Exploration of Co-Inoculation of Lactiplantibacillus plantarum and Kazachstania bulderi for Potential Use in Mushroom Pleurotus eryngii Pickle Fermentation

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Abstract: This study investigated the impact of Lactiplantibacillus plantarum PC-004 (PL) and Kazachstania bulderi PY-1 (PY), selected from home-made Paocai, on the physicochemical properties and volatile flavor compounds of fermented Pleurotus eryngii. P. eryngii, fermented by PY, was maintained at about pH 5 throughout fermentation, while co-fermentation exhibited a higher acid production rate and lower post-acidification value compared to mono-fermentation by PL. The viable cell counts of PY exhibited no difference between the mono- and co-fermentation, while PL significantly decreased in the co-fermentation after 168 h. Compared to mono-fermented P. eryngii, co-fermentation had lower levels of certain biogenic amines. Co-fermented P. eryngii had a higher value of nitrite in the early fermentation stage, but this decreased during mono-fermentations. Additionally, among the three different groups, PY fermentation had more esters, PL fermentation had more ketones and aldehydes, and co-fermentation had more abundance and diverse volatile flavor compounds than mono-fermentations. Our results suggest that co-fermentation with K. bulderi and L. plantarum is suitable for producing fermented P. eryngii, highlighting their potential for the fermentation of edible mushrooms.

Keywords: co-inoculation; L. plantarum; K. bulderi; fermented P. eryngii; physicochemical properties

1. Introduction

Pleurotus eryngii, also known as the king oyster mushroom, is widely cultivated and consumed for its unique flavors, low caloric and high nutritional value, and medicinal properties. This mushroom species is rich in polysaccharides [1], which have been found to exhibit various biological activities and health benefits, including antioxidant, anti-hyperlipidemic, anti-tumor, immunoregulatory, bacteriostatic, genoprotective effects, the promotion of intestinal health and disease prevention [2–4]. In China, the production of P. eryngii is high, but the forms of P. eryngii that are consumed are primarily limited to fresh and dried products because of the high perishability of fresh edible mushrooms, which have a high water content, a fast respiration rate, and a tendency to spoil quickly due to the presence of microflora [5,6]. Therefore, it is necessary to increase the consumption patterns of king oyster mushrooms by developing new processing technologies and value-added products so as to address the imbalance between its high production volume and limited consumption forms.

Fermentation adds new sensory qualities to food products and protects them from microbial spoilage [7]. Additionally, fermentation enhances the health-promoting properties of the product by introducing beneficial microflora and metabolites [8]. This traditional
Fermentation 2024, 10, 428

preservation technique has been used to preserve the fruiting bodies of wild-growing and cultivated edible fungi [9,10]. However, despite its widespread use for this purpose in various regions of the world, many of these mushroom fermentation processes remain rooted in home-based lactic acid fermentation practices and are unsuitable for industrial-scale production [9].

Previous studies demonstrated that lactic acid bacteria with documented health-beneficial properties can be used as a starter culture for edible mushroom fermentation, and this technology can effectively control spoilage and pathogenic microorganisms, increase the content of organic acids, decrease the content of nitrite, improve the sensory characteristics of final products, and exhibit relative probiotic activities [11–14]. Therefore, lactic fermentation can be applied to manufacturing new edible mushroom products. In addition, the core microbiota in traditional Chinese pickles revealed that, besides lactic acid bacteria, a large number of yeasts are present in all the samples investigated [15], which implies that yeasts may have a crucial role in the fermentation process of vegetables. The presence of yeasts in pickles contributes to quality, flavor, texture, and improved shelf life [16]. Under anaerobic conditions, yeasts perform alcoholic fermentation and produce ethanol that forms esters with organic acids, which are the primary end product of lactic acid bacteria. This enhances the presence of aromatic compounds and ethyl acetate in fermented products [17,18].

Considering that fermentation carried out by a single strain of lactic acid bacteria limits organoleptic properties [19] versus the potential co-fermentation by lactic acid bacteria and yeast for manufacturing fermented products [20–22], there has been limited focus on the co-fermentation of industrial mushrooms using different microflora species. In this study, we explored the physiochemical characteristics of *P. eryngii* fermented by starter cultures that were composed of *L. plantarum* and *K. bulderi*. The dynamics of pH, viable cell counts, organic acids, biogenic amines, nitrites, and volatile flavor compounds were investigated to determine the differences between co-fermentation and mono-fermentation. The findings from this research are expected to contribute to a more comprehensive understanding of the effects of the co-fermentation of lactic acid bacteria and yeast on edible mushroom fermentation, which was helpful in contributing to future products based on edible mushrooms.

2. Materials and Methods

2.1. Microorganisms and Raw Materials

*L. plantarum* PC-004 (PL) and *K. bulderi* PY-1 (PY) were used in this study. These lactic acid bacteria (LAB) and yeast were isolated from homemade Sichuan paocai and identified by sequencing 16S rRNA and the ITS1-5.8S-ITS4 region of rRNA, respectively. The two strains were selected for this study since they were the dominant genera and species isolated from homemade Sichuan paocai. Our preliminary experimental results indicated that *P. eryngii*, fermented by the above two strains, had better sensory tasting, so the two strains were initially used as single starter cultures and later as mixed starter cultures.

The fresh king oyster mushrooms *P. eryngii* used in this study were purchased from the local market (Kunming, China).

2.2. Preparation of Fermented Mushrooms

The mushrooms were sorted, cleaned, and washed under cold running water to remove surface impurities. Next, the mushrooms were cut into slices, and 150 g of the treated mushrooms were placed into screw-capped glass jars before 150 mL of water was added with a final concentration of 2% salt (w/v) and 1% sucrose (w/v). Then, the jars were placed into boiling water for 20 min. After cooling to room temperature, the starter cultures, including the single LAB strain (PL), single yeast strain (PY), and the mixed starter culture (PYL) composed of LAB and yeast, were added to initial viable cell counts of LAB (7.00 log CFU/mL) and yeast (6 log CFU/mL) without any inoculation as the control. All treatments were fermented at 25 °C for 7 days.
2.3. Microbiological Analyses and pH Measurement

The viable cell counts of PL and PY in fermented mushrooms were determined by the plate count method. Fermentation juice aseptically taken from glass jars was sequentially decimal-diluted, after which 100 µL was spread onto agar media. The De Man, Rogosa, and Sharpe (MRS) agar media for PL in all treatments were incubated at 37 °C for 48 h. Yeast extract peptone dextrose (YEPD) agar media was used for PY in mono-fermented *P. eryngii* and YEPD agar with 100 µg/mL erythromycin for PY in the co-fermented (PYL) *P. eryngii* were incubated at 25 °C for 48 h. The pH values were measured using a digital pH meter (Mettler-Toledo Instruments Co., Shanghai, China).

2.4. Determination of Organic Acids

The lactic acid and acetic acid in the fermented *P. eryngii* were analyzed by HPLC method. The samples (2 g, containing 1 g of solid substances and 1 mL of fermentation solution) at different fermentation times (0 h, 24 h, 72 h, 120 h, and 168 h) were homogenized. The homogenization was ultrasonically extracted for 1 h, then centrifuged at 8000 × g for 10 min. The supernatant was diluted using water at 7 mL, which was then filtered with a 0.45 µm filter membrane. The lactic acid and acetic acid analyses were carried out using an HPLC system (Agilent 1100, Boblingen, Germany) containing a reversed-phase C18-BP column (250 mm × 4.6 mm, 5 µm) at a column temperature of 30 °C and a detection wavelength of 214 nm. The mobile phase was 0.1 mol/L of the phosphate solution at pH 3.0, and the sample was eluted for 30 min. The flow rate was set at 0.8 mL/min, and the injection volume was 10 µL. The standard curve was constructed with organic acid standard solutions (0.01–10 mg/mL).

2.5. Determination of Nitrates

A solution of 0.5 mL of the saturated sodium borate solution (dissolved 25.0 g of Na₂B₄O₇·10H₂O in 500 mL hot distilled water) was added to 0.2 g of homogenized final products, followed by a boiling water bath for 15 min. After cooling to room temperature, 0.5 mL of the 0.25 mol/L potassium ferrocyanide solution (dissolved 21.5 g of potassium ferrocyanide in 200 mL distilled water) was used with shaking for 15 s. Then, 0.5 mL of the zinc acetate solution (22 g of zinc acetate in 3 mL of acetic acid with 100 mL of distilled water) and 1 mg of activated charcoal were added sequentially. After remaining at 25 °C for 30 min, the mixture was centrifuged at 8000 × g for 15 min. For the nitrite analysis and calibration control, 70 µL of the supernatant or diluted water was mixed with 65 µL of 0.4% (m/v) sulfanilic acid solution and 65 µL of the 0.2% (m/v) N-(1-Naphthyl)-ethylenediamine dihydrochloride solution, before shaking the mixture uniformly. After this, the mixture remained at 25 °C for 15 min, and the absorbance was read at 540 nm. The analysis of each sample was performed in triplicate.

2.6. Biogenic Amine Analysis

The determination of biogenic amine was carried out using the HPLC method. Briefly, a mixture of 1 g of solid substance and 1 mL of fermentation solution was homogenized. After that, the homogenization was ultrasonically extracted for 1 h and centrifuged to obtain the supernatant, diluted with water to 7 mL, and the treatments were filtered through a needle filter. The supernatant (0.2 mL) was placed in a brown centrifuge tube and, sequentially, 40 µL of the 8% (m/v) NaOH solution, 60 µL of the saturated sodium bicarbonate aqueous solution, and 0.2 mL of 10 mg/mL of dansulfonyl chloride with acetone were added. After a light-avoiding reaction at 50 °C for 40 min in a water bath, mixtures were cooled to room temperature. Then, 100 µL of ammonium hydroxide was added and kept at room temperature for 30 min. Mixtures were diluted with methanol to 1 mL and filtered through a 0.45 µm needle filter prior to HPLC analysis. The biogenic amines of fermented mushroom were carried out by an HPLC system (Waters 2695, Milford, CT, USA) using a reversed-phase C18 column (Compass C18 column; Waters, Milford, CT, USA) (250 mm × 4.6 mm, 5 µm) at a column temperature of 30 °C. A gradient elution
program consisting of methanol (A) and water (B) was used as follows: 0 to 8 min, 80% to 80% A; 8 to 18 min, 80% to 100% A; 18 to 22 min, 100% to 100% A; 22 to 25 min, 100% to 80% A; and 25 to 35 min, 80% to 80% A. The flow rate was set at 1 mL/min, and the injection volume was 10 µL.

2.7. Determination of Volatile Flavor Compounds

Volatile compounds were quantified by headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GCMS). The solid phase microextraction head was aged at the gas chromatographic inlet for 16 min at 250 °C. Then, 2 g of the fermented P. eryngii sample saturated with 1.125 g of NaCl was filled into 20 mL of a headspace vial fitted with polytetrafluoroethylene–silicone septa in an aluminum cap with a reaction at 60 °C for 40 min. The volatiles were collected with an extraction fiber (SUPELCO 50/30 µm DVB/CAR/PDMS, Darmstadt, Germany) into the headspace bottle for 40 min. After extraction, the fiber was immediately inserted into the injection port of the GC-MS at 250 °C for 0.5 min. GC-MS was carried out on an Agilent 7890AGC 5975C system coupled with a quadrupole mass filter for mass spectrometric detection (Agilent Technologies, Santa Clara, CA, USA), and chromatographic separation was carried out on an HP-5MS column (30 m × 250 µm × 0.25 µm, Agilent, Santa Clara, CA, USA). The analysis parameters were as follows: inlet temperature was 280 °C, column flow rate was 3 mL/min, and injection volume was 1 µL. The column temperature was held at 40 °C for 1.0 min, then increased to 160 °C with a 3 °C/min rate, then increased to 230 °C with a 15 °C/min rate, and held for 1 min. The temperature of the ion source of electron ionization was 230 °C, the temperature of the quadrupole was 150 °C, and the mass scanning range was 50–550 m/z. The compounds were tentatively identified by matching the mass spectra with the NIST14 mass spectral database (National Institute of Standards and Technology, Gaithersburg, MD, USA). The analysis of each sample was performed in triplicate.

2.8. Statistical Analysis

One-way analysis of variance (ANOVA) with Tukey's test (p < 0.05) was performed using GraphPad Prism (version 5.01 for Windows, Graphpad Software, San Diego, CA, USA). The data are shown as the mean value ± standard deviation. The unsupervised principal component analysis (PCA) was adopted to visualize the grouping of the fermented P. eryngii, whereas the supervised partial least squares discriminant analysis (PLS-DA) was implemented to determine potential volatile makers. Volatile flavor compounds, which were responsible for the discrimination of fermented P. eryngii, were determined by the varying importance in projection scores (VIP > 1) calculated through the PLS-DA models. The heatmap was constructed using ClustVis (version 2.0) (https://biit.cs.ut.ee/clustvis/) (accessed on 17 August 2023).

3. Results and Discussion

3.1. Dynamics of Microbial Counts and pH during Fermentation

First, we monitored the pH dynamics in P. eryngii fermented by different starter cultures (Figure 1B). The initial pH ranged from 5.60 to 5.80 in all fermented samples. For the fermented P. eryngii using PY, there were no clear changes in pH value in the early fermentation stage (0–4 h). After 4–24 h of fermentation, the pH value decreased from 5.78 to 4.91 and was kept at a stable range of 4.78–4.95 until the end of fermentation. For the fermented P. eryngii by PL, the pH value sharply declined to a level of 4.58 after the first 8 h of fermentation, and then the pH value kept decreasing to 3.88 after the next 8 h of fermentation. After that, the pH value slowly decreased from 3.88 to 3.48 in the 16–48 h fermentation stage and remained stable until the end of fermentation. For the co-fermented P. eryngii, the pH value decreased more sharply than the two groups that were fermented by a single strain, which declined to a level of 4.29 at the early 8 h fermentation process and kept decreasing to 3.89 at 12 h of fermentation. Then, the pH value decreased from 3.89 to 3.58 with a low reduction rate in the 12–24 h fermentation stage and remained
stability until the end of fermentation. We found that the co-fermentation of yeast and lactic acid bacteria could reduce the pH level more quickly than mono-fermentation, but the pH level of co-fermentation was higher than the single LAB strain of fermentation at the end fermentation stage, which meant that co-fermentation had the capacity of a high acid production rate and weak post-acidification, which is positively related to the textural properties and flavor during storage [23]. In addition to the result of viable cell count dynamics in different fermented values of P. eryngii, we thought that the introduction of yeast could not only decline the pH value sharply to form a suitable environment for the later fermentation process but could also be beneficial for post-acidification control. However, the result was slightly different from the studies on the co-fermentation of yeast and lactic acid bacteria in sourdough and soybean paste [24,25].

We investigated the dynamics of the two strains in different fermentation groups (Figure 1C). There was no significant difference in the viable cell counts of PY between P. eryngii fermented by PY and PYL. In the first 24 h of the fermentation stage, the viable cell counts of PY in the two fermentation groups increased to 8.20 log CFU/mL and kept a stable range from 8.10 to 8.50 log CFU/mL in the fermentation stage of 24–120 h. After that, the viable cell counts of PY decreased to a level lower than 8.0 log CFU/mL. In the first 24 h fermentation process, there was no significant difference in the viable cell counts of PL between P. eryngii fermented by PY and PYL, which both increased to 9.00 log CFU/mL. After that, the viable cell counts of PL in P. eryngii fermented by PY kept a stable range from 9.00 to 9.20 log CFU/mL in the fermentation stage of 48–120 h and slowly decreased until 120 h. However, the viable cell counts of PL when fermented by PYL sharply decreased from 9.20 to 7.90 log CFU/mL in the fermentation stage of 48–96 h, even though there was no inhibition between PY and PL on agar plates (Figure 1D).

Figure 1. States of fermented P. eryngii by different starter cultures (A) and inhibition activity between L. plantarum and K. bulderi (D), and dynamics of pH (B) and viable cell counts (C) in different fermented P. eryngii. Black lines and gray lines in Figure 1C represent mono-fermentation and co-fermentation, respectively. Error bars represent the standard errors of the means of three replications.
3.2. Organic Acids during Fermentation

Organic acids are directly related to pH in the fermentation process and affect the organoleptic properties and shelf life of the fermented products [24]. Lactic acid and acetic acid are the main organic acids produced by lactic acid bacteria and yeast in the fermentation process, respectively [26,27]. So, the dynamics of the content of lactic acid (Figure 2A) and acetic acid (Figure 2B) in the fermentation process were monitored. There was no apparent acetic acid accumulation in all the samples. *P. eryngii* fermented by PL and PYL accumulated to about 0.2 mg/g of lactic acid, but this was not the same when fermented by PY. Lactic acid had the highest accumulation rate in the first 24 h of the fermentation stage in both *P. eryngii* fermented by PL and PYL and increased from 0.12 mg/g to 3.25 mg/g, 0.13 mg/g to 3.20 mg/g, respectively. After that, the lactic acid content in *P. eryngii* fermented by PL kept increasing to 5.13 mg/g until the fermentation ended, and it remained stable in co-fermentation. Also, we noticed that some unidentified spoilage microflora disturbed the fermentation process in the control jars (Figure 1A). Lactic acid bacteria inhibit the spoilage microflora growth primarily by means of a decrease in pH [28], and yeasts cause this by producing a complex array of aroma compounds, such as esters, higher alcohols, carbonyls, phenolic compounds, fatty acid derivatives, and sulfur compounds [29].

![Figure 2](image-url). Contents of lactic acid (A), acetic acid (B), biogenic amines (C) and nitrite (D) in different fermented *P. eryngii*. Error bars represent the standard errors of the means of three replications. The means followed by different lowercase letters for the different fermented *P. eryngii* indicate significant differences at \( p < 0.05 \), and means followed by different capital in each treatment for the different fermentation time indicate significant differences at \( p < 0.05 \).
3.3. Biogenic Amines in Different Fermented P. eryngii

Biogenic amines (BAs) are produced by microbial decarboxylases on free amino acids in raw materials and may cause food poisoning and produce undesirable effects, such as influencing the freshness and other organoleptic characteristics of fermented foods [30,31]. Tryptamine, putrescine, cadaverine, beta-phenethylamine, spermidine, spermine, histamine, and tyramine were detected in P. eryngii fermented by different starter cultures. As the results show in Figure 2C, there was no clear evidence of tyramine and beta-phenethylamine accumulation in all fermented P. eryngii. The highest BA was tryptamine in mushrooms fermented by PY (650.72 ± 41.00 µg/g) and was significantly higher than in the PL fermentation (56.27 ± 6.01 µg/g) and the co-culture (294.07 ± 21.11 µg/g). Except for this, the other BAs in P. eryngii fermented by PL were significantly higher than PY alone and the co-fermentation. The total BAs in P. eryngii fermented by PL (387.90 ± 2.14 µg/g) was lower than those fermented by PY (744.42 ± 24.62 µg/g) and PYL (489.12 ± 24.99 µg/g) because of the main contributor of tryptamine and spermidine. The exposure assessment of tryptamine from the European Food Safety Authority (EFSA) calculated the highest exposure levels of tryptamine for 'cheese' (0.1–12.3 mg/day), 'fish sauce' (0.2–12.2 mg/day), and 'fermented sausages' (1.9–10.7 mg/day) [32]. According to the exposure assessment, P. eryngii fermented by PL and PYL are safer than that fermented by PY. The other main contributor of total BAs was spermidine in P. eryngii fermented by PY, PL, and PYL, which were 74.85 ± 15.83 µg/g, 296.68 ± 5.16 µg/g, and 174.27 ± 22.30 µg/g, respectively. However, spermidine is theorized to prolong human lifespan, improve anti-tumor immunity, and postpone the manifestation of various age-associated disorders, such as cardiovascular disease and neurodegeneration [33,34]. As the main culprits of BAs in food poisoning in Japan and Europe [35], the content of histamine and tyramine was low in all fermented P. eryngii. The results demonstrated that the mixed starter culture of yeast and lactic acid bacteria could neutralize BA production in fermented mushrooms compared to mono-fermentation. Several studies [36–39] have shown that a mixed starter culture could decrease the levels of some biogenic amines.

3.4. Dynamics of Nitrite in Fermentation Process

We observed nitrite level changes in the fermentation process of different P. eryngii and its formation and accumulation may be a food safety risk and affect human health [40]. As the results show in Figure 2D, the nitrate content of P. eryngii fermented by PL remained at a lower level than that fermented by PY and PYL during the whole fermentation process, and its value ranged from 0.21 to 0.40 mg/kg. The nitrite content of the mushroom fermented by PY increased from 0.21 to 1.05 mg/kg after 120 h of fermentation and then decreased to 0.88 mg/kg at the end of fermentation. During co-fermentation, nitrite increased to the highest level (1.46 mg/kg) after the first 24 h of fermentation, then decreased with prolonged fermentation time and reduced to 0.87 mg/kg at the end of fermentation. The interactions between lactic acid bacteria and yeast could facilitate nitrite accumulation in short-time fermentation, and yeast may have the ability of nitrite decomposition. Nitrite concentration significantly increases at the early fermentation stage but decreases over time in fermented foods, especially in spontaneously fermented productions because this nitrite can be degraded as the sole nitrogen source for yeast [41] or by acids, enzymes, and other metabolites produced by lactic acid bacteria [42].

3.5. Volatile Flavor Compounds in Different Fermented P. eryngii

The volatile flavor compounds of P. eryngii, fermented by different starter cultures, were detected by HS-SPME-GCMS. A total of 60 kinds of typical flavor compounds were detected, including 17 alcohols, 13 esters, 12 aldehydes, 7 ketones, 4 alkanes, 4 organic acids, and 3 other compounds. Among the volatile flavor compounds, 12 of them were commonly present in all the fermented P. eryngii, and 10, 11, and 6 of them were uniquely detected in P. eryngii fermented by PY, PL, and PYL, respectively (Figure 3A). In these unique volatile flavor compounds, 3 alcohols, 6 esters, and 1 organic acid existed in P.
P. eryngii fermented by PY. In total, 2 alcohols, 1 ester, 1 alkenoic acid, 2 aldehydes, 4 ketones, and 1 organic acid existed in P. eryngii fermented by PYL. In total, 3 aldehydes, 2 alkenes, and 1 organic acid existed in P. eryngii fermented by PYL. Unsupervised PCA was applied to distinguish the volatile flavor compounds of samples fermented by different strains. A clear separation was observed (Figure 3B) among the three different fermented P. eryngii. The first two main components (PC1 and PC2, with contributions of 70.21% and 27.65%, respectively) explained 97.86% of the total variance. Compared to P. eryngii fermented by PYL, P. eryngii fermented by PY had fewer aldehydes, alkenes, and ketones, and that fermented by PL had fewer esters (Figure 3C). Specifically, there were 12 and 6 esters in yeast fermentation and co-fermentation, respectively. However, there were only two esters that were fermented by PL. We noticed that of all the fermented P. eryngii, ethyl esters predominated in the PY-fermented P. eryngii but were not detected in the PL fermentation. A similar result has been observed [43] in which lactic acid bacteria may depress the production of fatty acid precursors in yeast due to the fatty acid precursor level rather than the activity of the biosynthetic enzymes, which is the major limiting factor for ethyl ester production [44]. Aldehydes and ketones, which are the significant volatile compound compositions in fermented foods [45–47], were rarely detected in PY-fermented P. eryngii but were high in that fermented by PL and PYL. Compared to the mono-fermentations, co-fermentation exhibited more types of flavor compounds. The common substances between co-fermentation and mono-fermentations, which were more than those between the two mono-fermentation groups, implied that the flavor substances in co-fermented P. eryngii might be predominantly derived from the superposition of flavor substances fermented by lactic acid bacteria and yeast rather than new formations by these two strains during fermentation. This also showed that co-fermentation can enhance the complexity of flavor compounds in the fermented products.

Figure 3. Differences of volatile flavor compounds in different fermented P. eryngii. (A)—Venn map of volatile flavor compounds in the three fermented P. eryngii, (B)—PCA analysis of volatile flavor compounds in the three fermented P. eryngii, (C)—Heatmap of volatile flavor compounds in the three fermented P. eryngii.
The potential volatiles that could be identified to discriminate fermented *P. eryngii* by different microbial cultures, and we performed supervised PLS-DA on the volatile data set (Figure 4). According to the PLS-DA model, the VIP scores of volatiles were calculated, and important volatile flavor compounds were selected based on the VIP values, which are not less than one. Univariate analysis was also carried out on the same data set to identify the significant volatiles that were helpful in distinguishing the three groups of fermented *P. eryngii*. A total of 23 with both VIP > 1 and *p* < 0.05 were determined, including 7 aldehydes, 5 alcohols, 4 esters, 3 alkenes, 2 ketones, and 2 organic acids. There were only two compounds, 3-methyl-1-butanol and hexanal, that had VIP scores > 2.0. According to the “National food safety standard—Standard for uses of food additives” (GB 2760-2014) [48], these two compounds are mainly used for the preparation of apple, banana, and tomato flavors.

![Figure 4](image.png)

**Figure 4.** PLS-DA analysis of different *P. eryngii*. (A)—Score chart of partial least squares discriminant analysis (up) and validation of diagnostic statistics for PLS-DA models (down), (B)—Diagram with chromatographic peaks of samples with VIP > 1 (*p* < 0.05) of different fermented *P. eryngii*.

4. Conclusions

We performed a comprehensive investigation to characterize and distinguish *P. eryngii* fermented by a single lactic acid bacterium, single yeast, and a mixed starter culture. We investigated the characteristics of the mixed starter culture of lactic acid bacteria and yeast to ferment *P. eryngii*. The co-fermentation of lactic acid bacteria and yeast increased the acidification rate of *P. eryngii* at the early fermentation stage and remained at a higher level at the end stage. Additionally, all the fermented *P. eryngii* under investigation were distinct in terms of the composition and abundance of volatile compounds observed. Our results indicated that *P. eryngii* fermented by *L. plantarum* PL and *K. bulderi* PY had the most abundant volatile compounds. Therefore, edible fungi fermented by these combinations of different microorganism strains have a very good application potential. Despite this, we think that it is still a challenge to design an adaptable starter culture for different edible fungi by means of a bottom-up strategy.

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