

Quality Characteristics of Fermented Edible Mushroom with Black Glutinous Rice Product

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Abstract The fermented mushroom with pigmented rice was developed by mixing edible mushrooms with Thai pigmented rice, including black glutinous rice, Riceberry rice, and Rai Dok Kha rice, and evaluated sensory analysis. The physicochemical properties investigated the fermented mushroom with black glutinous rice, which was the most favorable sensory evaluation. The increasing lactic acid bacteria and decreasing in pH and total anthocyanin were observed during fermentation. The color value of brightness (L^*) decreased, whereas the redness (a^*) and yellowness (b^*) increased with longer storage time. The proximate compositions of 15-day fermentation rose from fresh preparation of 0-day fermentation. All microbial indices were within the Thai standard required for the fermented mushroom product. The fermented mushroom with black glutinous rice product was further evaluated the consumer test compared with commercial fermented mushroom. The developed product was obtained a significantly higher score of 7.47 than the commercial product with a score of 7.13 ($p \leq 0.05$).

Keywords: fermented mushroom, pigmented rice, sensory analysis, fermentation

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1. Introduction

Agaricomycetes are edible mushrooms that include Indian Oyster mushrooms *Pleurotus Ostreatus*, and Jelly ear mushrooms *Auricularia auricula-Judae* [1]. They are widely consumed in Thailand due to their nutritional content component, including carbohydrates, proteins, dietary fiber, and oxidative compounds [2,3]. The mushrooms are alternative flavor enhancer to monosodium glutamate due to their umami flavor [4,5]. Therefore, mushrooms are used for cooking and added to food products [6]. Among mushroom-food products, a fermented mushroom called *Nham Hed* is one of the most popular appetizers in Thailand. The fermented mushroom is generally produced by mixing minced mushrooms, glutinous rice, salt, sugar, garlic together. These mixture ingredients are wrapped in a bundle allowing fermented process until the sour taste occurs. The fermentation process typically takes 3-5 days at ambient temperature, relying on different microorganisms to initiate the fermentation [7]. During fermentation, lactic acid bacteria (LAB) is a prominent microorganism that produces organic acids from carbohydrates, leading to decreased pH and the inhibition of undesirable microorganisms

[8]. Another microorganism, *Staphylococcus aureus* is also a source of lipolytic and proteolytic enzymes that attribute to flavor formation [9]. In addition, to increase the nutritional value, the addition of pigmented rice into fermented mushroom is an attractive alternative approach. Pigmented rice in Thailand, such as black glutinous rice, Riceberry rice, and Rai Dok Kha rice, are commonly consumed since they consist of high levels of phenolic compounds with anthocyanins. It is a good antioxidant source that inhibits the growth of several cancer cells such as lung cancer and prevents anemia [10,11]. Moreover, pigmented rice is also composed of minerals and vitamins such as iron, vitamin A, vitamin E, vitamin B [12].

In this study, the edible mushroom and pigmented rice were combined to produce the fermented mushroom. Different pigmented rice included black glutinous rice, Riceberry rice, and Rai Dok Kha rice mixed with fermented mushrooms were first evaluated sensory analysis. Subsequently, the most favorable fermented mushroom with pigmented rice was investigated physicochemical properties and effect on shelf-life period on the amount of lactic acid bacteria, pH, and anthocyanin during 15-day fermentation. The favorable consumer test of the developed fermented mushroom was also compared with commercial fermented mushrooms.

2. Materials and Methods

2.1. Materials

White glutinous rice (Raitip brand), black glutinous rice (Raitip brand), Riceberry rice (Royal project brand), Rai Dok Kha rice (Thai Mueang city, Phang Nga, Thailand), Indian oyster mushroom, jelly ear mushroom, garlic, chili, and salt were obtained from local markets.

2.2. Preparation of Cooked White Glutinous Rice and Pigmented Rice

The 100 g of white glutinous rice was immersed into the 200 mL water at room temperature ($30 \pm 2^\circ\text{C}$) for 6 h, then steamed for 20 min. ($105 \pm 2^\circ\text{C}$) [13]. The 100 g of black glutinous rice was immersed into the 200 mL hot water ($90 \pm 2^\circ\text{C}$) for 6 h, then steamed for 40 min. The 100 g of Riceberry rice was immersed into the 200 mL water at room temperature for 30 min., then steamed for 40 min. [14]. The 100 g of Rai Dok Kha rice was immersed into the 200 mL water at room temperature ($30 \pm 2^\circ\text{C}$) for 30 min., then steamed for 30 min. [15]. All types of rice were cooled down to ambient temperature before further experiment.

2.3. Preparation of Fermented Mushroom

Both Indian oyster mushrooms and Jelly ear mushrooms were washed, shredded and steamed at 105°C for 20 min. Subsequently, a 40 g of steamed Indian oyster mushroom was squeezed to obtain mushroom juice for the fermented mushroom mixture solution. The preparation of fermented mushroom in this study was adapted from TISI (2004) [7]. All ingredients including steamed Indian oyster mushroom and Jelly ear mushroom, cooked pigmented rice, mushroom juice, garlic, chili, and salt were mixed. A 15 g of the mixture was packed into a plastic bag (5x6 cm), then tightly tied with a rubber band. Samples were incubated for 72 h at $30 \pm 1^\circ\text{C}$ and $50 \pm 2\%$ relative humidity (Figure 1A).

2.3.1. Sensory Analysis

The controlled fermented mushroom of white glutinous rice and 3 types of pigmented rice fermented mushroom were evaluated by a 9-point hedonic liking scale. This study was reviewed and approved by the Research Ethics Committee Walailak University (approval number: WUEC-21-050-01 and date 02/04/2021). Informed verbal consent was obtained from the 30 untrained panels. Each fermented mushroom was placed in a cup and individually served with 2.0 g of cabbage. The liking scale points were ranged from 1 - extremely dislike to 9 - extremely like with the sensory characteristics of appearance, color, smell, flavor, taste, texture, and overall liking [16]. The fermented mushroom with different types of pigmented rice that was obtained the highest score was then selected for further study.

2.3.2. Physicochemical Analysis

The fermented mushrooms were investigated the chemical and physical characteristics of energy, fat,

protein, carbohydrate, sugar, sodium, ash, and moisture content using AOAC methodology AOAC. (2016) [17]. The Color value (CIE $L^*a^*b^*$) was investigated using Spectrophotometer Cm-350d (Minolta, Japan). The color variations of each sample were determined by recording the average of three readings taken on the sample's surface. Color measurement was carried out in triplicate. The percentage of expressible moisture was measured as the weight loss resulting from the compression of the sample. The method to measure the weight loss was modified from Nakao (1991) [18]. Samples were cut into cylinders (1.5 x 3.0 cm), then placed between double layers of filter papers (Whatman No. 4), and subjected to the compression using a texture analyzer with a cylindrical aluminum probe (50 mm diameter). The measurement was performed at a crosshead speed of 3 mm sec⁻¹ to 70% strain for 60 seconds. The expressible moisture content (%) was calculated as the ratio of the apparent expressible moisture to the total moisture content of the fermented mushroom determined by a moisture analyzer MA 30 (Sartorius, Germany). The percentage of released water was measured according to Funami et al. (1998) [19] as the following equation:

$$\text{Released water} = \frac{[(A - B) - C]}{A - C} \times 100$$

Where A is the sample with peel weight (g), B is the sample without peel weight (g), and C is empty peel weight (g).

The freshly prepared fermented mushroom and 3, 6, 9, 12, and 15-day fermented mushroom were investigated the shelf life by analysis of pH using a pH meter (Mettler Teledo 320, Switzerland), amount of anthocyanin [17], lactic acid bacteria (LAB) count. [20] and microbial quantities including *Salmonella* spp. [21], *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli*, yeast, and mold [22].

2.3.3. Consumer Acceptance Test

The most favorable fermented mushroom was evaluated consumer acceptance test compared to the commercial fermented mushroom. This study was reviewed and approved by the Research Ethics Committee Walailak University (approval number: WUEC-21-050-01 and date 02/04/2021). Informed verbal consent was obtained from 120 untrained consumers who were randomly selected as an accidental sampling varying in age and education level [23]. Each fermented mushroom was served with 2.0 g of cabbage. The preference scores were collected, including appearance, color, smell, flavor, taste, texture, and overall liking based on a 9-point hedonic scale from extremely dislike to extremely like.

2.3.4. Statistical Analysis

The statistical data analysis was performed using Statistical Package for Social Sciences (SPSS) version 21 (SPSS Inc., Chicago, IL, USA). The data were analyzed using ANOVA, followed by Duncan's new multiple range test to evaluate the significant difference for the sensory test. The developed fermented mushroom was compared the commercial fermented mushroom by statistical t-test at the 5 % level of significance [24].

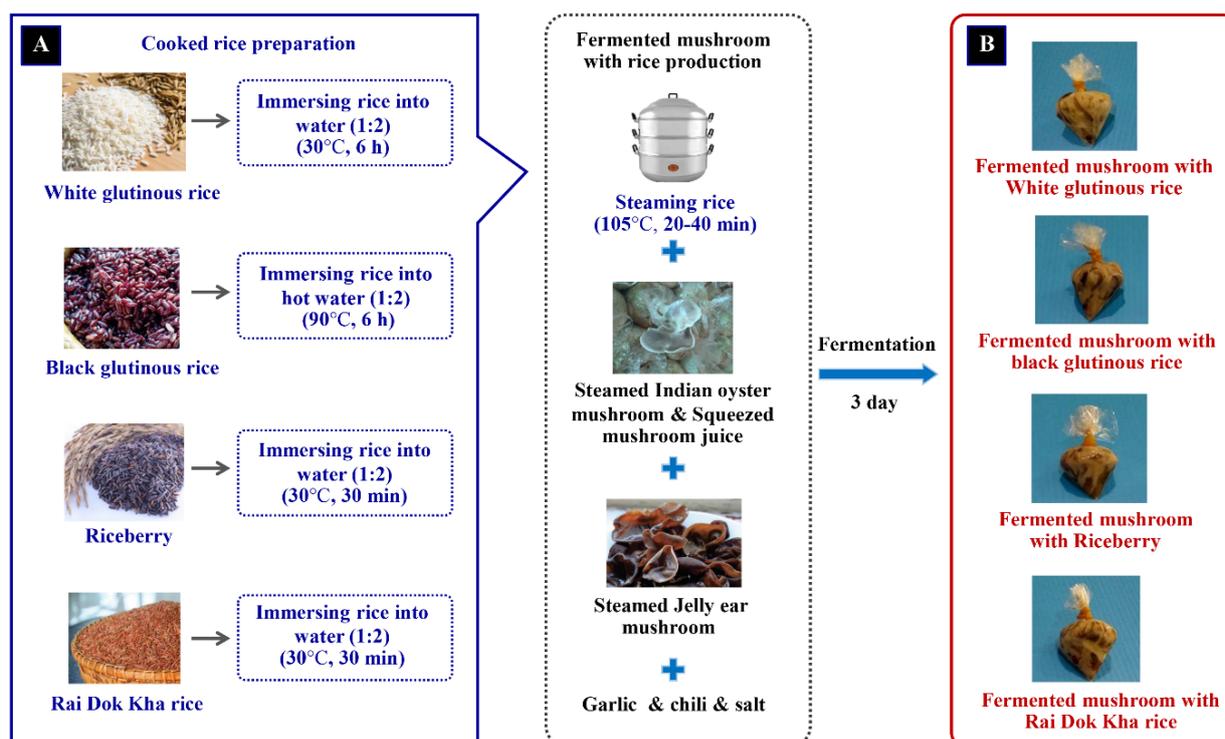


Figure 1. Fermented mushroom with pigmented rice preparation procedure (A) and obtained products (B)

3. Results and Discussion

3.1. Sensory Evaluation of Fermented Mushroom Mixing Pigmented Rice Substituted Non-pigmented Glutinous Rice

Three types of pigmented rice, including black glutinous rice, Riceberry rice, and Rai Dok Kha rice were substituted with white glutinous rice to produce the fermented mushroom. The product of different pigmented rice for fermented mushrooms is demonstrated in Figure 1B. The sensory test evaluated the effect of other pigmented rice on fermented mushrooms. As shown in Table 1, black glutinous fermented mushroom was the most favorable for all sensory characteristics ($p \leq 0.05$), with the score ranging from 7.47 ± 1.01 to 8.30 ± 0.53 . This might be due to its smell and texture. In addition, the black glutinous rice is composed of abundant amylopectin resulting in slower decomposition of the fermentation process [15]. Our study

is consistent with Tubbiyam et al. (2019) [25]. This could be confirmed that black glutinous rice can be used as an alternative ingredient in fermented mushroom with no difference. Therefore, the black glutinous rice was chosen as the pigmented rice for fermented mushroom for further study.

3.2. Physicochemical Properties of Fermented Mushroom with Black Glutinous Rice

The freshly prepared fermented mushroom and 15-day of fermented mushroom were investigated for chemical and physical compositions. As shown in Table 2, the brightness (L^*) was decreased with the increase in the fermented day from 43.54 ± 0.09 to 42.85 ± 0.10 , whereas the redness (a^*) and yellowness (b^*) were increased with the decrease in the fermented day from 3.78 ± 0.10 to 4.33 ± 0.06 and 11.11 ± 0.03 to 13.27 ± 0.03 , respectively. The higher a^* and b^* might be because the color of black glutinous rice changes from black purple to red-purplish during the fermentation [26,27].

Table 1. The acceptance level of white glutinous rice fermented mushroom and various kinds of pigmented rice fermented mushrooms.

Sensory Characteristics	Fermented mushroom			
	White glutinous rice (Control)	Black glutinous rice	Riceberry rice	Rai Dok Kha rice
Appearance	6.34 ± 0.88^b	7.68 ± 0.77^a	6.93 ± 1.12^b	6.53 ± 0.23^b
Color	6.97 ± 1.04^b	8.03 ± 0.81^a	7.07 ± 0.91^b	6.73 ± 0.38^b
Smell	6.50 ± 1.01^b	7.70 ± 1.24^a	6.53 ± 1.20^b	6.37 ± 1.03^b
Flavor	6.33 ± 1.03^b	7.47 ± 1.01^a	6.30 ± 1.58^b	6.17 ± 0.79^b
Taste	6.00 ± 1.11^b	7.73 ± 0.91^a	6.47 ± 1.66^b	6.00 ± 1.02^b
Texture	6.20 ± 0.89^b	7.76 ± 0.99^a	6.60 ± 1.22^b	6.50 ± 0.94^b
Overall liking	6.37 ± 0.81^c	8.30 ± 0.53^a	6.97 ± 1.25^b	6.47 ± 0.78^c

Remark: ^{a-c} Mean followed by different letters within the same row significantly differ ($p \leq 0.05$).

Table 2. Analysis results of the chemical composition and physical characteristics of 100 g of black glutinous rice fermented mushroom

Physicochemical properties	0-Day	15-Day
Energy (Kcal)	65.79	69.38
Fat (g)	0.27 ± 0.01 ^b	0.38 ± 0.03 ^a
Protein (g)	4.12 ± 0.01 ^b	4.24 ± 0.03 ^a
Total Carbohydrate (g)	11.72 ± 0.08 ^b	12.25 ± 0.01 ^a
Total sugar (g)	0.82 ± 0.02 ^a	0.39 ± 0.04 ^b
Sodium (mg)	9,221.50 ± 0.56 ^b	10,269.66 ± 0.58 ^a
Ash(%) ^{ns}	2.93 ± 0.02	3.01 ± 0.03
Moisture content (g)	80.96 ± 0.06 ^a	80.12 ± 0.05 ^b
Color value		
L*	43.54 ± 0.09 ^a	42.85 ± 0.10 ^b
a*	3.78 ± 0.10 ^b	4.33 ± 0.06 ^a
b*	11.11 ± 0.03 ^b	13.27 ± 0.03 ^a
Weight loss(%) ^{ns}	0.00 ± 0.00	0.14 ± 0.06
Percentage of Syneresis(%) ^{ns}	0.20 ± 0.00	0.40 ± 0.02

Remark: ^{a-b} Mean followed by different letters within the same row significantly differ ($p \leq 0.05$). ^{ns} no significant difference ($p > 0.05$).

The freshly prepared fermented mushroom preserved at $30 \pm 2^\circ\text{C}$ had the pH value of 4.97, while the 15-day fermented mushroom kept at $4 \pm 2^\circ\text{C}$ had the pH value of 3.86. Since the longer storage time provided the increase in acid level which resulted in the color change [28]. The percentage of weight loss and release of 15-day fermented mushrooms have risen. This might be attributed to the proteolysis of the muscle of protein at the acid condition leading to released water [29]. The energy, fat, protein, carbohydrate, sodium, and ash of 15-day fermented mushrooms rose from the freshly prepared fermented mushroom (0-day) since the fermentation could enhance protein solubility [30]. The sugar composition of 15-day fermented mushrooms decreased due to the metabolism of lactic acid bacteria [31,32]. The moisture contents of fermented mushrooms also decreased because of the influence of ionic strength on osmotic pressure [33].

3.3. The Effect of Shelf-life of Fermented Mushroom

The shelf life of black glutinous rice fermented mushroom was investigated at 0, 3, 6, 9, 12, 15 fermented days. As shown the Figure 2, the pH value was consistently decreased with the increase in the fermented day due to lactic acid bacteria production under anaerobic conditions [34]. The lactic acid could produce the flavor of the fermented mushroom. The pH of 3-day fermented

mushroom was lower than the limit of maximum permissible pH of 4.60 [35]. Considering the effect of shelf life on anthocyanin content as shown in Table 3. Anthocyanin decreases that is no significant difference ($p > 0.05$) with increased fermented days, probably because of anthocyanin degradation under acidic conditions [36]. On the other hand, anthocyanin provides good antioxidative activity at low pH [37].

The effect of shelf life on microbial quantity was investigated including *Salmonella* spp., *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli*, yeast, and mold varying in the fermented days from 0 to 15. The 0-3 days fermented mushrooms were stored at room temperatures and fermented mushrooms were kept at $4 \pm 2^\circ\text{C}$ during 4-15 days. As shown in Table 4, *Salmonella* spp. was not found in the developed fermented mushroom for the whole 15 days, which was accepted as the standard permission [7]. *Staphylococcus aureus* and *Clostridium perfringens* were found at lower than 10 CFU g^{-1} . *Escherichia coli* was found at lower than 10 MPN g^{-1} , which was below the established limit [7]. The amount of yeast and mold was detected at more than 10 CFU g^{-1} at longer 9 days, which exceeds the TISI standard. This indicates that the developed fermented mushroom could be stored for 9 days. Mutukwa et al. (2019) [38] reported that blanching alone does not use chemicals. This may reduce the initial microbial count. This research steamed mushrooms alone also do not use chemicals, therefore, it can cause spoilage after 9 days of storage.

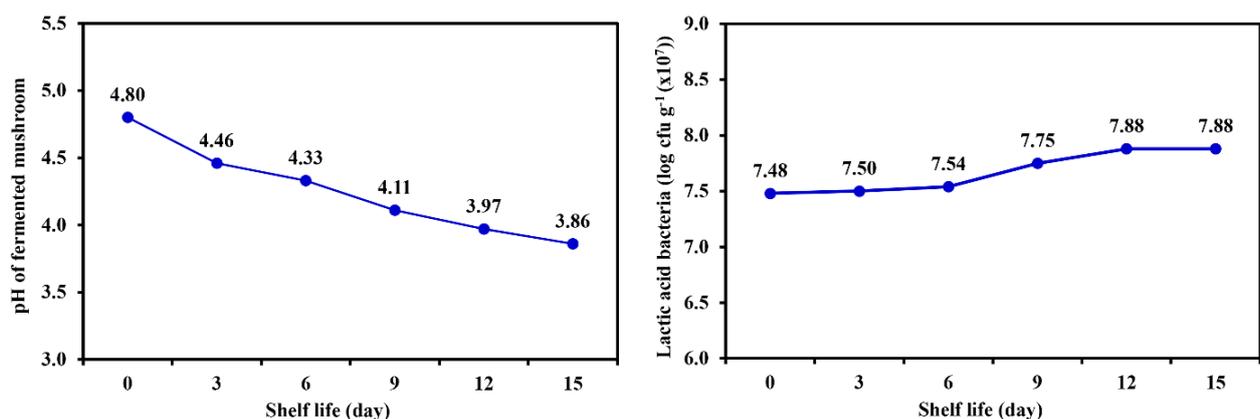


Figure 2. pH values (A) and amount of lactic bacteria ($\log \text{cfu g}^{-1}$) of fermented mushroom during 0 to 15-day of fermentation

Table 3. Anthocyanin content, pH value, and lactic acid bacteria content of 100 g of fermented mushroom

Day/Properties	Anthocyanin(mg) ^{ns}	pH ^{ns}	Bacterial group Lactic acid (colonies g ⁻¹)
Day 0	14.49 ± 0.05 ^a	4.80	7.48 × 10 ⁷
Day 3	13.72 ± 0.01 ^b	4.46	7.50 × 10 ⁷
Day 6	13.71 ± 0.03 ^b	4.33	7.54 × 10 ⁷
Day 9	13.58 ± 0.04 ^c	4.11	7.75 × 10 ⁷
Day 12	13.56 ± 0.03 ^c	3.97	7.88 × 10 ⁷
Day 15	13.39 ± 0.04 ^d	3.86	7.88 × 10 ⁷

Remark: ^{a-d} Mean followed by different letters within the same row significantly differ (p≤0.05).

^{ns} no significant difference (p>0.05).

Table 4. Microbial Analysis of fermented mushroom

type	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
<i>Salmonella</i> spp. (colonies g ⁻¹)	Not found					
<i>Staphylococcus aureus</i> (colonies g ⁻¹)	<10	<10	<10	<10	<10	<10
<i>Clostridium perfringens</i> (colonies g ⁻¹)	<10	<10	<10	<10	<10	<10
<i>Escherichia coli</i> (MPN g ⁻¹)	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0
Yeast and mold content (colonies g ⁻¹)	<10	<10	<10	<10	>10	>10

Table 5. Consumer test of the black glutinous fermented mushroom (n = 120)

Sensory attributes	Black glutinous fermented mushroom		Commercial fermented mushroom	
	Scores	Preference level	Scores	Preference level
Appearance	7.45 ± 1.14 ^a	Like Moderately	7.10 ± 1.29 ^b	Like Moderately
Color	7.45 ± 1.30 ^a	Like Moderately	7.24 ± 1.25 ^b	Like Moderately
Smell ^{ns}	6.91 ± 1.43	Like Slightly	6.74 ± 1.38	Like Slightly
Flavor ^{ns}	7.05 ± 1.28	Like Moderately	6.86 ± 1.35	Like Slightly
Taste	7.12 ± 1.27 ^a	Like Moderately	6.87 ± 1.22 ^b	Like Slightly
Texture ^{ns}	7.04 ± 1.31	Like Moderately	6.90 ± 1.16	Like Slightly
Overall liking	7.47 ± 1.14 ^a	Like Moderately	7.13 ± 1.19 ^b	Like Moderately

Remark: ^{a-b} Mean followed by different letters within the same row significantly differ (p≤0.05).

^{ns} no significant difference (P > 0.05).

3.4. Consumer Test

The consumer test was evaluated from 120 untrained consumers from Walailak University canteen. The age range included below 20 years (27 %), 21 - 30 years (57 %), 31 - 40 years (10 %), 41 - 50 years (4 %) and over 50 years (2 %). Most of educational levels was bachelor's degrees (82 %). The result is shown in Table 5. The score of appearance, smell, color, flavor, taste, texture, and overall liking was at the level of slightly like to moderately like. The consumers gave an overall liking score of 7.47 ± 1.14 which was higher than the expected score of 7.00. The sensory acceptability of the black glutinous fermented mushroom was compared with the commercial fermented mushroom showed that the black glutinous fermented mushroom was obtained a higher preferably significant overall liking score than the commercial fermented mushroom (p≤0.05). In addition, 77.50% of the untrained consumers were willing to purchase this product.

4. Conclusion

The pigmented rice included black glutinous rice, Riceberry rice, and Rai Dok Kha rice, were successfully compared to non-pigmented glutinous rice to produce the fermented mushroom. The fermented mushroom with black glutinous rice had the most favorable sensory evaluation. The storage time affects the physical and chemical properties of the fermented mushroom including the decrease in pH, color change, and anthocyanin content

reduction in the microbial quantities. The developed fermented mushroom could be stored at 4 ± 2°C for 9 days. In addition, the developed fermented mushroom had a statistically significant favorable consumer test compared with the commercial fermented mushroom. Therefore, this product has the potential to be developed as the commercial product for future development.

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