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Effects of salt concentration and *Lactobacillus plantarum* population on grey oyster mushroom fermentation

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ABSTRACT

This study aimed to evaluate the effectiveness of different conditions (i.e., different salt concentrations from 3.5 to 4.5%, and initial *Lactobacillus plantarum* populations from 10^4 to 10^6 CFU/mL) on the quality of fermentative grey oyster mushrooms at 15°C during 25 days. The quality parameters i.e., total color difference, texture, total soluble solid, pH, lactic acid bacteria counts, total polyphenol content (TPC) and the free radical scavenging activity were measured during the fermentation. The results illustrated that the fermentation of grey oyster mushrooms would be in NaCl (3.5%) and initial *Lactobacillus plantarum* (10^4 CFU/mL). The obtained data showed that although no significant difference was observed among different salt concentrations and inoculum starter culture on the quality of fermented mushrooms, the fermentation conditions enhanced the rising of TPC resulting in an increase in antioxidant activities of fermented oyster mushrooms. This study reveals that fermented grey oyster mushrooms can be used as a source of healthy plant-based foods by vegetarians to improve their nutrition diet.

1. INTRODUCTION

The grey oyster mushroom (*Pleurotus sajor-caju*) is rich in protein, fiber, vitamins, and minerals. It possesses unique nutritional and medicinal values, along with a distinct aroma and taste (Dunkwal et al., 2007). Antioxidants present in mushrooms are likely to be protective agents due to their ability to reduce oxidative damage (Rashidi & Yang, 2016).

In addition, oyster mushrooms are a great source of dietary fiber and β -glucan, which help reduce blood cholesterol levels and glycemic response (Manzi & Pizzoferrato, 2000). The dietary fibers in oyster mushrooms can increase the transit time of bowel contents, enhancing bulk, frequency, and ease of fecal voiding. Consequently, they may protect the body from irritable bowel syndrome and colon cancer (Wan Rosli et al., 2011). Therefore, oyster

mushrooms are considered a potential food source for consumers, particularly those following a vegetarian diet (Ahmed et al., 2016). Fresh mushrooms are easily dehydrated, damaged and therefore they have a short shelf-life (Mishra & Mani, 2019). In Viet Nam, the price of fresh mushrooms is generally unstable. The products of grey oyster mushrooms are not diversified, mainly fresh and dried forms. On the other hand, lactic acid fermentation is usually used to preserve and prolong the shelf-life of vegetables while fermented products were considered as favorite traditional foods (Swain et al., 2014). Utilizing *Lactobacillus plantarum* in lactic acid fermentation methods for mushroom preservation indicates that the inoculated fermentation method is superior for long-term preservation compared to the traditional heavy salting technique (Zheng et al., 2018).

Generally, there are several ways to prepare vegetables for fermenting: grating, shredding, chopping, slicing, or leaving them whole. Most importantly, the fermentation process is mainly affected by technological conditions such as salt concentration and temperature. Salt plays a crucial role in improving salty taste and contributing to the extraction of nutrients from the vegetables such as sucrose, glucose, and fructose (Xiong et al., 2016). Additionally, the salt could inhibit a wide range of undesired microorganisms during the fermentation. According to research by Chun et al. (2020), the effects of salt concentrations at four levels (9%, 12%, 15%, and 18%) on the fermentation of doenjang were investigated. However, the drawbacks of natural fermentation at home were unstable, easily spoiled, and uncontrolled due to the fluctuation of natural background microflora existing on vegetables (Di Cagno et al., 2015). The natural fermentation of vegetables may result in spoilage if desired lactic acid bacteria are not present in sufficient numbers (Van Garde & Woodburn, 1994). To improve the progress of fermentation and the quality of the end product, in this work, the effect of different conditions in fermenting grey oyster mushrooms in terms of salts and starter culture concentrations was studied to gain insight into the changes in quality parameters during fermentation.

2. MATERIALS AND METHOD

2.1. Material

Grey oyster mushroom (GOM) was purchased from the local market (Can Tho City, Viet Nam) in the early morning and transported to the Department of Food Technology (Can Tho University, Viet Nam) within one hour.

The *Lactobacillus plantarum* strain (Institute of Food and Biotechnology, Can Tho University) was sub-cultured in 10 mL of De Man, Rogosa, and Sharpe (MRS) broth (Merck, Germany) at 37°C for 48 h. The suspension was then centrifuged at 6,000 rpm for 10 minutes and the pellet obtained was washed twice with sterilized water to collect bacterial cells. The pellet was re-suspended in 10 mL of sterilized distilled water and vortexed for five minutes (MX-S, EMC LAB, Germany) for further use in the fermentation.

2.2. Preparing sample

The raw edible GOM portion underwent washing, draining, and blanching steps in which the ratio of mushrooms and water to 1:2 along with 0.3% CaCl₂

at 85-90°C for 6 min. Next, mushrooms were cooled drained, and then weighed 200 g of blanched mushrooms in cylindrical plastic jars (7.1 x 9.2 cm). The fermentation solutions, maintaining a consistent sucrose concentration of 2% as recommended by Montet et al. (2014), were combined with varying salt (3.5%, 4.0%, and 4.5%). Before being added to the fermentation jars, (diameter: 16 cm, height: 24 cm), these solutions underwent pasteurization at 100°C for 3 minutes.

Lactobacillus plantarum strain was set up at a final concentration of 10⁴ and 10⁶ CFU/mL. The fermentation was carried out in the ratio of mushrooms and the solution to 1:1; the fermentation process was at 15±2°C for 25 days in the cooling room (Viet Nam).

2.3. Analytical methods

Total color difference (ΔE): Color coordinates were determined in the CIE-LAB system with lightness (L^*), redness (a^*) and yellowness (b^*) using a colorimeter (FRU, China). Total color difference (ΔE) was calculated by $\Delta E = [(L^* - L_{ref})^2 + (a^* - a_{ref})^2 + (b^* - b_{ref})^2]^{1/2}$ (Jung, Ghoul, & de Lamballerie-Anton, 2003; Saidatul, Noriham, Zainal, Khairusy, & Nurain, 2013). The color values of samples before fermentation (day 0) were used as a reference in the ΔE calculation. The texture (gf) of mushroom mycelium was measured using a texture analyzer (Rheotex, Japan).

The pH of the fermentation solution was determined by an electronic pH meter (Vernier, USA). The total soluble solid (TSS) of the fermentation solution was measured using a refractometer (0-32%, Japan). Lactic acid bacteria (LAB) count was determined by pour-plating with an over layer on De Man, Rogosa and Sharpe agar (MRS, Merck, Germany) at 37°C for 48-72 h. All measurements were performed in duplicate. The results of microbiological analysis were expressed as log CFU/mL.

The total polyphenol content (TPC, mg GAE/g) of mushrooms was determined spectrophotometrically according to Foline-Ciocalteu method (Huynh et al., 2014) with slight modifications. In brief, 1 mL of deionized water was added to 1 mL of the sample. After thorough mixing, the Foline-Ciocalteu reagent (0.5 mL) was added, and the tube was shaken vigorously. After 6 min, 1.5 mL of 20% sodium carbonate solution was added, followed by adding deionized water to a final volume of 5 mL and mixing well again. The solution was then allowed to stand in the dark for 2 h at ambient temperature. The

absorbance of the solution was measured at $\lambda = 760$ nm against a blank sample consisting of 1 mL of 90% methanol instead of the sample solution. Total phenolic content was expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g DW) by using a standard curve of gallic acid in the range of 0-50 mg/L. The free radical scavenging activity of the GOM extracts was evaluated using the stable free radical diphenylpicrylhydrazyl (DPPH) as described by Brand-Williams, Cuvelier, and Berset (1995). The DPPH solution was prepared by dissolving 3.94 mg DPPH in 100 mL pure methanol. In a test tube, 100 L of extract was allowed to react with 2 mL of the DPPH solution for 30 min in the dark at room temperature. Then, the absorbance was measured at 517 nm and results are expressed in g TE per 100 g DM.

2.4. Statistical analysis

All experiments were performed in triplicate. Results are reported as mean value \pm standard deviation of these duplicate analyses. Statistically significant differences were performed at a 5% significance level using Statgraphics Centurion 18.1.12 (Statgraphics Technologies, Inc., The Plains, Virginia).

3. RESULTS AND DISCUSSION

Color is one of the most important quality attributes of fermented food since it affects overall quality (Deng et al., 2019). Table 1 illustrates the total color difference (ΔE) of GOM during fermentation using different concentrations of *L. plantarum* and NaCl. The results show that the average ΔE significantly increased from 6.4 ± 3.6 to 21.2 ± 1.7 (GOM'cap); and from 3.3 ± 0.9 to 4.5 ± 0.7 (GOM'mycelium) from day 9 to 25, respectively.

Table 1. Total color difference (ΔE) of grey oyster mushrooms during fermentation

Cap	¹ 3.5%- ² 10 ⁴	3.5%-10 ⁶	4.0%-10 ⁴	4.0%-10 ⁶	4.5%-10 ⁴	4.5%-10 ⁶	Mean
9	4.67 \pm 2.11	7.16 \pm 4.23	6.64 \pm 3.28	8.11 \pm 4.92	6.27 \pm 3.58	5.44 \pm 3.27	6.4 \pm 3.6 ^a
18	7.96 \pm 2.52	10.1 \pm 6.17	10.6 \pm 6.35	6.58 \pm 2.65	8.02 \pm 2.59	8.39 \pm 4.59	8.6 \pm 4.2 ^a
25	20.3 \pm 1.20	21.6 \pm 1.03	23.6 \pm 1.38	22.3 \pm 1.67	19.0 \pm 1.18	20.0 \pm 3.45	21.2 \pm 1.7 ^b
Mean	8.3 \pm 1.9 ^a	9.8 \pm 3.8 ^a	10.2 \pm 3.7 ^a	9.3 \pm 3.1 ^a	8.3 \pm 2.5 ^a	8.5 \pm 3.8 ^a	
Mycelium	¹ 3.5%- ² 10 ⁴	3.5%-10 ⁶	4.0%-10 ⁴	4.0%-10 ⁶	4.5%-10 ⁴	4.5%-10 ⁶	Mean
9	4.42 \pm 1.95	3.97 \pm 1.89	3.20 \pm 1.42	3.01 \pm 0.96	1.97 \pm 1.05	3.03 \pm 1.10	3.3 \pm 0.9 ^a
18	4.01 \pm 1.10	4.00 \pm 0.62	4.51 \pm 0.70	3.61 \pm 2.02	3.45 \pm 1.20	3.31 \pm 1.49	3.8 \pm 0.4 ^{ab}
25	4.43 \pm 0.81	4.29 \pm 0.96	5.43 \pm 0.70	5.07 \pm 0.85	4.67 \pm 0.71	3.32 \pm 1.20	4.5 \pm 0.7 ^b
Mean	3.2 \pm 0.13 ^{ab}	3.1 \pm 1.2 ^{ab}	3.3 \pm 0.9 ^b	2.9 \pm 1.3 ^{ab}	2.5 \pm 1.0 ^{ab}	2.4 \pm 1.3 ^a	

Notes: ^{1, 2} salt concentration and lactic acid bacteria count in fermentation solution; mean values with the same superscript letter within one column or one row are not significantly different at the level of 5%.

The rise in these values representing a shift toward lighter coloration could be attributed to changes in mushroom pigment or the inhibition of enzymatic and non-enzymatic browning in high acid and low pH conditions. However, Liu et al. (2016) showed that the lightness of the three kinds of oyster mushrooms decreased during fermentation, whereas redness and yellowness increased, indicating that mushrooms turned brown during fermentation, it could be attributed to enzymatic and non-enzymatic browning. Firmness is an important sensory property of fresh and processed GOM. The texture of grey oyster mushrooms during fermentation is shown in Table 2. The results of fermented GOM

shown in Table 2 indicated that the texture of GOM significantly decreased after the fermentation process, from 91.3 ± 33.9 to 74.0 ± 18.1 at day 0 and day 25, respectively. The results are not consistent with the research by other authors, who reported a significant increase in firmness of fermented mushrooms (Zivanovic & Buescher, 2004). It is suggested that the different types of mushrooms and fermentation conditions may contribute to variations in their texture. Furthermore, there was no clear positive correlation between salt concentration, and starter culture with the firmness of fermented GOM in this study.

Table 2: Texture of grey oyster mushrooms during fermentation

Texture (gf)	13.5%-210 ⁴	3.5%-10 ⁶	4.0%-10 ⁴	4.0%-10 ⁶	4.5%-10 ⁴	4.5%-10 ⁶	Mean
0	91.0±34	91.3±33.9	91.3±33.9	91.3±33.9	91.3±33.9	91.3±33.9	91.5±33.9 ^b
9	67.0±27.0	91.2±58.0	65.3±21.5	52.2±28.0	79.5±43.2	77.5±26.7	72.2±13.5 ^a
18	58.9±19.8	48.3±17.5	62.6±19.1	68.9±24.3	75.0±17.6	80.8±43.9	65.8±11.7 ^a
25	58.4±13.7	74.4±17.6	61.6±16.0	75.4±18.3	108.2±58.4	66.2±33.1	74.0±18.1 ^a
Mean	68.9±15.2 ^a	76.4±31.7 ^{ab}	70.5±22.6 ^a	72.0±26.1 ^a	88.9±38.3 ^b	79.0±34.4 ^{ab}	

Notes: ^{1, 2} salt concentration and lactic acid bacteria count in fermentation solution; mean values with the same superscript letter within one column or one row are not significantly different at the level of 5%.

The pH is a critical indicator for the fermentation process. As shown in Table 3, the initial pH values of fermented GOM with different salt and starter inoculum were between 6.48 and 6.77. The pH values decreased significantly on day 9 (pH 3.82 ± 0.02) (p<0.05) followed by the lowest value of 3.70±0.07 on day 18 (p<0.05). Subsequently, there was an increase observed on day 25, with the pH reaching 3.91 ± 0.01 (p<0.05). This finding aligns with a study by Jabłońska-Ryś¹ (2022) on fermented mushrooms (*Agaricus bisporus*), which observed fluctuations in pH, decreasing and then increasing during the final stage of fermentation. Moreover, there were no significant differences in pH value

between fermented samples by the different salt concentrations and inoculum starter cultures. The results were not consistent with other studies, which reported that an increase in salt concentration resulted in a decrease in pH (Xiong et al., 2016). All pH values of fermented samples were lower than 4.0. According to Steinkraus (2002), fermenting vegetables to maintain a pH below 4.0 ensures their stability while also preserving anaerobic conditions. It is suggested that the fermentation of grey oyster mushrooms would be in salt concentration (3.5%) and initial *Lactobacillus plantarum* (10⁴ CFU/mL) at 15°C for 9 days.

Table 3. pH of oyster mushrooms during fermentation

pH	13.5%-210 ⁴	3.5%-10 ⁶	4.0%-10 ⁴	4.0%-10 ⁶	4.5%-10 ⁴	4.5%-10 ⁶	Mean
0	6.77±0.19	6.48±0.05	6.66±0.14	6.72±0.16	6.73±0.13	6.70±0.14	6.68±0.14 ^c
9	3.87±0.01	3.79±0.01	3.88±0.09	3.75±0.04	3.82±0.01	3.78±0.03	3.82±0.02 ^b
18	3.71±0.11	3.65±0.04	3.75±0.09	3.65±0.04	3.69±0.04	3.68±0.07	3.70±0.07 ^a
25	3.93±0.01	3.93±0.01	3.90±0.01	3.92±0.01	3.90±0.01	3.86±0.01	3.91±0.01 ^b
Mean	4.57±0.08 ^a	4.46±0.04 ^a	4.55±0.08 ^a	4.51±0.06 ^a	4.54±0.01 ^a	4.50±0.06 ^a	

Notes: ^{1, 2} salt concentration and lactic acid bacteria count in fermentation solution; mean values with the same superscript letter within one column or one row are not significantly different at the level of 5%.

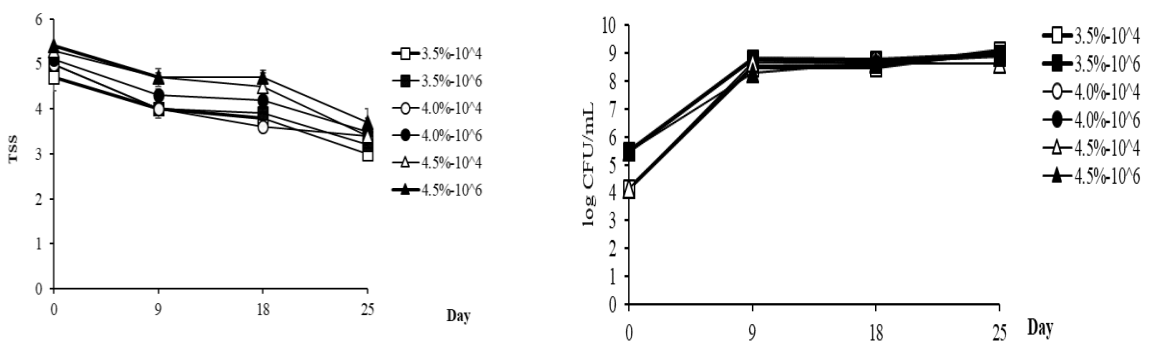


Figure 1: TSS and counts of LAB changes during grey oyster mushroom fermentation

Bars represent the standard deviation from triplicate determinations

The TSS decreased significantly by fermentation time (from day 0 of 5.1±0.2 to day 25 of 3.4±0.1) (p<0.05) (Fig. 1). The positive relationship between

TTS and pH was seen as the use of nutrition by LAB induced by the decrease of pH during fermentation. As shown in Fig. 1, the original population of LAB

was 4.15 and 5.48 log CFU/g, then increased to log 8.0 CFU/g and remained at this level in all fermented samples after day 25 of fermentation. Similar results were also reported by Liu et al. (2016), showing that the population of LAB of fermented oyster mushrooms increased during 3 first day and then remained at 7.5 log CFU/mL on the 18th day. The rapid rise in LAB in the beginning was most likely owing to the anaerobic environment that developed over time when aerobic microorganisms acting on fresh mushrooms consumed oxygen in the pickle jars. *L. plantarum* is a homofermentative LAB, which uses the Embden-

Meyerhof-Parnas pathway for glucose catabolism and produces lactic acid as the primary by-product (Giacon et al., 2021). High production amounts of lactic acid in the fermentation process had considerable effects on sensory characteristics and the storability of the product. Moreover, during the late fermentation stage in this study, the amount of LAB still exceeded 8 log CFU/g, which was a good indication. Since high LAB population was a critical parameter in controlling spoilage and pathogenic microorganisms in fermented food (Gao, Li, & Liu, 2014).

Table 4. Total polyphenol contents (TPC) of grey oyster mushrooms during fermentation (mg GAE/100 g DB)

TPC	¹ 3.5%- ² 10 ⁴	3.5%-10 ⁶	4.0%-10 ⁴	4.0%-10 ⁶	4.5%-10 ⁴	4.5%-10 ⁶	Mean
0	0.74±0.10	0.74±0.10	0.74±0.10	0.74±0.10	0.74±0.10	0.74±0.10	0.74±0.10 ^a
9	0.71±0.12	0.76±0.13	0.97±0.19	0.75±0.04	0.77±0.16	0.74±0.15	0.79±0.13 ^a
18	0.73±0.13	0.84±0.20	0.85±0.17	0.70±0.06	0.73±0.12	0.58±0.16	0.74±0.15 ^a
25	1.24±0.05	1.13±0.04	1.29±0.03	0.96±0.03	0.83±0.05	1.13±0.02	1.10±0.04 ^b
Mean	0.86±0.10 ^{ab}	0.87±0.12 ^{ab}	0.77±0.12 ^b	0.79±0.06 ^a	0.77±0.11 ^a	0.80±0.11 ^a	

Table 5. DPPH (mg TE/g sample) of grey oyster mushrooms during fermentation

DPPH	¹ 3.5%- ² 10 ⁴	3.5%-10 ⁶	4.0%-10 ⁴	4.0%-10 ⁶	4.5%-10 ⁴	4.5%-10 ⁶	Mean
0	1.00±0.74	1.00±0.74	1.00±0.74	1.00±0.74	1.00±0.74	1.00±0.74	1.26±0.74 ^{ab}
9	2.36±0.78	2.19±0.68	2.18±1.00	1.51±1.04	1.45±0.65	2.08±1.10	1.96±0.88 ^b
18	1.07±0.01	1.07±0.01	0.96±0.45	0.79±0.46	1.10±0.22	0.98±0.16	0.94±0.39 ^a
25	0.77±0.15	4.28±0.62	0.91±0.17	2.88±0.02	3.32±0.22	5.52±0.68	2.95±0.31 ^b
Mean	1.30±0.42 ^{ab}	2.14±0.50 ^{bc}	1.24±0.60 ^a	1.53±0.57 ^{ab}	1.72±0.46 ^{ab}	2.78±0.11 ^b	

Notes: ^{1, 2} salt concentration and lactic acid bacteria count in fermentation solution; mean values with the same superscript letter within one column or one row are not significantly different at the level of 5%

The total polyphenol contents (TPC) and DPPH free radical scavenging capacity (expressed in mg TE/g) of grey oyster mushrooms during fermentation are shown in Tables 4 and 5, respectively. The results showed that the average TPC of fermented GOM significantly increased from 0.75±0.00 to 1.10±0.04 mg GAE/g DW on day 9 and day 25, respectively. Similar results in the DPPH scavenging ability were observed from 1.26 ± 0.74 to 2.95 ± 0.31 (mg TE/g) on day 9 and day 25, respectively. Moreover, there were no significant differences among fermented samples in this study. The obtained results are in agreement with previous studies that showed the rise of TPC in fermented vegetables in comparison with raw materials (Di Cagno et al., 2008; Dueñas, Fernández et al., 2005; Filannino et al., 2013; Marazza et al., 2012). The enzymatic depolymerization of phenolic compounds by LAB accounts for the majority of this increase (Hur, Lee, Kim, Choi, & Kim, 2014), resulting in more efficient uptake and bioavailability of plant

polyphenols (Fessard & Remize, 2017). Moreover, the activity of several enzymes *Lactobacillus* spp. has been linked to the increase in antioxidant activity that occurs during lactic acid fermentation, such as β-glucosidase, tannase, p-coumaric acid decarboxylase or feruloyl esterase (Fritsch et al., 2017; Jiménez et al., 2014; Pyo et al., 2005; Rodríguez et al., 2009; Rodríguez et al., 2008). In this study, the obtained results suggest that fermented GOM posed a higher amount of TPC and good scavenging effect against DPPH radical compared to the raw materials even though further investigations are necessary to characterize the bioactive compounds in fermented GOM.

4. CONCLUSION

In conclusion, the results revealed the effect of salt concentrations and starter cultures on the physicochemical properties of fermented grey oyster mushrooms. Fresh grey oyster mushrooms can be fermented with NaCl (3.5%) and *L.*

plantarum (10⁴ CFU/g) to enhance their antioxidant activities. Moreover, further studies are necessary to investigate the effect of added salt on flavor, shelf-life, sensory properties, and the acceleration of harmful bacteria in the products when combined with other strains of lactic acid bacteria.

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