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Quality of beef *longissimus dorsi* muscle marinated in water extract of shiitake mushrooms (*Lentinula edodes*)

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Abstract

The physicochemical and sensory characteristics are the main factors influencing meat quality during storage time. This investigation aimed to determine the effect of marinating water extract from shiitake mushrooms (*Lentinula edodes*) on the physicochemical and sensory characteristics of beef *longissimus dorsi* muscle over storage time. The study used a completely randomized experimental design with a split-plot pattern. It was observed in 4 subplot concentrations (0%, 25%, 50%, and 75%) and 3 main plots of cold storage time (4, 8, and 12 days) in 2-3℃. The results showed that marinating shiitake mushroom water extract significantly affected ($p<0.05$) the protein, fat, water holding capacity (WHC), cooking loss, pH, bacterial load, color, smell, and taste. The meat protein content, WHC, pH, and cooking loss reach optimal concentration at 25%. The fat content increases, aligning with the concentration of water extract from shiitake mushrooms in storage time. Similarly, the bacterial load decreased in parallel with the concentration of shiitake mushroom extract. Panelists significantly liked ($p<0.05$) the meat color, aroma, and taste. This study concluded that shiitake mushroom water extract potentially preserves physicochemical and sensory characteristics through its antimicrobial and antioxidant activities. This research suggested using a 25% concentration of shiitake mushroom water extraction to maintain beef quality.

1. Introduction

Natural resources are an invaluable part of biodiversity, can be utilized for biotechnological innovation, and promote the search for stabilizers to maintain the physicochemical and sensory characteristics of food that can be used for certain industrial purposes. Many of the diverse mushroom species, including shiitake mushrooms (*Lentinula edodes*), which are available in nature, have not yet been explored to produce certain enzymes that can influence the physicochemical properties of food and can be used commercially to provide meat color stabilizers.

Oxidation and microorganism activity are problems faced by the meat industry because they can cause a decrease in nutritional, physicochemical, and sensory quality, such as off-flavor, rancidity, texture degradation, and unpleasant color changes during storage and processing. Inhibition of oxidation processes, especially those that cause changes in meat color, using synthetic antioxidants, is known to cause health problems.

Physicochemical and sensory quality and color are essential indicators that influence the quality of fresh beef and greatly determine consumers' purchasing decisions. The interaction of exogenous and endogenous factors with post-mortem muscle myoglobin determines the quality and stability of meat color (Faustman and Cassenf, 1990). The red color of meat easily changes to an undesirable brown due to the formation of metmyoglobin in the oxidation process during storage and processing (Fan *et al*., 2019). Oxidative damage to meat is manifested in the form of discoloration, development of bad taste, formation of toxic compounds, decreased shelf life, and nutrient loss (Lorenzo *et al*., 2014; Shah *et al*., 2014). Antioxidants are compounds that can delay, slow down, and prevent oxidation (Kim *et al*., 2013). To date, synthetic antioxidants such as butylated hydroxyl anisole (BHA) have been commonly used to prevent oxidation in meat, which can cause potential health risks to humans and increase product toxicity (Venkatesh and Sood, 2011).

The use of synthetic antioxidants has the potential for toxicological effects and food safety, which causes the demand for natural antioxidants by consumers and

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the meat industry to continue to increase (Kitzberger *et al*., 2007). Incredibly natural antioxidants derived from plants that contain various bioactive compounds and have beneficial effects on health consumers (Faustman and Cassenf, 1990; Liu *et al*., 2013; Kozarski *et al*., 2014; Hawashin *et al*., 2016). Plants rich in antioxidants include vegetables and fruits such as olives, pomegranates, grapes, and berries (Khatua *et al*., 2013; Kozarski *et al*., 2014), as well as herbs and spices such as rosemary, oregano, broccoli, sage, black cumin, thyme or turmeric (Liu *et al*., 2013; Fogarasi *et al*., 2018).

Consumers and the meat industry must search for natural antioxidants derived from food components. In recent years, the consumption of mushrooms has been known to have activity as a natural antioxidant (Venkatesh and Sood, 2011). Additionally, edible mushrooms also have biological activities such as antitumor, antiviral, anticomplementary, anticoagulant, antidiabetic, hypolipidemic, hepatoprotective, immunostimulant, and immunological activities. This causes consumable mushrooms to be used in food, cosmetic ingredients, biomedicine, agriculture, environmental protection, and wastewater management (Mwangi *et al*., 2022). Edible mushrooms provide many sources of highly bioactive metabolites and have a wide pharmacological range, including antioxidants (Wardah *et al*., 2023). Shiitake mushrooms are edible mushrooms that are reported to have many pharmacological activities as antioxidants and anti-inflammatories (Kalyoncu *et al*., 2010). Therefore, the use of natural antioxidants from shiitake mushroom water extract on beef's color and physicochemical stability is essential to know.

Consumable mushrooms are fascinating to research as a source of natural antioxidants. Besides having sensory properties, Shiitake mushrooms also have good nutritional value. They are beneficial for health because of their bioactive compounds, especially the high beta-glucan content, as an antioxidant. The ergothioneine (ESH) compound from shiitake mushroom extract is reported to stabilize the color of tuna meat (Bao *et al*., 2010).

Improving fresh meat's physicochemical and sensory stability significantly benefits the processing industry and final products. Minced meat or cuts of meat are susceptible to changes due to oxidation activity and spoilage microorganisms compared to whole meat because grinding and cutting treatments expose the meat's surface to more air and microbial contamination (Patel and Goyal, 2012). This study aimed to explore the effect of using water extract from shiitake mushrooms on beef's protein and fat content, number of bacteria, physicochemical, and sensory stability.

2. Materials and methods

The research was carried out using a completely randomized design with a split-plot pattern with 4 treatments of water extract concentration of shiitake mushrooms, each repeated 5 times. Research variables were observed at storage times of 4, 8, and 12 days at a storage temperature of 2-3°C.

2.1 Shiitake mushroom extraction

Shiitake mushrooms were purchased from mushroom cultivators in Surabaya, Indonesia. A total of 10 kg of shiitake mushrooms were washed, air-dried indoors, sliced, crushed in a blender, added with 100 L of distilled water then filtered. The clear part was shaken at 150 rpm for 24 h, filtered using Whatman No. 40 paper, and the supernatant was taken to be used as a marinating agent.

2.2 Marinated the meat in shiitake mushroom water extract

A 2.4 kg of fresh beef *longissimus dorsi* muscle purchased from the supermarket was cut into 2×2 cm pieces and then mixed evenly to distribute the fat homogeneously. Next, the meat was mixed evenly with salt (1.5%, w/w) and divided into 4 portions of 600 g each. The first part was used as a control, and the remaining 3 parts were marinated in shiitake mushroom water extract for 10 min, respectively, 42 mL (25%, g/mL), 84 mL (50%, g/mL), and 125 mL (75%, g/mL). A total of five portions, each of 120 g were divided and placed in in polypropylene plastic, covered tightly and stored in the refrigerator at 2-3°C for 12 days. Observations of protein and fat content, water holding capacity (WHC), cooking loss, acidity (pH), number of bacteria, color, aroma, and taste of meat were observed on days 4, 8, and 12 of storage.

2.3 Determination of fat content

Determination of meat fat content was carried out using the Mojonnier method (Sallam *et al*., 2004). A total of 10 g of the meat sample was crushed, and the mixture was put into a Mojonnier tube. A total of 2.5 mL of NH_4OH and 10 mL of 95% ethanol were added, then shaken for 90 s. Then 25 mL of ethyl ether was added, shaken for 90 s, and centrifuged at 600 rpm for 30 s. The ether solution was decanted from a Majonnier plate flask of known weight. Then 5 mL of ethanol was added to the sample and shaken vigorously for 15 s, and 15 mL of ethyl ether was added and shaken again for 60 s. Then 15 mL of petroleum ether was added and shaken for 60 s, then continued with centrifugation at 600 rpm for 30 s. The solution was decanted into a Mojonnier dish, after which 15 mL of diethyl ether was introduced and the

https://doi.org/10.26656/fr.2017.9(5).150

mixture was agitated for 60 s. Subsequently, 15 mL of petroleum ether was added and the system was again agitated for 60 s. The resulting mixture was centrifuged at 600 rpm for 30 s. The solvent phase was then transferred to a Mojonnier dish and evaporated in a fume cupboard at 95°C. The dish was dried in an oven at 100°C until a constant weight was obtained. Residue weight is expressed as the weight of fat/oil in the material.

2.4 Determination of protein content

The protein content of meat was quantified using the AOAC Official Method 984.13 (titrimetric), a Kjeldahl-based procedure. A total of 1 g of meat sample from each treatment was put into a 300 mL Kjeldahl flask containing 1 g of a mixture of selenium (7 g potassium sulfate and 0.8 g cupric sulfate) and 12 mL of concentrated sulfuric acid, then digested at 420°C for 1 h. After cooling to room temperature, the sample's Kjeldahl flask was added with 50 mL of distilled water and 50 mL of 40% sodium hydroxide. Distillation was carried out in an Erlenmeyer flask (250 mL) containing 25 mL of 4% boric acid. The distillation process was stopped when the color change was observed from red to green. The distillate was then titrated with 0.2 N hydrochloric acid until the color changed from green to red.

2.5 Determination of cooking losses

Measurement of meat cooking loss from each treatment and replication used the method described by Lee *et al*. (2006). A total of 10 g of meat samples from each treatment and replication were steamed at 80°C for 15 min. The meat samples were cooled and drained with tissue paper without applying pressure and then weighed. The difference in weight before and after steaming is the percentage of cooking loss divided by the weight before steaming. The formula for measuring cooking loss is as follows:

$$\text{Cooking losses (\%)} = \frac{(\text{weight before steaming} - \text{weight after steaming})}{\text{weight before steaming}} \times 100\%$$

2.6 Determination of water holding capacity

Measuring the water-holding capacity (WHC) of meat was carried out using the method described by Hereu *et al*. (2012). A total of 2 g of meat samples from each treatment and replication were crushed and put into a 20 mL centrifuge tube containing 10 mL of distillate water. The tube was then centrifuged at 3000 rpm for 20 min. The supernatant was taken, and the volume was measured. The WHC calculation follows the following formula:

$$\text{WHC (\%)} = \frac{(\text{volume before contrifugation} - \text{supernatant volume})}{\text{volume before centrifugation}} \times 100\%$$

2.7 Measuring the acidity of meat

The acidity (pH) of meat was measured using a pH meter that has been calibrated with a phosphate buffer solution with a pH of 4.0 and 7.0. A total of 10 g of meat samples from each treatment and replication were taken, crushed, added with 10 mL of distilled water, and homogenized. The probe of the pH meter is dipped in the mixture, and the pH is read.

2.8 Enumeration of bacterial counts

The total bacteria count in meat samples was determined using the spread plate method following AOAC Official Method 966.23 (AOAC INTERNATIONAL, 2023). A total of 25 g of sample from each treatment and replication was put into an Erlenmeyer flask (500 mL) and added with 225 mL of phosphate buffer solution. The serial dilution of the broth culture was carried out by transferring 1 mL into 9 mL of phosphate buffer solution. After thorough homogenizing, 0.1 mL of each dilution is spread evenly over the entire surface of the nutrient agar media in the petri dish. All inoculated plates were then incubated at 35-37°C for 48 h. Colonies that grow in the media were counted using a colony counter. Plates with 30 – 300 colonies were selected to determine the total viable count (CFU/g) with the formula:

$$\text{Total Viable Count (CFU/g)} = \frac{\text{Number of colonies}}{\text{Inoculated volume (mL)}} \times \text{Dilution factor}$$

2.9 Sensory characteristics

The sensory characteristics of meat were assessed by twenty semi-trained panelists consisting of students and lecturers. All panelists have a taste threshold of 10% sugar in tea water, do not smoke, and are not color blind. Panelists assessed the level of preference for meat that had been stored in the refrigerator for 12 days, and the panelists' level of choice was: color and aroma based on the Linkert scale, namely 1 = very dislike (ashen color and very abnormal aroma), 2 = do not like (pale color and abnormal aroma), 3 = neutral (less bright color and less than usual aroma), 4 = like (bright color and distinctive smell of meat), and 5 = very like (very bright color and very characteristic aroma of meat). Taste assessment using the same method and scale is carried out on steamed meat.

2.10 Statistical analysis

Observational data have been analyzed using a one-way analysis of variance according to a completely randomized experimental design at a significance level of 0.05. Data on bacterial and sensory counts were transformed into log 10 and log + 0.5, respectively, before analysis of variance was carried out. Further tests

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were carried out using an honest significant difference test at a significance level of 0.05 to determine the location of the differences between treatments if the results of the analysis of variance showed a significant effect ($p<0.05$).

3. Results and discussion

3.1 Meat protein content

This study (Figure 1) showed that meat protein content decreased during cold storage, especially when marinated with 75% shiitake mushroom water extract. At a shelf life of 4 days, the protein content of beef marinated in 25-75% water extract of shiitake mushrooms was not significantly different ($p>0.05$) from the protein content of beef that was not marinated in shiitake mushroom extract (control). However, at storage times of 8 and 12 days, the protein content of meat marinated with shiitake mushroom water extract was 50% (16.05±0.52%) and 75% (15.37±0.21%) significantly ($p<0.05$) lower than beef that was not marinated (18.67±0.26%). Meanwhile, the protein content of beef marinated in 25% shiitake mushroom water extract (17.41±0.14%) was no difference significantly ($p>0.05$) compared to beef that was not marinated. The decrease in protein content due to the marination of shiitake mushroom water extract at high concentrations is thought to be caused by changes in the texture of the meat to make it softer. Shiitake mushroom powder increases the juiciness and decreases the toughness and chewiness of pork (Diallo *et al*., 2020). This study aligns with Wang *et al*. (2009) who reported that changing the texture to tend to be softer can reduce the protein content of meat sausages. Beef marinated in shiitake mushroom water extract is thought to undergo a gelation process, namely when the protein absorbs water and undergoes a coagulation process. Proteins could experience gelation, which occurs when proteins aggregate into networks that form texture and juiciness (Wahono *et al*., 2019).

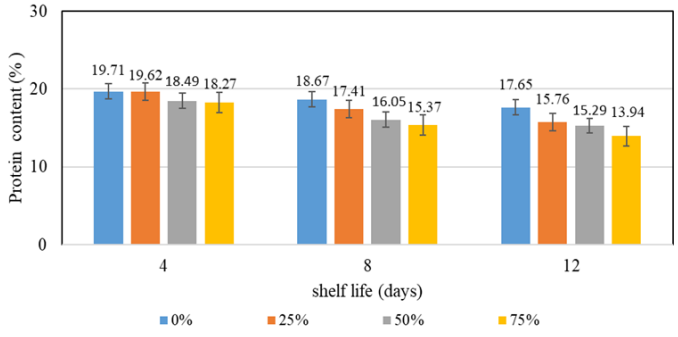

Figure 1. Protein content of beef marinated in shiitake mushroom water extract and stored cold at 2-3°C for 12 days.

3.2 Fat level

This study (Figure 2) showed that the fat content of beef that was marinated in shiitake mushroom water extract was significantly more stable than beef that was not marinated in shiitake mushroom water extract. During the 12-day shelf life, the fat content of beef marinated in shiitake mushroom water extract was 25-75% significantly ($p<0.05$) compared to the average fat content of meat that was not marinated (0%). The stability of beef fat content increased along with the concentration of shiitake mushroom water extract. The strength of the fat content of beef marinated in shiitake mushroom extract is thought to be due to the activity of antioxidant compounds in shiitake mushrooms. Venkatesh and Sood (2011) reported that shiitake mushrooms contain phenolic compounds known as potent antioxidants. Kim *et al*. (2013) said that shiitake mushrooms have several therapeutic actions, including antioxidant properties. Kitzberger *et al*. (2007) stated that antioxidant components can delay, slow down, and prevent oxidation.

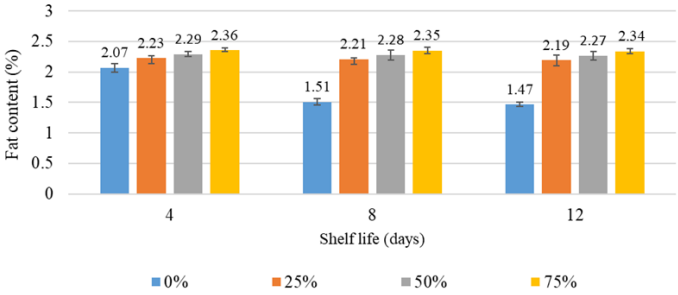

Figure 2. The fat content of beef marinated in shiitake mushroom water extract and stored cold at 2-3°C for 12 days.

3.3 Water holding capacity

This study (Figure 3) showed that the water-holding capacity (WHC) of beef decreases as the concentration of shiitake mushroom water extract increases during cold storage. The WHC of beef marinated in shiitake mushroom water extract with a concentration of 50-75% was significantly ($p<0.05$) lower than the WHC of beef marinated in 25% shiitake mushroom water extract and which was not marinated. However, there was no significant difference ($p>0.05$) between the WHC of beef marinated with 25% water extract of shiitake mushrooms and the WHC of beef that was not marinated (0%). The decrease in WHC of beef due to marination in shiitake mushroom water extract is thought to be due to changes in the structure of the meat to make it soft, so that the ability of the meat to bind water decreases. The WHC of meat is influenced by the state of the meat protein, especially water, which binds directly to the hydroxyl groups of meat protein (Buckle *et al*., 1985). WHC is the ability of meat to retain water and is an integral part of product quality in terms of juiciness and tenderness (Buckle *et al*., 1985). Kitzberger *et al*. (2007) reported

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that shiitake mushroom extract can hydrolyze protein, increasing protein hydrolysis causes an increase in protein solubility but reduces water-holding capacity. Protease enzymes can reduce the amount of connective tissue, degrade collagen and elastin in connective tissue, and reduce meat hardness (Ryder *et al*., 2015).

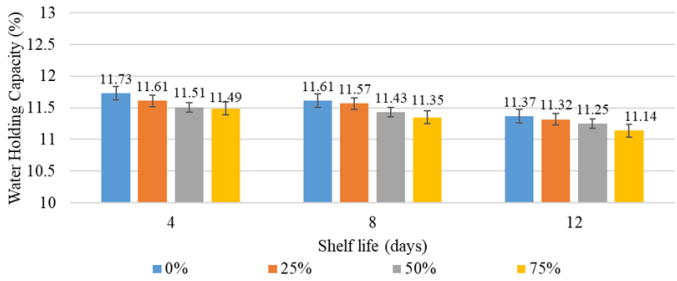

Figure 3. Water holding capacity (WHC) of beef marinated in shiitake mushroom extract and stored cold at 2-3°C for 24 h.

3.4 Cooking loss

This study demonstrated that marination of beef with shiitake mushroom water extract significantly ($p < 0.05$) increased cooking loss (Figure 4). The cooking loss of beef further increased with higher concentrations of shiitake mushroom water extract and longer storage times. Cooking loss of beef that was not marinated (0%) was significantly ($p<0.05$) lower than beef that was marinated in 50% and 75% shiitake mushroom water extract at all storage times, but was not significantly different ($P>0.05$) with marination in 25%. The increase in cooking loss of beef marinated in shiitake mushroom water extract is thought to be due to decreased water-holding capacity due to protein hydrolysis by the protease enzyme contained in shiitake mushrooms, which changes the structure so that the dissolved protein comes out of the meat (Hilario *et al*., 2004; Hereu *et al*., 2012). The temperature and length of the cooking process also influence the cooking loss value. High cooking temperatures and long cooking times will prevent a lot of juice from being lost, and vice versa. Processing food at high temperatures and for a long time can cause liquid and essential nutrients to be lost from the food (Sunayardi, 2021).

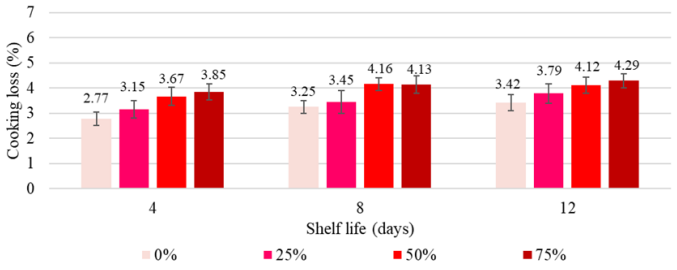

Figure 4. Cooking loss of beef marinated in shiitake mushroom water extract and stored cold at 2-3°C for 12 days.

3.5 Acidity level

This study (Figure 5) showed that the marination of meat in water extract of shiitake mushrooms can maintain the pH of meat during cold storage. The pH of beef marinated in water extract of shiitake mushrooms at a shelf life of 8 and 12 days was significantly ($p<0.05$) higher than the pH of meat that was not marinated in shiitake mushroom extract (0%). The pH stability of meat marinated in shiitake mushroom water extract is thought to be because shiitake mushrooms contain antimicrobial components. Kitzberger *et al*. (2007) reported that shiitake mushrooms contain antimicrobial compounds; Shiitake mushroom extract has broad antimicrobial activity, including bacteria, yeast, and mold. Borch *et al*. (1996) said that various bacteria could cause spoilage of refrigerated beef, including lactic acid-producing bacteria such as *Lactobacillus* spp., and *Leuconostoc* spp. This research aligns with Hereu *et al*. (2012) and Dermiki *et al*. (2013), who reported that shiitake mushroom extract did not affect the pH of chopped beef.

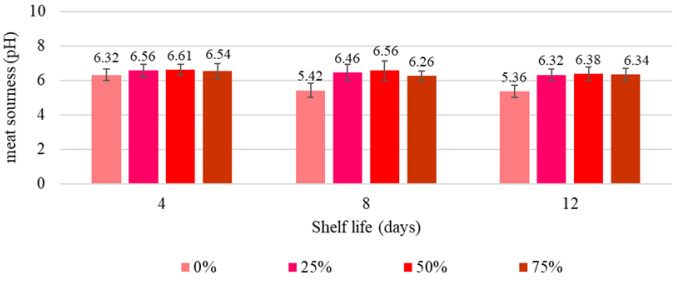

Figure 5. Acidity (pH) of beef marinated in shiitake mushroom extract and stored cold at 2-3°C for 12 days.

3.6 Bacterial count

This study (Figure 6) showed that the marination of meat in water extract of shiitake mushrooms can inhibit the number of bacteria in meat during cold storage. The number of bacteria in beef that was marinated in water extract of shiitake mushrooms for a shelf life of 4, 8, and 12 days was significantly ($p<0.05$) lower than the number of bacteria in meat that was not marinated in shiitake mushroom extract. The inhibition of the number of bacteria in marinated meat in shiitake mushroom water extract is thought to be because shiitake mushrooms contain antibacterial components. This data on the number of bacteria confirms that the pH stability

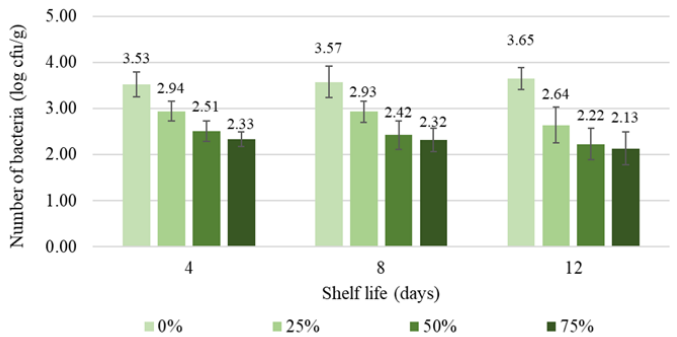

Figure 6. Number of bacteria in beef marinated in shiitake mushroom extract and stored cold at 2-3°C for 12 days.

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of the meat marinated in the water extract of shiitake mushrooms was caused by the inhibition of bacterial growth by the antimicrobial components in shiitake mushrooms.

3.7 Sensory characteristic

This study showed that panelists significantly ($p<0.05$) preferred the color (Figure 7), aroma (Figure 8), and taste (Figure 9) of beef marinated in shiitake mushroom extract compared to the color of meat that was not marinated in shiitake mushroom extract during storage for 12 days at 2-3°C. This research also indicates that the marination of beef in shiitake mushroom water extract can stabilize the color brightness, distinctive smell, and taste of beef. The use of shiitake mushroom water extract is thought to be because shiitake mushrooms can act as antioxidants and antimicrobials. The brownish color change in meat occurs due to the formation of metmyoglobin in the oxidation process during storage (Fan *et al*., 2019). Oxidative activity in meat is manifested in the form of color changes, development of off-flavors, formation of toxic compounds, decreased shelf life, and loss of nutrients (Lorenzo and Gómez, 2012; Shah *et al*., 2014). The natural antioxidants found in shiitake mushrooms are considered compounds that can delay, slow down, and prevent the oxidation process (Kim *et al*., 2013).

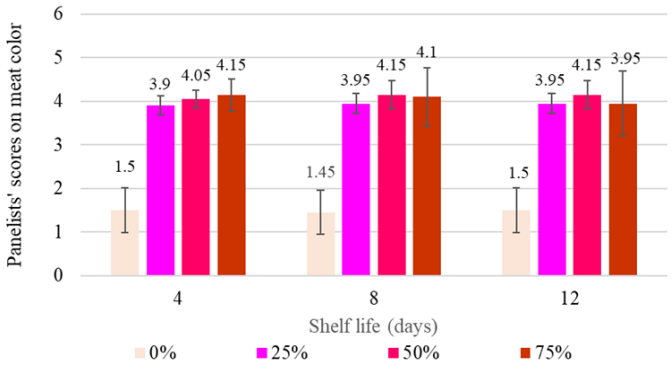

Figure 7. Color of beef marinated in shiitake mushroom extract and stored cold at 2-3°C for 12 days.

Several researchers report that lipid oxidation can cause a decrease in the flavor quality and rancidity of meat and meat products (Lorenzo and Gómez, 2012). Lipid oxidation affects color, texture, nutritional value, taste, and aroma, which are unacceptable to consumers (Júnior *et al*., 2013). Meanwhile, microbial spoilage causes unpleasant odors and often the formation of mucus, which makes the product undesirable for human consumption (Hilario *et al*., 2004). Kupcová *et al*. (2018) reported that shiitake mushroom water extract contains antimicrobial, cytotoxic, anti-inflammatory, and antioxidant properties. However, this research also shows that maceration of beef in shiitake mushroom water extract at high concentrations can cause a decrease in the level of taste preference. The reduction in taste preference is thought to be due to the influence of the components in shiitake mushrooms. Chaipoot *et al*. (2023) reported that shiitake mushrooms taste delicious because they contain volatile and non-volatile compounds such as glutamic acid, aspartic acid, and monosodium glutamate.

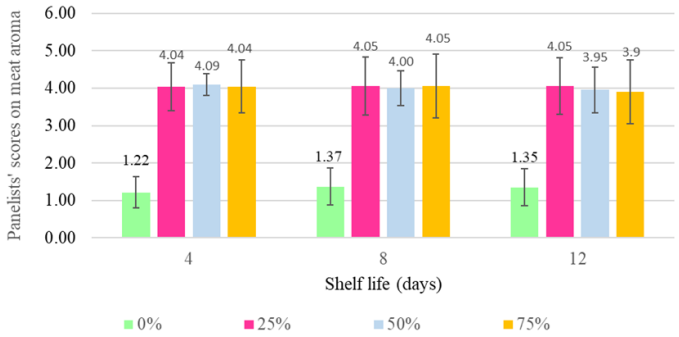

Figure 8. The aroma of beef marinated in water extract of shiitake mushrooms and stored cold at 2-3°C for 12 days.

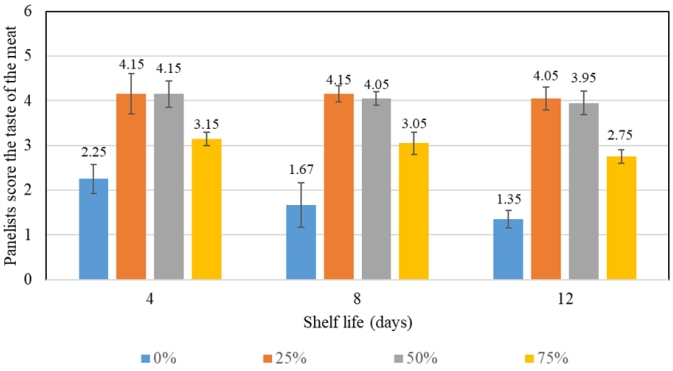

Figure 9. The flavor of beef marinated in water extract of shiitake mushrooms and stored cold at 2-3°C for 12 days.

4. Conclusion

The current study concludes that the marination of water extract of shiitake mushrooms at a concentration of 25% can maintain the quality of meat, including physicochemical and sensory characteristics. Shiitake mushrooms have the potential to be used as a natural preservative for food and processed food products and are helpful for society and the food industry in processing to reduce the use of chemicals.

Conflict of interest

All authors declare that there is no conflict of interest in any aspect of this study.

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