

Research Article

Physicochemical Characteristics and Storage Stability of Hybrid Beef Patty Using Shiitake Mushroom (*Lentinus edodes*)

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Received 2 August 2022; Revised 11 January 2023; Accepted 20 January 2023; Published 6 February 2023

Academic Editor: Ali Akbar

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This study evaluated the physicochemical characteristics and storage stability (at 0, 3, and 7 days) of hybrid beef patties with different amount of shiitake mushrooms (*Lentinus edodes*) added. Shiitake mushrooms contain healthy ingredients such as ergosterol and β -glucan. Four proportions of shiitake mushrooms were added to beef patties (T1, 20%, T2, 40%, T3, 60%, T4, 80%) as a substitute for beef and compared with a control group (CON 0%). Chemical composition, water holding capacity (WHC), cooking loss, pH, color, texture profile analysis, and sensory properties of the products were compared on day 0. As a storage stability experiment, volatile basic nitrogen (VBN), 2-thiobarbituric acid reactive substances (TBARS), and total microbial count were compared (0, 3, and 7 days). The results revealed that replacement with shiitake improved the WHC and cooking loss of patties but had a negative effect on sensory properties and storage stability. These results indicate that shiitake mushrooms can be added along with beef to produce hybrid patties; however, the usage amount must be considered.

1. Introduction

As the economy and population are growing proportionally, per capita meat consumption and total meat consumption worldwide are increasing [1]. Meat is one of the most nutritious natural foods and is considered essential for humans to maintain a healthy and balanced diet [2]. Recently, due to consumer interest in health and nutrition, meat products as functional foods are being developed [3]. Although meat is a nutritious food, it does not have sufficient complex carbohydrates such as dietary fiber, and most of the fat is composed of monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) [4–6]. Therefore, there have been efforts to develop functional products by adding dietary fiber or nonmeat ingredients to meat products [7–9]. Mushrooms have been used as a food and medicine since ancient history and are considered a novel source of dietary fiber [10, 11]. Mushrooms have a protein content ranging from 18.87% to 36.96% on a dry basis, depending on the species [12]. Plant

protein has attracted attention as a substitute for animal protein due to its low cost, abundant supply, and excellent nutritional value [13]. Mushrooms are rich in fat-soluble vitamins along with ergosterol content, rich in polyunsaturated fatty acids that are beneficial to health, and nutritionally high-quality food with ingredients such as β -glucan and triterpenoids [14–16]. Ergosterol has health-promoting effects such as anti-inflammatory and anticancer and reduces the risk of cardiovascular diseases [17]. The dietary fiber present in mushrooms is mainly composed of chitin and β -glucan, which are water-insoluble dietary fibers, and contains less than 10% of soluble dietary fiber [18]. The beneficial effects of β -glucan, such as improvement in constipation, lowering of glycemic index, and lowering of cholesterol level, have been demonstrated in many studies [19]. Mushrooms impart an umami taste due to the presence of glutamic acid, aspartic acid, and 5' ribonucleotides [18, 20, 21]. Although more than 2,000 species of mushrooms exist in nature, about 25 species are recognized as

food suitable for consumption [22]. Among them, the shiitake mushroom is the second most-produced mushroom in the world after the button mushroom [23]. The components ergosterol and β -glucan in shiitake mushrooms impart beneficial effects on health [24]. Currently, studies on the application of mushrooms to meat products are dealing with the addition of mushroom powder to meat products [25, 26]. Studies on the direct addition of mushrooms to sausages have previously been conducted [27, 28]. However, there exists only a few studies on the addition of mushrooms directly to meat patties. So, we considered adding shiitake mushrooms directly to the patties and focused on making a hybrid patty. The purpose of this study was to evaluate the effect of the level of inclusion of shiitake mushrooms on the physicochemical characteristics, storage stability, and sensory properties of hybrid patties.

2. Materials and Methods

2.1. Hybrid Patty Preparation. Five formulations groups were designed with different levels of shiitake mushroom: control (CON) 0%; T1, 20%; T2, 40%; T3, 60%; and T4, 80% ($n=3$ by each formulation). The formulations of hybrid patties are shown in Table 1. Surface moisture of beef (top round) and shiitake mushrooms (Yeongdong, Korea) were removed using a kitchen paper. Then, both ingredients were ground in a grinder (M-12S, Fujee, Korea). Next, textured soybean protein (Hokyong-tech, Ansong, Korea), chickpea powder (Jangmung Food, Seoul, Korea), and lentil bean powder (52Food, Gwangju, Korea) were added to the minced mixture of beef and shiitake mushrooms. Then, ice, NaCl (OCI Company Ltd., Seoul, Korea), sodium tripolyphosphate (Samchun Chemical, Seoul, Korea), pepper (Ottogi Corp, Ltd., Seoul, Korea), and sunflower oil (Sayınlar gıda maddeleri sanayi ve ticaret A. Ş., Gaziantep, Turkey) were added to the mixture and mixed thoroughly for 4 min to form an emulsion. After adding wheat gluten powder (Edentown F & B, Incheon, Korea), pumpkin powder (Health Based, Gimcheon, Korea), oat powder (Ssdfood, Seoul, Korea), lance asiabell powder (Syherb, Seoul, Korea), yam powder (Jangsoomanse, Seoul, Korea), almond powder (The Almond Farmer, Taylorville, Australia), dried radish powder (Sandeulnongsan, Gwangju, Korea), and Arabic gum (Namyung Commercial Co., Ltd., Seoul, Korea), the mixture was mixed thoroughly for 3 min to complete the process of dough formation. Typically, 100 g each of the emulsion was molded into a patty shape. The patties were vacuum-packed and stored at $4 \pm 1^\circ\text{C}$ for 7 days.

2.2. Chemical Composition. The moisture, protein, ash, and fat content (%) of 0-day patties were determined according to association of official analytical chemists (AOAC) [29]. For crude fat estimation, a 0.5 g sample was homogenized in 25 ml of Folch solution (chloroform: methanol, 2:1, bv/v) and left in a refrigerator at 4°C for 24 h. The sample was filtered through Whatman No. 2 paper and cleaned with 5 ml of Folch solution. After mixing 10 ml of distilled water

TABLE 1: The formulations of a hybrid beef patty with shiitake mushroom (*Lentinus edodes*).

Ingredients (%)	CON	T1	T2	T3	T4
Top round	80	60	40	20	0
Shiitake mushroom (<i>Lentinus edodes</i>)	0	20	40	60	80
Textured soy protein	2	2	2	2	2
Chickpea powder	1	1	1	1	1
Lentil bean powder	1	1	1	1	1
Wheat gluten powder	0.3	0.3	0.3	0.3	0.3
Sunflower oil	1	1	1	1	1
Pumpkin powder	0.5	0.5	0.5	0.5	0.5
Oat powder	0.5	0.5	0.5	0.5	0.5
Lance asiabell powder	0.1	0.1	0.1	0.1	0.1
Yam powder	0.1	0.1	0.1	0.1	0.1
Almond powder	0.1	0.1	0.1	0.1	0.1
Purple sweet potato powder	0.1	0.1	0.1	0.1	0.1
Dried radish powder	0.5	0.5	0.5	0.5	0.5
Ice	10	10	10	10	10
NaCl	1.5	1.5	1.5	1.5	1.5
Sodium triphosphate	0.2	0.2	0.2	0.2	0.2
Pepper	0.1	0.1	0.1	0.1	0.1
Arabic gum	1	1	1	1	1
Total	100	100	100	100	100

into the filtrate, the sample was centrifuged at 3000 rpm at room temperature for 20 min. After removing the separated upper layer consisting of water and ethanol using a pipette, chloroform was evaporated overnight in a hood, and the weight was measured. Proteins were measured using the Kjeldahl method. Briefly, 0.5 g of sample and 25 mL of 98% sulfuric acid (12080.100, Merck, USA) were heated together in a flask, and then, the flask was connected to a distillation apparatus, and the ammonia component of the sample was adsorbed using boric acid in the flask. Then, titration with 0.1 N sulfuric acid was performed. In the case of moisture measurement, 1 g samples are placed on aluminium weighing boats and heated in a dry oven (SH-DO-100 FG, Samheung, Korea) at 105°C for 16 hours. Moisture content was calculated using the weight after heating and the weight before heating. In the case of ash measurement, a 0.3 g sample was placed in a weighed crucible. After that, the crucible was put into a 540°C muffle furnace (MF2-12 GF, Jeio tech, Korea) and taken out after 10 hours. It was then cooled for 1 hour and weighed. The incineration content was calculated using the preincineration weight and the incinerated weight.

2.3. Total Dietary Content Analysis. The total dietary fiber content was analyzed using a dietary fiber analyzer (TDFI, Ankom technology, Macedon, NY, USA) to which the AOAC analysis method was applied. For each sample, 0.5 g of each sample was placed in two total dietary fiber (TDF) bag A (IDF flow-thru, Ankom technology). Then, 40 mL of mes-tris buffer (Sigma, St. Louis, Mo, USA) was mixed and 50 μL of α -amylase (Megazyme, Wicklow, Ireland) was added. It was then stirred at 97°C for 30 minutes. After cooling to 60°C , 100 μL of protease (Megazyme) was added. After stirring at 60°C . for 30 minutes, the pH of the sample

was adjusted to 4.0–4.7 using 0.561 N HCl (OCI Company Ltd, Seoul, Korea) and 6 N NaOH (OCI Company Ltd.). 300 μ L of amyloglucosidase (Megazyme) was added to the sample and stirred at 60°C for 30 minutes. Each sample after the reaction was transferred to a TDF bag B (SDF filter bag, Ankom technology) to which 1 g of celite (Sigma) was added. After that, 225 mL of 95% (w/v) ethanol (OCI Company Ltd.) was added to stop the enzyme reaction and precipitate total dietary fiber, and then filtered. Then, washing and filtration were repeated twice in the order of 15 mL of distilled water, 95% (w/v) ethanol, and 78% (w/v) ethanol, respectively. Then, after drying with a dryer (WFO-450PD, Eyela, Tokyo, Japan) for 90 minutes at 105°C, the weight was measured. The protein content of one of the two samples measured by weight was analyzed by the Kjeldal method, and the ash content of the other was measured after incineration at 540°C for 10 hours. The blank was performed in the same way as the total dietary fiber analysis method without adding a sample, and the total dietary fiber (TDF)

content was calculated using equation (1) and expressed as g/100 g.

2.4. Water Holding Capacity (WHC) and Cooking Loss. Cooking loss was measured by the following method. A sample was placed into a polypropylene bag and cooked for 40 minutes at 70°C in a water bath (SW-90 MW, Sangwoo Scientific, Korea). After transferring to a cooling rack and cooling for 30 minutes, the weight (g) lost after heating was measured as the weight ratio (%) of the initial sample. Water holding capacity was measured by modifying the centrifugation method of Laakkonen [30]. After measuring 0.5 g of the sample in a tube, it was heated at a constant temperature water bath at 80°C for 20 min. After allowing to cool for 10 min, the sample was centrifuged for 10 min (10°C) at 2,000 rpm, and the weight was measured and calculated as follows:

$$\text{Water holding capacity (\%)} = \frac{\text{total moisture} - \text{free moisture}}{\text{total moisture}} * 100, \quad (1)$$

$$\text{Free moisture (\%)} = \left[\frac{\text{weight before centrifugation} - \text{weight after centrifugation}}{\text{weight of sample} * \text{fat coefficient}} \right] * 100, \quad (2)$$

$$\text{Fat coefficient} = 1 - \frac{\text{Fat (\%)}}{100}. \quad (3)$$

2.5. pH. The pH of the sample was measured after adding 50 mL of distilled water to 5 g of the sample. All samples were homogenized for 30 sec using a homogenizer (Stomacher® 400 Circulator, Seward, UK), and pH was measured with a pH meter (Mettler Delta 340, Mettler-Toledo, Ltd., UK) calibrated in phosphate buffer at pH 4 and 7.

2.6. Color. The color was measured with a spectro colorimeter (Model JX-777, Color Techno. System Co., Japan) standardized on a white plate (L^* , 89.39; a^* , 0.13; b^* , -0.51), directly applied to the patty at three times per sample. The white fluorescent lamp (D65) was used as a light source. Color values were expressed as L^* (lightness), a^* (redness), and b^* (yellowness).

2.7. Texture Profile Analysis. Texture profile analysis was performed using a rheometer (Model Compac-100, SUN SCIENTIFIC Co., LTD., USA). Before the measurement, the temperature of the sample was equilibrated to room temperature. The size of the sample was cut into 1 cm \times 1 cm \times 1 cm. Force versus time curves were obtained from two compression cycle measurements. The area of the probe used for measurement was 3.14 cm². The weight of the load cell was 10 kg, and the speed of the cross-head was

measured to be 200 mm/min. The parameters of hardness, cohesiveness, and chewiness were calculated based on the curves described by Bourne [31]. Hardness is defined as the maximum force of the first compression and cohesiveness is the curve area of the second compression divided by the first compression curve area. The chewiness was calculated as gumminess \times springiness.

2.8. Sensory Properties. For the sensory evaluation, seven trained panelists were subjectively evaluated for six items of color, flavor, off-odor, softness, juiciness, and total preference of patty products with different levels of addition of shiitake mushroom and beef on 0 day. Using a 5-point scale, color (1 = light, 5 = dark), flavor (1 = bad, 5 = good), off-flavor (1 = many, 5 = absent), year (1 = tough, 5 = soft), juiciness (1 = dry, 5 = juicy), and total preference (1 = bad, 5 = very good) were evaluated.

2.9. 2-Thiobarbituric Acid Reactive Substances (TBARS). TBARS was measured by modifying the extraction method of Witte et al. [32]. Cold 10% perchloric acid (15 mL) and 25 mL of tertiary distilled water were added to 10 g of the sample and homogenized at 10,000 rpm for 10 sec in a homogenizer. The homogenate was filtered using a Whatman No. 2 filter paper. The filtrate (5 mL) and 5 mL of 0.02 M TBA solution were mixed thoroughly and left in a cool and

dark place for 16 h. The absorbance was measured at a wavelength of 529 nm using a spectrophotometer (DU-650, Beckman, USA). Tertiary distilled water was used as a blank. TBARS levels were expressed as mg malonaldehyde (mg malonaldehyde/kg) per 1,000 g of sample. The standard curve used at this time measured by malonaldehyde was $y = 0.1975x - 0.0011$ ($r = 0.999$) and was calculated as $y = \text{absorbance}$, $x = \text{TBARS value}$.

2.10. Volatile Basic Nitrogen (VBN). The method of Pearson [33] was used to measure the VBN content. Distilled water (90 mL) was added to 10 g of the sample and homogenized at 10,000 rpm for about 30 sec. The homogenate was filtered using a Whatman No. 2 filter paper. The filtrate (1 mL) was placed in the outer chamber of the Conway unit, and 1 mL of 0.01 N boric acid solution and 3 drops of the indicator (0.066% methyl red + 0.066% bromocresol green) were added to the inner chamber. After applying white Vaseline to the adhesive part of the lid and closing the lid, 1 mL of 50% K_2CO_3 was injected into the outer chamber, immediately sealed, and the vessel was stirred horizontally and incubated at 37°C for 120 min. After incubation, the boric acid solution in the inner chamber was titrated with 0.02 N H_2SO_4 . The VBN level was expressed in terms of mg (mg/100 g) per 100 g sample.

$$\text{VBN} \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{(a - b) * F * 28.014 * 100}{\text{amount of sample}}, \quad (4)$$

where a is the amount of sulfuric acid injected (mL), b is the amount of H_2SO_4 injected into the blank (mL), F is the 0.02 N H_2SO_4 standardized index, and 28.014 = amount of N required to titrate 1 mL of 0.02 N H_2SO_4 .

2.11. Total Microbial Count (TMC). The total microbial count was followed the serial dilution method of Park et al. [34]. A 0.1% peptone solution (90 ml) was added to 10 g of the sample and homogenized for 30 seconds with a stomacher bag. Then, the serially diluted samples were inoculated onto plate count agar (PCA) medium and incubated at 37°C for 48 h. After the incubation was completed, the colonies were counted using a colony counter. The total number of microorganisms was expressed as log cfu/g.

2.12. Ergosterol Measurement. The ergosterol content analysis method followed method of Koo et al. [35]. 100 mg of dried shiitake fruiting bodies were ground and 10 mL of 10% KOH was added and treated at 80°C for 1 h. After treatment, 10 mL of hexane was added and stirred using a vortex. After stirring, the supernatant was separated, and hexane was evaporated using nitrogen gas in the separated layer. The remaining white crystals were dissolved in 99.9% methanol and filtered through a 0.2 μm filter (Sartorius Stedim Biotech, Germany). To measure the ergosterol content, an HPLC system (YL9100 Plus HPLC System, YoungIn Chromass, Korea) was used with a C18 column (4.6 mm \times 250 mm, 5 μm), and 99.9% methanol was used as a mobile phase. The flow rate was 1.0 mL/min, the injection

volume was 5 μL , and the wavelength was 282 nm. Under this condition, the peak of ergosterol appeared after 7.42 minutes. A calibration curve was prepared by diluting ergosterol (Sigma-Aldrich, St. Louis, USA) at 10, 25, 50, and 100 $\mu\text{g/g}$, and the ergosterol content of the shiitake fruit body was calculated using this calibration curve (Figure 1).

2.13. β -Glucan Measurement. Glucan extraction was performed using Megazyme β -glucan assay kit (Mushroom and Yeast β -glucan Assay Procedure K-YBGL, Megazyme, Ireland). To 100 mg of dried and ground shiitake fruiting bodies, 2.0 mL of 12 M HCl was added, vortexed until dissolved, and treated on ice for 2 h. Next, 4 mL of distilled water was added and mixed well, followed by adding 6 mL more of distilled water and then treated in a 100°C water bath for 2 h. After the reaction solution was cooled to room temperature, 0.2 M sodium acetate buffer (pH 5.0) was added to achieve a final volume of 100 mL. After that, 6 mL of 8 M NaOH was added and centrifuged at 13,000 rpm for 5 min. After centrifugation, 0.1 mL of exo-1,3- β -glucanase (20 U/mL) plus β -glucosidase dissolved in 200 mM sodium buffer was added to 0.1 mL of the supernatant and reacted in a water bath at 40°C for 60 min. Typically, 3 mL of GOPOD (Megazyme) was added to the reaction solution, the reaction was allowed to take place at 40°C for 20 min, and the absorbance was measured at 510 nm using a spectrophotometer (Mobi, Microdigital, Korea). The obtained values were used to calculate the total glucan content. In addition, 2 mL of 1.7 M NaOH was added to 100 mg of the sample and treated on ice for 20 min. To this reaction solution, 8 mL of 1.2 M sodium acetate buffer (pH 3.8) and 0.2 mL of amyloglucosidase (1630 U/mL) + invertase (500 U/mL) solution were added and treated in a water bath at 40°C for 30 minutes. Next, the mixture was centrifuged for 5 min at 13000 rpm. To 0.1 mL of the supernatant, 0.1 mL of 0.2 M sodium acetate buffer (pH 5.0) and 3 mL of GOPOD reagent were added, reacted at 40°C for 20 min, and the absorbance was measured at 510 nm. The obtained values were used to calculate the α -glucan content. The β -glucan content was calculated by subtracting the α -glucan content from the total glucan content.

2.14. Statistical Analysis. All measurements were repeated at least 3 times, and the statistical processing program SAS (9.4 for Windows, USA) was used to test the significance of the results. To compare significant differences between the measured values, a significance test ($p < 0.05$) was performed using the Duncan multiple range test.

3. Results and Discussion

3.1. Chemical Composition. Table 2 shows the results of chemical composition of the hybrid patties. The moisture content ranged from 63.06% (CON) to 71.54% (T4), with a significantly higher value in T4 ($p < 0.05$). Fat content ranged from 1.46% (T4) to 9.43% (CON) and was significantly higher in CON and significantly lower in T4 ($p < 0.05$). Ash content was significantly lower in T4. Protein content

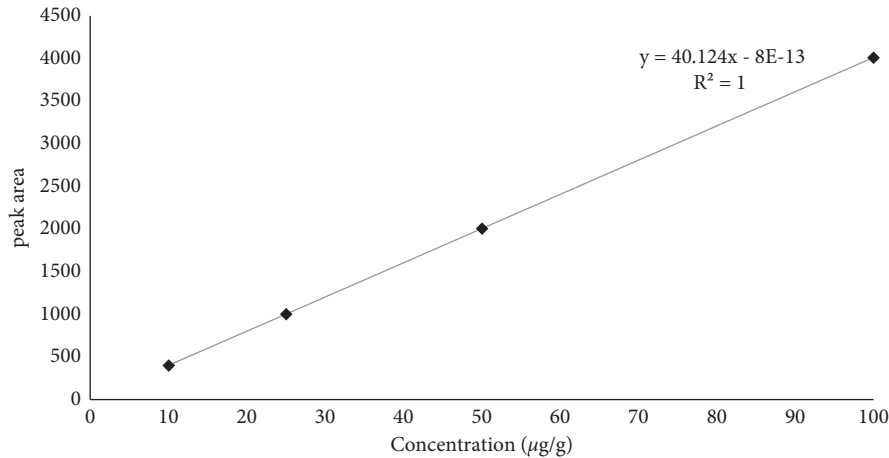


FIGURE 1: Ergosterol calibration curve.

TABLE 2: Chemical composition of the hybrid beef patty with shiitake mushroom (*Lentinus edodes*).

	Treatments ⁽¹⁾				
	CON	T1	T2	T3	T4
Moisture (%)	63.06 ± 1.04 ^B	64.01 ± 2.29 ^B	65.13 ± 2.37 ^B	65.77 ± 2.71 ^B	71.54 ± 1.97 ^A
Fat (%)	9.43 ± 0.78 ^A	7.56 ± 0.75 ^B	5.70 ± 0.62 ^C	3.76 ± 0.57 ^D	1.46 ± 0.19 ^E
Ash (%)	2.42 ± 0.08 ^A	2.32 ± 0.03 ^A	2.28 ± 0.02 ^A	2.40 ± 0.08 ^A	2.05 ± 0.25 ^B
Protein (%)	21.93 ± 1.67 ^A	17.93 ± 2.91 ^{AB}	16.20 ± 3.28 ^{BC}	15.65 ± 2.76 ^{BC}	11.18 ± 2.40 ^C
Dietary fiber (%)	3.16 ± 0.15 ^E	8.18 ± 0.21 ^D	10.69 ± 0.73 ^C	12.42 ± 0.45 ^B	13.77 ± 0.30 ^A

^{A-E} Means with different superscriptions within the same row differ ($p < 0.05$, $n = 3$). ⁽¹⁾Treatments: CON (*Lentinus edodes* 0%), T1 (*Lentinus edodes* 20%), T2 (*Lentinus edodes* 40%), T3 (*Lentinus edodes* 60%), and T4 (*Lentinus edodes* 80%).

was 11.18% (T4)–21.93% (CON) and was significantly higher in CON and significantly lower in T4 ($p < 0.05$). Dietary fiber was 3.16% (CON)–13.77% (T4) and was significantly higher in T4 and significantly lower in CON ($p < 0.05$). Shiitake mushroom consists of 90.7% water, 0.8% ash, and 2.6% protein and consists of dietary fiber glucan and chitin [36, 37]. On the contrary, the beef top round may consist of 69.5% water, 1.0% ash, 22.1% protein, and 9.2% fat [38]. Therefore, it is thought that higher content of shiitake mushroom would contribute to a higher level of moisture and dietary fiber, and lower fat, ash, and protein content.

3.2. Water Holding Capacity (WHC), Cooking Loss, and pH.

Table 3 shows the results of WHC, cooking loss, and pH of the hybrid patties. WHC ranged from 68.85% (CON) to 89.29% (T4), which was significantly higher in T4 and significantly lower in CON ($p < 0.05$). The cooking loss was 2.34% (T4)–11.01% (CON) and was significantly higher in T4 and significantly lower in CON ($p < 0.05$). Mushrooms have a high-water retention capacity due to cell wall materials such as chitin and beta-glucan [39]. Therefore, it is considered that higher mushroom content would contribute to higher water holding capacity and lower cooking loss. The pH ranged from 5.72 (CON) to 6.58 (T4), indicating a significantly higher value in T4. Depending on the processing process, the pH of dried shiitake mushrooms is lower than that of raw shiitake mushrooms, but the pH of raw shiitake is

about 6.55 and that of the rump is about 5.59 [40, 41]. Therefore, it is thought that higher content of mushrooms during patty production might contribute to higher pH levels.

3.3. Color. Table 4 shows the results of color characteristics of the hybrid patties. The L^* (lightness) showed a tendency to increase as the mushroom content increased and showed a significantly higher value in T4 ($p < 0.05$). The A^* (redness) showed a significantly higher value in CON and a significantly lower value in T3 ($p < 0.05$). The b^* (yellowness) showed a significantly higher value in CON ($p < 0.05$). Since all parts of the shiitake mushroom are bright except for the dark outer surface of the cap, the brightness is high when the mushrooms are crushed. In this experiment, it is thought that the higher mushroom content led to higher L^* . In the case of a^* and b^* , it is considered that the CON showed significantly higher values due to high meat content along with fewer mushrooms.

3.4. Texture Profile Analysis. Table 5 shows the results of texture profile analysis of the hybrid patties. The texture profile analysis was greatly affected by the type and amount of protein. In the case of meat products kneaded with emulsions such as sausages and patties, the amount of soluble protein extracted by NaCl or sarcoplasmic protein during kneading was greatly affected [42–44]. As the content

TABLE 3: Water holding capacity, cooking loss, and pH of the hybrid beef patty with shiitake mushroom (*Lentinus edodes*).

	Treatments ⁽¹⁾				
	CON	T1	T2	T3	T4
Water holding capacity (%)	68.85 ± 3.54 ^D	77.55 ± 2.85 ^C	82.20 ± 1.80 ^{BC}	86.97 ± 3.89 ^{AB}	89.29 ± 1.94 ^A
Cooking loss (%)	11.01 ± 0.45 ^A	6.43 ± 0.91 ^B	5.69 ± 0.48 ^B	4.29 ± 0.58 ^C	2.34 ± 0.71 ^D
pH	5.72 ± 0.12 ^E	5.95 ± 0.06 ^D	6.18 ± 0.06 ^C	6.31 ± 0.03 ^B	6.58 ± 0.03 ^A

^{A-E}Means with different superscriptions within the same row differ ($p < 0.05$, $n = 3$). ⁽¹⁾Treatments: CON (*Lentinus edodes* 0%), T1 (*Lentinus edodes* 20%), T2 (*Lentinus edodes* 40%), T3 (*Lentinus edodes* 60%), and T4 (*Lentinus edodes* 80%).

TABLE 4: Color of the hybrid beef patty with shiitake mushroom (*Lentinus edodes*).

	Treatments ⁽¹⁾				
	CON	T1	T2	T3	T4
L^*	43.95 ± 1.74 ^D	50.36 ± 0.85 ^C	52.25 ± 1.01 ^{BC}	55.94 ± 1.94 ^{AB}	58.57 ± 3.90 ^A
a^*	28.30 ± 0.94 ^A	16.97 ± 2.14 ^B	9.18 ± 0.92 ^C	5.87 ± 1.65 ^D	8.61 ± 1.18 ^E
b^*	17.57 ± 0.79 ^A	12.35 ± 2.61 ^{AB}	10.22 ± 4.06 ^B	12.04 ± 2.17 ^B	13.41 ± 1.14 ^B

^{A-E}Means with different superscriptions within the same row differ ($p < 0.05$, $n = 3$). ⁽¹⁾Treatments: CON (*Lentinus edodes* 0%), T1 (*Lentinus edodes* 20%), T2 (*Lentinus edodes* 40%), T3 (*Lentinus edodes* 60%), and T4 (*Lentinus edodes* 80%).

TABLE 5: Texture profile analysis of the hybrid beef patty with shiitake mushroom (*Lentinus edodes*).

	Treatments ⁽¹⁾				
	CON	T1	T2	T3	T4
Hardness (kg)	2.09 ± 0.64 ^A	1.83 ± 0.23 ^{AB}	1.62 ± 0.48 ^{AB}	1.36 ± 0.24 ^{BC}	0.78 ± 0.41 ^C
Cohesiveness (%)	55.05 ± 4.36 ^{AB}	52.16 ± 3.27 ^B	60.56 ± 4.48 ^{AB}	58.38 ± 7.17 ^{AB}	6.23 ± 3.70 ^C
Chewiness (kg)	1.20 ± 0.19 ^A	0.96 ± 0.12 ^{AB}	0.94 ± 0.22 ^{AB}	0.86 ± 0.15 ^B	0.04 ± 0.01 ^C

^{A-C}Means with different superscriptions within the same row differ ($p < 0.05$, $n = 3$). ⁽¹⁾Treatments: CON (*Lentinus edodes* 0%), T1 (*Lentinus edodes* 20%), T2 (*Lentinus edodes* 40%), T3 (*Lentinus edodes* 60%), and T4 (*Lentinus edodes* 80%).

of shiitake mushroom increased, the hardness and chewiness values tended to decrease, and all values were significantly lower in the treatment group T4 without meat ($p < 0.05$). It is thought that the binding property decreases as the content of meat decrease depending on the type and amount of protein extracted during kneading. In addition, since the amount of protein extracted in the T4 treatment group without added meat was very small, and there was no binding property, the shape was not properly captured when making the patties and was disturbed during the cooking process.

3.5. Storage Stability. The storage stability of the hybrid patties was tested based on storage under refrigeration for 0 days, 3 days, and 7 days. To evaluate the storage stability experiments, VBN, TBARS, and TMC were performed, and the data are shown in Table 6. VBN showed a significantly lower value in the T4 treatment group at 0 days ($p < 0.05$). On day 3, T1 and T2 treatments showed significantly higher values, but on day 7, T2 and CON showed significantly lower values ($p < 0.05$). On the contrary, T1 and T3 showed significantly higher values on day 7 ($p < 0.05$). However, Kim and Shin [45] reported that when the VBN value content is 15 mg%, humans can percept the rotten odor. In addition, the Ministry of Food and Drug Safety (MFDS) [46] stipulates that the VBN value should be 20 mg% or less for edible meat and packaged meat so that it should be ingestible. TBARS showed significantly lower values in CON on days 0, 3 and 7 ($p < 0.05$). On day 0, it was found that the higher level of

mushrooms led to a higher TBARS level. In the experiments of Yang et al. [47] and Cheung and Cheung [48], the presence of shiitake mushrooms showed lower antioxidant activity than other mushrooms. Also, in the experiment of Cerón-Guevara et al. [26], as the content of mushroom powder increased, the TBARS value showed a tendency to increase. The content of unsaturated fatty acids in shiitake mushrooms is about 76% of the fat, and unsaturated fatty acids are easily oxidized [49, 50]. Therefore, in this result, shiitake mushrooms are considered to have an effect on the increase in TBARS values. Greene and Cumuze [51] considered that the TBARS value was in the range of the smallest detectable level for oxidized flavor when ingested at 0.6–2 mg/kg. In this experiment, the highest level was noted on day 7 (0.31 mg/kg), so it is considered that the oxidized flavor cannot be detected when ingested. TMC showed that the number of microorganisms increased as the level of mushrooms increased from day 0. Over time of 0, 3, and 7 days, a significantly higher value was observed in T4 ($p < 0.05$), and there was no significant difference between CON, T1, and T2 treatment groups ($p > 0.05$).

3.6. Sensory Properties. Table 7 shows the results of sensory properties of hybrid patties. Color tends to receive a lower score as the mushroom content increases and showed a significantly lower value in T4. This observation shows a similar trend to the result of increasing the brightness as shown in the color measurement. Flavor showed

TABLE 6: Volatile basic nitrogen (VBN), total microbial count (TMC), and 2-thiobarbituric acid reactive substances (TBARS) of the hybrid beef patty with shiitake mushroom (*Lentinus edodes*).

	Storage (days)	Treatments ⁽¹⁾				
		CON	T1	T2	T3	T4
VBN (mg%)	0	7.15 ± 0.42 ^A	7.33 ± 0.82 ^A	7.06 ± 0.73 ^A	7.15 ± 0.63 ^A	5.04 ± 0.16 ^B
	3	8.61 ± 0.16 ^B	11.54 ± 0.42 ^B	11.54 ± 0.96 ^A	9.16 ± 0.57 ^B	8.15 ± 0.48 ^B
	7	9.87 ± 0.16 ^{BC}	13.34 ± 0.73 ^A	9.22 ± 0.55 ^C	12.98 ± 0.42 ^A	10.51 ± 0.42 ^B
TMC (log cfu/g)	0	4.14 ± 0.24 ^B	3.86 ± 0.24 ^B	3.96 ± 0.10 ^B	4.07 ± 0.16 ^B	4.43 ± 0.14 ^A
	3	4.15 ± 0.21 ^B	4.15 ± 0.21 ^B	4.65 ± 0.49 ^{AB}	5.15 ± 0.39 ^{AB}	4.72 ± 0.17 ^A
	7	5.18 ± 0.26 ^C	5.10 ± 0.42 ^C	5.10 ± 0.25 ^C	5.83 ± 0.23 ^B	6.79 ± 0.45 ^A
TBARS (mg MDA/kg)	0	0.10 ± 0.00 ^C	0.13 ± 0.00 ^C	0.14 ± 0.02 ^C	0.20 ± 0.01 ^B	0.32 ± 0.06 ^A
	3	0.12 ± 0.01 ^C	0.15 ± 0.01 ^B	0.16 ± 0.01 ^B	0.14 ± 0.01 ^B	0.46 ± 0.01 ^A
	7	0.10 ± 0.01 ^D	0.25 ± 0.01 ^A	0.31 ± 0.02 ^B	0.18 ± 0.02 ^C	0.31 ± 0.02 ^A

^{A-D}Means with different superscriptions within the same row differ ($p < 0.05$, $n = 3$). ⁽¹⁾Treatments: CON (*Lentinus edodes* 0%), T1 (*Lentinus edodes* 20%), T2 (*Lentinus edodes* 40%), T3 (*Lentinus edodes* 60%), and T4 (*Lentinus edodes* 80%).

TABLE 7: Sensory properties of the hybrid beef patty with shiitake mushroom (*Lentinus edodes*).

	Treatments ⁽¹⁾				
	CON	T1	T2	T3	T4
Color	4.21 ± 1.07 ^A	3.64 ± 0.63 ^{AB}	3.43 ± 0.53 ^{AB}	3.07 ± 0.61 ^B	2.14 ± 0.63 ^C
Flavor	4.29 ± 0.49 ^A	3.71 ± 0.49 ^{AB}	3.50 ± 0.50 ^{BC}	3.14 ± 0.69 ^{BC}	2.93 ± 0.84 ^C
Off-odor	3.86 ± 1.07	3.64 ± 1.03	3.43 ± 0.98	3.71 ± 1.11	3.00 ± 1.00
Softness	2.71 ± 0.95	3.29 ± 0.76	3.21 ± 0.39	3.29 ± 0.49	3.29 ± 0.95
Juiciness	2.21 ± 1.15	2.93 ± 0.73	2.86 ± 0.69	3.00 ± 0.58	3.21 ± 0.99
Total preference	4.00 ± 0.76 ^A	3.57 ± 0.45 ^A	3.36 ± 0.48 ^A	2.36 ± 0.48 ^B	2.50 ± 0.96 ^B

^{A-C}Means with different superscriptions within the same row differ ($p < 0.05$, $n = 3$). ⁽¹⁾Treatments: CON (*Lentinus edodes* 0%), T1 (*Lentinus edodes* 20%), T2 (*Lentinus edodes* 40%), T3 (*Lentinus edodes* 60%), and T4 (*Lentinus edodes* 80%).

a significantly higher value in CON and a significantly lower value in T4. The nonvolatile taste compounds and volatile aromatic compounds present in shiitake impart flavor to the mushrooms [52]. Among them, 1-octen-3-ol, which is of the highest content, imparts a sweet but earthy smell to the mushrooms [53, 54]. Therefore, it is considered that a higher level of mushrooms would lead to a stronger soil odor and lower flavor. O'Quinn et al. [55] reported that consumers preferred beef with flavors characterized as beefy and nutty rather than grassy. Therefore, it is considered that lower the flavor score, lower the total preference. There were no significant differences in off-flavor, softness, and juiciness.

3.7. Ergosterol and β -Glucan. Table 8 shows the results of the content of ergosterol and β -glucan in shiitake mushrooms. The ergosterol content of shiitake mushroom fruiting body was 34.09 $\mu\text{g/g}$ and beta glucan content was 28.13 g/mg . Several studies have reported that ergosterol and beta glucan have beneficial effects on humans [24, 56–58]. Therefore, adding shiitake mushrooms to patties is thought to have beneficial effects on health.

4. Conclusion

This study was conducted to investigate the physicochemical and storage characteristics of hybrid beef patties depending on the amount of shiitake mushrooms added and to determine the appropriate addition amount of shiitake

TABLE 8: Ergosterol and β -glucan content of shiitake mushroom (*Lentinus edodes*).

Ergosterol content ($\mu\text{g/g}$)	Glucan content (g/mg)		
	Alpha	Beta	Total
34.09 ± 4.45	0.36 ± 0.02	28.13 ± 0.48	28.50 ± 0.50

All measurements were performed in triplicate ($n = 3$).

mushrooms in hybrid beef patty production. The addition of shiitake mushrooms led to an increase in moisture and dietary fiber content, thereby enhancing water holding capacity and reducing cooking loss. However, the protein content, cohesiveness, and hardness of the patties were decreased. The addition of shiitake mushrooms showed a tendency to increase the values than control in the VBN, TMC, and TBARS experiments on day 7, which had a negative effect on storage. Concerning the sensory properties, the characteristic earthy smell of shiitake mushrooms had poor scores in flavor and total preference. However, the VBN and TBARS values were in the edible range. Shiitake mushrooms contain good ingredients such as ergosterol and β -glucan, which are thought to have a beneficial effect on human health. The obtained results indicate that shiitake mushrooms should be added in half the quantity when preparing hybrid beef patties in consideration of storage stability, texture profile analysis, and flavor deterioration. In this study, only the ratio of beef and shiitake mushrooms was different for the analysis. In future studies,

research on the addition of substances that can improve the storage stability and flavor of beef patties is needed.

Data Availability

All data generated in the current study are included within the article. Further datasets can be obtained from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by a grant (715003-07) from the Research Center for Production Management and Technical Development for High Quality Livestock Products through Agriculture, Food, and Rural Affairs Convergence Technologies Program for Educating Creative Global Leader, Ministry of Agriculture, Food and Rural Affairs. This research was supported by the Korean Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries (IPET) through the Agri-Bioindustry Technology Development Program, funded by Ministry of Agriculture, Food, and Rural Affairs (MAFRA) (Project No. 321028-5), Korea. This study was supported by “Regional Innovation Strategy (RIS)” through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (MOE). This work was supported by the research grant of the Chungbuk National University in 2019.

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