermoScientific, Waltham, MA, USA). The separation was achieved using a C18 reversed-phase column (1.7 µm, 2.1 × 2.2 mm, Inertsil ODS-3, GL Sciences, Eindhoven, The Netherlands). A 10 µL injection volume was used. The mobile phase system consisted of phase A and phase B, adjust to pH 3. Phase A was a 10% of 20 mM ammonium formate (Merck, Darmstadt, Germany) and 90% of water, whereas phase B was a 10% of 20 mM ammonium formate plus 90% of acetonitrile (RCl Labscan, Bangkok, Thailand). The flow rate of mobile phases was 0.15 mL/min in the gradient program. The electrospray ionization (ESI) source was used positively with the following conditions: the spray voltage was 3500 V; the ion transfer tube temperature was 325 °C; vaporizer temperature was 275 °C.

2.9. Determination of Reducing Sugar

Reducing sugar during the fermentation was performed using the dinitrosalicylic acid (DNS) reagent method of Goncalves et al. [30]. The DNS reagent was dissolved in 5 g of DNS (Himedia, Mumbai, India) with 250 mL of distilled water at a temperature of 80 °C. The reagent was then left standing at room temperature. Then, 100 mL of 2 N sodium hydroxide and potassium sodium tartrate-4-hydrate (Kemaus, Cherrybrook, New South Wales, Australia) was added to the reagent. The volume was adjusted to 500 mL with distilled water. The sample (25 µL) was placed in resistant microplates (crystal polyester plates). Then, the DNS reagent, 25 µL, was added. The reactions were placed in the oven for incubation at 105 °C for 5 min. Then, they were immediately placed in an ice bucket. The 250 µL of distilled water was added. The multi-mode microplate reader measured the reaction at a wavelength of 540 nm. The reducing sugar was calculated by comparison with the glucose standard curve. A distilled water was used as a blank.

2.10. Determination of Total Phenolic Content (TPC)

The TPC was performed using Folin–Ciocalteu method following Adebo et al. [31] with minor modifications. Briefly, 80 µL of fermented mushroom beverage was added into a 96-well microplate and reacted with 20 µL of Folin–Ciocalteu reagent (Merck, Darmstadt, Germany). Subsequently, 100 µL aqueous solution (20%, w/v) of sodium carbonate anhydrous (Merck, Darmstadt, Germany) was pipetted and mixed well with a vortex (Model G560E, Scientific Industries, New York, NY, USA). The mixture was wrapped with aluminum foil and incubated for 30 min at room temperature in the dark. The absorbance was read at 765 nm by a multi-mode microplate reader. The deionized water was used as blank. The amount of TPC was calculated as gallic acid equivalent (GAE) from the standard calibration curve of gallic acid (Millipore, MA, USA) and expressed as µg GAE/mL of sample.

2.11. Determination of Antioxidant Activity

Antioxidant activity was measured by using ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfate)) assay, which was a modified method of Pumtes et al. [32]. The corresponding
radical cation (ABTS•+) was prepared from a 7 mM aqueous stock solution of ABTS (Merck, Darmstadt, Germany) and 2.45 mM potassium persulfate (RCl Labscan, Bangkok, Thailand) in distilled water. The solution was left to stand in the dark at room temperature for 16 h. The working ABTS•+ was diluted with distilled water (1:20) to obtain an absorbance of 0.70 (±0.02). A 40 µL of fermented mushroom beverage and 160 µL of ABTS•+ solution was mixed in a 96-well microplate. The absorbance was measured at 734 nm using the multi-mode microplate reader. Pure ethanol (RCl Labscan, Bangkok, Thailand) was again used to calibrate the spectrophotometer. The ABTS radical cation scavenging activity or antioxidant activity was also quantified using trolox (Merck, Darmstadt, Germany) as a standard.

2.12. Enumeration of Microbial Population

The LAB, total bacteria count, and microorganisms (Bacillus cereus, Clostridium perfringens, Salmonella spp., Staphylococcus aureus, and Escherichia coli) of FMB were determined by the standard serial dilution technique and plate count technique following the method of Sirilun et al. [33]. Man Rogosa and Sharpe (MRS) agar medium were used for LAB. The plate count agar (PCA) was applied to an enumeration of the total bacterial count. The Phenol Red Egg Yolk Kanamycin (PREYK) agar, Tryptose-sulfite-cycloserine Egg Yolk (TSCEY) agar, Salmonella Shigella (SS) agar, and Mannitol Salt Phenol Red Egg Yolk (MSEY) agar were utilized for B. cereus, C. perfringens, Salmonella spp., and S. aureus, respectively. The load of E. coli was performed by multiple tube fermentation technique. The microbial counts were indicated as log colony-forming unit (CFU) per milliliter of a sample or most parable number (MPN) per 100 milliliters of sample.

2.13. Statistics Analysis

The analytical information was exhibited in terms of mean values with standard deviation. All experiments were executed in triplicate. The data were analyzed by analysis of variance (ANOVA) using SPSS (version 17, SPSS Inc., Chicago, IL, USA). Differences between mean values were proved using Tukey tests at a confidence level of 95%.

3. Results

3.1. Evaluation of L. plantarum SK15 on Amino Acid Decarboxylase Production

A modified method had detected the decarboxylase production of L. plantarum SK15 on ornithine decarboxylase and arginine dihydrolase broth. In this study, S. sonnei and S. typhi were selected as positive controls for ornithine decarboxylase and arginine dihydrolase broth, respectively. The absorbance of S. sonnei in ornithine decarboxylase broth decreased and then slightly increased at 24 h of incubation time (Figure 1a). For S. typhi in arginine dihydrolase broth, the absorbance decreased in the range of incubation 0–12 h and later increased, as shown in Figure 1b. The absorbance of L. plantarum SK15 decreased to 12 and 9 h for ornithine decarboxylase broth and arginine dihydrolase broth, respectively, and then the absorbance was rather stable. The absorbance was used as an indicator for the color change of the medium during the incubation time. S. sonnei and S. typhi were recommended as a positive control of ornithine decarboxylase and arginine dihydrolase broth, respectively. The microbes could generate the decarboxylase enzyme, yielding the biogenic amines. The amines accumulation resulted in an alkaline pH. The increased pH of the medium is detected by purple color in ornithine decarboxylase broth (Figure 1a). For arginine dihydrolase broth, the color changed from purple to yellow and then back to purple (Figure 1b). However, L. plantarum SK15 could not produce the enzyme to decarboxylate amino acid to a biogenic amine. As a result, the indicator color changed from purple (high absorbance) to yellow (low absorbance), as shown in Figure 1a,b.
The changes of biogenic amines during FMB fermentation were analyzed at 0, 3, 6, 9, 12, 18, 24, 48, 96, 144, 192, 240, 360, 480, 600, and 720 h in Figure 2. The PUT content was firstly detected at 6 h for FMB without *L. plantarum* SK15 starter and 0 h for FMB inoculated *L. plantarum* SK15. The initial detection of SPD content from FMB with and without *L. plantarum* SK15 starters were found in 18 and 96 h, respectively. The amines were gradually raised with the increasing fermentation time till the end of fermentation time in this study at 720 h. The PUT content of FMB with and without starter was in the range of 0.02–0.41 mg/L and 0.01–0.68 mg/L, respectively. While SPD content of FMB with and without *L. plantarum* SK15 starter was in the range of 0.57–3.75 mg/L and 0.98–5.38 mg/L, respectively.

3.2. Physical Observations of a Fermented Mushroom Beverage (FMB)

FMB without and with *L. plantarum* SK15 starter culture was observed for the change of physical properties during fermentation for 720 h. The physical properties of FMB samples were observed in terms of color, odor, taste, turbidity, and gas formation. The color of FMB with and without starter culture was brown because of the color of raw material and the presence of cane sugar. The color samples were later turned into a dark brown during fermentation. The odor of FMB with and without starter culture was initially the mushroom smell until 144 h of the fermentation, and then the smell of FMB became sour (pickle smell). These odors were the result of organic acid production during fermentation. In the beginning, plenty of cane sugar was the reason for a slightly sweet on both of starter addition processes. After that, the taste of the FMB samples was turned sour till the end of the process. Due to organic acid being produced, a reason to lead to acidity was decreased during the fermentation process. The turbidity of FMB samples was increasingly found while the fermentation time was expanded because of the rise of dissolved solid particles. The presence of gas in FMB was observed in some no adding starter cultures during 72 h of fermentation time. The gas formation was due to the presence of raw cane sugar as a carbon source. So, the fermentation process using cane sugar as one of the materials should follow the gas management protocol during the process. The FMB with starter culture was not found at a level of gas.

3.3. Biogenic Amines Content of a Fermented Mushroom Beverage (FMB)

The absorbance change of *L. plantarum* SK15 broth and positive control broth that relate to amino acid decarboxylase production for (a) ornithine decarboxylase broth; (b) arginine dihydrolase broth.

![Figure 1](image-url)
Figure 2. The change of biogenic amines content in a fermented mushroom beverage (FMB) with and without starter culture *L. plantarum* SK15 during fermentation time: (a) putrescine (PUT); (b) spermidine (SPD).

3.4. pH and Total Acidity of a Fermented Mushroom Beverage (FMB)

The pH of FMB samples was measured as shown in Figure 3a. The FMB with starter culture was exhibited a pH value of 2.52–3.35, whereas the samples without starter culture indicated a pH value of 3.74–5.10. The pattern of pH slightly decreased during fermentation time.

Figure 3. The change of acidity in a fermented mushroom beverage (FMB) with and without starter culture *L. plantarum* SK15 during fermentation time: (a) pH; (b) total acidity as the percentage of lactic acid equivalent.

The total acidity of FMB was represented as a lactic acid equivalent, as shown in Figure 3b. The FMB with starter culture exhibited a total acidity of 0.27–1.45% (w/v), whereas the total acidity of a sample without starter culture was in the range of 0.13–0.28% (w/v).

3.5. Alcohol Content in a Fermented Mushroom Beverage (FMB)

The methanol content was not found in all FMB products with and without *L. plantarum* SK15 starter culture during 720 h fermentation.

At the beginning of FMB with and without *L. plantarum* SK15, the trend of ethanol content was slightly increased. Then, the trend was stable after 480 h of fermentation time. The amount of ethanol of FMB with and without starter was in the range of 0.01–0.14% (v/v) and 0.01–0.54% (v/v), respectively (Figure 4). The maximum amount of ethanol was recorded in FMB without *L. plantarum* SK15 starter culture at 720 h.
Figure 4. The change of ethanol content in a fermented mushroom beverage (FMB) with and without starter culture *L. plantarum* SK15 during fermentation time.

### 3.6. Amino Acid Content of a Fermented Mushroom Beverage (FMB)

FMB samples measured the presence of amino acids at 0 and 720 h, as shown in Table 1. All of the amino acids were found in FBM, except cysteine, lysine, and methionine. There were four amino acids: alanine, isoleucine, leucine, and valine, which appeared in high amounts. The initial total amino acid content of FMB with and without *L. plantarum* SK15 was not significantly different. The total amino acid content increased from 991.35 to 1059.27 µg/L in FMB without *L. plantarum* SK15. In comparison, the total amino acid content decreased from 998.05 to 845.80 µg/L in the sample inoculated starter *L. plantarum* SK15.

Table 1. Total amino acid and free amino acid content of a fermented mushroom beverage (FMB) with and without starter culture *L. plantarum* SK15 during fermentation time at 0 h and 720 h.

| Amino Acid       | Amino Acid Content $^1$ (µg/L) |
|------------------|--------------------------------|
|                  | Without Starter   | With Starter   | Without Starter | With Starter   |
|                  | 0 h               | 720 h          | 0 h             | 720 h          |
| Alanine          | 138.65 ± 2.03 c   | 133.21 ± 1.84 b| 145.03 ± 2.21 d| 69.60 ± 1.06 a |
| Arginine         | 22.95 ± 1.72 a    | 23.30 ± 1.50 a | 28.97 ± 1.97 b | 23.40 ± 1.45 a |
| Aspartic acid    | 10.78 ± 2.85 b    | 9.91 ± 0.87 ab | 11.86 ± 1.99 b | 6.05 ± 0.26 a  |
| Cysteine         | ND                | ND             | ND              | ND             |
| Glutamic acid    | 65.09 ± 2.97 b    | 56.85 ± 2.52 a | 69.28 ± 0.85 b | 54.78 ± 1.23 a |
| Glycine          | 14.13 ± 0.84 a    | 17.58 ± 1.37 ab| 19.40 ± 2.38 b | 20.54 ± 0.96 b |
| Histidine        | 2.25 ± 0.20 b     | 2.68 ± 0.14 b  | 3.22 ± 0.24 c  | 1.39 ± 0.10 a  |
| Isoleucine       | 173.99 ± 2.08 b   | 176.22 ± 2.28 b| 178.86 ± 2.95 b| 165.44 ± 1.55 a|
| Leucine          | 184.96 ± 2.87 b   | 192.88 ± 2.08 c| 190.69 ± 1.76 c| 177.79 ± 1.64 a|
| Lysine           | ND                | ND             | ND              | ND             |
| Methionine       | ND                | ND             | ND              | ND             |
| Phenylalanine    | 101.81 ± 2.73 b   | 102.17 ± 1.59 b| 108.62 ± 1.17 c| 56.51 ± 1.55 a |
| Proline          | 38.01 ± 0.89 a    | 39.53 ± 0.59 ab| 42.07 ± 1.72 b | 47.50 ± 1.42 c |
| Serine           | 7.24 ± 0.24 c     | 5.72 ± 0.44 b  | 5.29 ± 0.36 b  | 2.30 ± 0.28 a  |
| Tyrosine         | 50.81 ± 1.11 c    | 46.26 ± 0.47 b | 57.62 ± 1.07 d | 16.70 ± 0.55 a |
| Threonine        | 18.62 ± 0.47 ab   | 19.73 ± 2.00 b | 24.54 ± 1.15 c | 16.17 ± 0.98 a |
| Valine           | 162.06 ± 1.51 a   | 172.04 ± 3.48 b| 173.83 ± 0.93 b| 187.65 ± 0.91 c|
| Total            | 991.35 ± 22.49 b  | 998.05 ± 21.16 b| 1059.27 ± 20.75 c| 845.80 ± 13.93 a|

$^1$ Mean value ± standard deviation with different letters (a, b, c, and d) in the same row indicate significant differences during fermentation time among FMB ($p < 0.05$). ND was not detected.
3.7. Reducing Sugar Content of a Fermented Mushroom Beverage (FMB)

The reducing sugar content in the FMB was exhibited, as shown in Figure 5. The amount of reducing sugar of FMB without *L. plantarum* SK15 was in the range of 0.39–1.03 mg/L, whereas reducing sugar content was in the range of 0.49–1.85 mg/L in a sample inoculated with *L. plantarum* SK15. After 480 h, the reducing sugar content decreased in natural fermentation. On the other hand, the reducing sugar content rose in FMB with a starter culture.

![Figure 5: The change of reducing sugar content in a fermented mushroom beverage (FMB) with and without starter culture *L. plantarum* SK15 during fermentation time.](image)

3.8. Total Phenolic Content (TPC) of a Fermented Mushroom Beverage (FMB)

Figure 6 exhibited TPC in FMB with and without starter *L. plantarum* SK15 as gallic acid equivalent. The trend of TPC was slightly increased in FMB with starter *L. plantarum* SK15. Its TPC was in the range of 42.04–63.92 µg GAE/mL of sample. TPC in a sample without starter decreased in the initial fermentation time and then increased after 96 h of fermentation time and was in the ranges of 36.38–60.35 µg GAE/mL of sample. TPC had a high level in FMB with starter *L. plantarum* SK15.

3.9. Antioxidant Activity of a Fermented Mushroom Beverage (FMB)

The ABTS assays assessed the antioxidant activity of the FMB with and without starter *L. plantarum* SK15 culture as trolox equivalent, as shown in Figure 7. There were in the ranges of 21.14–51.67 µg trolox equivalent/mL of FMB without starter. There was a trolox equivalent/mL of a sample with a starter in the ranges of 44.15–54.67 µg. The trend of antioxidant activity decreased in FMB without starter *L. plantarum* SK15. However, the antioxidant activity in FMB with starter *L. plantarum* SK15 was slightly increased and then stable.
3.9. Antioxidant Activity of a Fermented Mushroom Beverage (FMB)

The ABTS assays assessed the antioxidant activity of the FMB with and without starter culture *L. plantarum* SK15 as trolox equivalent, as shown in Figure 7. There were antioxidant activities in the ranges of 21.1–51.67 µg trolox equivalent/mL of FMB without starter. There was a decrease in trolox equivalent/mL of a sample with starter in the ranges of 44.15–54.67 µg. The trend of antioxidant activity decreased in FMB without starter *L. plantarum* SK15. However, the antioxidant activity in FMB with starter *L. plantarum* SK15 was slightly increased and then stable.

3.10. Microbial Load of a Fermented Mushroom Beverage (FMB)

FMB without starter culture did not observe LAB during 720 h fermentation time. A load of LAB decreased slightly, following fermentation time (7.3–8.6 log CFU/mL of the sample) in FMB with *L. plantarum* SK15 starter. The total bacteria load of FMB increased during fermentation time (Figure 8a). However, the bacteria count was not found for the duration of 360 h for FMB without starter culture; the maximum level of total bacteria was 2.64 log CFU/mL of a sample at 600 h of fermentation time. In the case of addition starter culture, the total bacteria count was in the range of 6.55–8.79 log CFU/mL of sample. *B. cereus*, *C. perfringens*, *S. aureus*, and *Salmonella* spp. were not detected in FPB samples throughout the fermentation process. *E. coli* were found less than 2.2 MPN/100 mL of sample.
3.10. Microbial Load of a Fermented Mushroom Beverage (FMB)

FMB without starter culture did not observe LAB during fermentation time. As a result, the presence of free amino acid decarboxylase was an important factor affecting the production of biogenic amines. The effectiveness of \textit{L. plantarum} SK15 as a non-amino acid decarboxylase-producing stain was examined in the reduction of biogenic amine accumulation during fermentation of FMB. The addition and no addition of \textit{L. plantarum} SK15 in the FMB fermentation process was studied on the alteration of PUT and SPD, including safety and quality. In the natural fermentation process (without \textit{L. plantarum} SK15 as a starter culture), the PUT content of FMB was higher than FMB with \textit{L. plantarum} SK15. Similarly, SPD content of FMB without \textit{L. plantarum} SK15 was at a high level compared with the inoculated \textit{L. plantarum} SK15 as a starter. This proves that starter culture \textit{L. plantarum} SK15 could reduce the accumulation of the amines in FMB. Nowadays, the biogenic amine study has a limitation for adding starter culture in FMB from \textit{H. erinaceus}. However, a few researchers exhibited other fermentation products. Wu et al. [22] studied biogenic amine (PUT, CAD, and TYR) reduction by mixed starter culture of \textit{L. plantarum} Shanghai brewing 1.08 and Zygosaccharomyces rouxii CGMCC 3791 in pickled Chinese sauerkraut (cabbage pickles and radish pickles). The production of biogenic amines decreased during fermentation with co-culture as a starter in both sauerkraut pickles. Similarly, Zaman et al. [35] demonstrated that HIS content was reduced by 27.7% and 15.4% by the addition of starter \textit{Staphylococcus carnosus} FS19 and \textit{Bacillus amyloliquefaciens} FS05, respectively, and that the overall BA content was 15.9% and 12.5% less in inoculated \textit{S. carnosus} FS19 and \textit{B. amyloliquefaciens} FS05, respectively, compared with the control in fish sauce product. A review of Mah and Hwang [36] showed the same result. They studied biogenic amine formation in a salted and fermented anchovy by \textit{Staphylococcus xylosus} as a starter culture. \textit{S. xylosus}, applied as a protective culture in salted and fermented anchovy, could reduce histamine and overall amines by 18% and 16%, respectively, compared with the control.

The content of PUT and SPD in FMB was gradually raised with the increasing fermentation time till the end of fermentation time in this study at 720 h. It might have occurred from proteolysis during fermentation time. As a result, the presence of free amino acid was increased, which led to the formation of biogenic amine in a fermented sample [37]. FMB samples with starter culture had lower biogenic amine contents than samples without a starter culture. The starter culture factor influenced the production of biogenic amine.

![Figure 8](image-url)  
**Figure 8.** The change of microbial load in a fermented mushroom beverage (FMB) with and without starter culture \textit{L. plantarum} SK15 during fermentation time: (a) total bacteria count; (b) lactic acid bacteria (LAB).

4. Discussion

In the present study, the selected \textit{L. plantarum} SK15 strain showed a negative reaction that led to a low accumulation of biogenic amine in a fermentation process. There was a study of \textit{L. plantarum} N8 on agmatine (AGM) and PUT production using amino acid decarboxylase. Arginine hydrochloride and ornithine monohydrochloride decarboxylase medium were used as detection. The result showed that \textit{L. plantarum} N8 strain did not produce AGM and PUT [34]. Thus, the suitable microorganism that did not produce decarboxylase was an important factor affecting the accumulation of biogenic amines.
in fermented foods. A possible hypothesis explained that specific starter culture did not produce an amino acid decarboxylase enzyme, which changed the amino acid to a biogenic amine. Furthermore, the starter culture may inhibit biogenic amine forming bacteria by competition in the environment, rapid nutrient usage, depletion, or production of a bacteriocin-like inhibitory substance [36].

Analysis of acid content could indicate the safety and quality of FMB. The pH level slightly decreased during fermentation time in both FMB with and without *L. plantarum* SK15. The acceptable pH was less than or equal to 4.3 for fermented beverages, according to Thai Community Standard Product (TCPS) [38]. All of the pH of FMB with starter culture was in the range of TCPS regulation from the beginning of fermentation onward. However, the pH of FMB without starter culture was less than pH 4.3 after fermentation 200 h. Total acidity as a percentage of lactic acid equivalent was increased in FMB with a starter. *L. plantarum* SK15 was one of LAB that could produce lactic acid from the anaerobic microbial breakdown of carbon source (sugar cane) during the process [39].

The level of methanol in a beverage is one of the strictest regulations for the food industry and consumer safety. The permissible level of methanol in fermented plant beverages is 240 mg/L according to TCPS. Another confirmation was used as an indicator of the safe methanol level. The presence of methanol should be less than 8% in spirit beverages [28]. In this study, the methanol level was not found in FMB samples. It might be that the mushroom contained a low level of pectin, which is located at the cell wall during ripening. Pectin can produce methanol by removing methyl groups with pectin methylesterase [26].

The permissible level of ethanol in beverages was less than 3% (v/v) with reference to TCPS. All of FMB were within the acceptance limit of ethanol. In the natural fermentation process (without *L. plantarum* SK15 as a starter culture), the ethanol content of FMB was higher than FMB with *L. plantarum* SK15. This proves that the use of starter culture *L. plantarum* SK15 could reduce the ethanol content in FMB. The possible reason for the elevated level of ethanol might be the contamination of some microorganisms that might contaminate from soil or water in raw mushroom material during fermentation and production. For example, yeast, which is contaminated with *H. erinaceus*, could change the sugar to alcohol by aerobic reaction [33]. Excess intake of ethanol leads to high blood alcohol concentration, increases osmolality, and the accumulation of metabolites can cause high anion gap metabolic acidosis, acute renal failure, or unexplained neurologic disease [40].

In general, amounts of amino acid precursors were related to their corresponding biogenic amines. The total amino acid decreased from 998.05 to 845.80 µg/L in a sample with *L. plantarum* SK15 starter culture. Because amino acid was the precursor of biogenic amine, which could be metabolized during the fermentation process, the fermented beverages added starter had higher proteolytic activity. It enhanced catabolite in free amino acids to other products [37]. The decreased amino acid (precursors of biogenic amine) affects the reduction of biogenic amine accumulation. The present report is related to the previous study of *L. sakei* BCC102 and Debaryomyces Hansenii BCC 106 as a starter culture in Nham fermentation process for five days. The content of total free amino acid content in Nham inoculated *L. sakei* BCC102 and mixed *L. sakei* BCC102, and *D. hansenii* BCC 106 culture decreased by 13.3% (from 31.83 to 27.43 mg/100 g sample) and 19.73% (from 30.97 to 24.86 mg/100 g sample) [37]. From previous literature, arginine is a precursor of PUT and SPD. The arginine in the sample with *L. plantarum* SK15 starter culture was 23.30 and 23.40 µg/L at 0 and 720 h, respectively. The nonsignificant change of the arginine content might be due to other PUT and SPD formation pathways from ornithine [14].

The changes in amino contents may be due to these amino acids being used for the growth of the microorganisms and might be metabolized to flavors [41]. The report confirmed that amino acids can reform to other compounds. Aspartic acid could be converted to sweeteners (aspartame). Leucine, threonine, and valine can change to volatile compounds. Serine transforms to glycine. Glutamic acid is well-known to exhibit umami-
type flavor and is widely used in the food industry. Aspartic acid also imparted umami taste in foods.

The starter culture addition affects a reducing sugar content. The natural fermentation process involved lower reducing sugar content than FMB with *L. plantarum* SK15. The possible reason was that the starter increased the ability of β-glucosidase, which destroys the glycosidic bond of polysaccharides [42]. Furthermore, the total sugar hydrolysis caused the change of reducing sugar content by acid present in raw material, which might affect the degradation of disaccharides to monosaccharides [43].

The TPC (gallic acid equivalent) trend in FMB with *L. plantarum* SK15 had a high level compared to the sample without *L. plantarum* SK15. Sample with *L. plantarum* SK15 had low pH, which helps to enhance the availability of the phenol extract. Bautista-Expósito et al. [44] reported that the low pH condition had a high level of total soluble phenolic compound in the hydrolysis of wheat bran. The antioxidant activity in FMB with *L. plantarum* SK15 had a clearly higher level than the sample without *L. plantarum* SK15. Because of *L. plantarum* SK15 as LAB. The previous work presented a fermented beverage with antioxidant properties using the lactic acid bacteria as a starter culture [39]. Furthermore, some data report that mushroom is the source of prebiotic that can support the activity of probiotic. β-glucan is one of the polysaccharide molecules found in many sources, including *H. erinaceus*. β-glucan is linked with its ability to show significant immunomodulative properties, possess better antioxidant activities, and exhibit scavenging capacities against free radicals [45].

FMB without starter culture did not observe for the LAB during 720 h fermentation time. A load of LAB decreased slightly along fermentation time in FMB with *L. plantarum* SK15 starter. It might be that the starter *L. plantarum* SK15 is one of LAB; it was used in the fermentation process. The total bacteria and microbes, such as *B. cereus*, *C. perfringens*, *S. aureus*, *Salmonella* spp., and *E. coli*, were found with the values within the acceptance range following the TCPS, due to the partial pasteurization being applied to the production of FMB fermentation. Thus, there were no assessed pathogenic microbes in the FMB sample with or without *L. plantarum* SK15 starter culture at the beginning and after the fermentation.

5. Conclusions

The present study studied the effectiveness of starter *L. plantarum* SK15 on biogenic amine (PUT and SPD), pH, total acidity, alcohol content (methanol and ethanol), amino acid, reducing sugar content, bioactive compounds (TPC and antioxidant activity), and microbial loads in FMB samples with and without starter. *L. plantarum* SK15 did not produce amino acid decarboxylase. Therefore, it was used as a starter culture in the process of FMB fermentation. PUT and SPD were slightly increased following the fermentation time. The FMB with starter culture contained a low PUT and SPD content when compared with the FMB without starter culture. The addition of starter culture in FMB had lower pH and less alcohol content than FMB without starter culture. The pH and alcohol values were acceptable, according to TCPS. The total amino acid of the FMB with *L. plantarum* SK15 was decreased after 720 h fermentation. After 480 h, the reducing sugar content decreased in natural fermentation, while the level of reducing sugar increased in the FMB with *L. plantarum* SK15. In addition, FMB with *L. plantarum* SK15 had a high level of TPC and antioxidant activity compared to FMB without *L. plantarum* SK15. The results suggested that the use of specific starter culture (*L. plantarum* SK15) had the potential to be a starter culture in the process of FMB fermentation.

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