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Full Length Article

Utilization of Button Mushroom (*Agaricus bisporus*) Water Extract as a Culling Hens Meat Tenderizer

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Abstract

The invention of a meat tenderizer that is cheap, easy to obtain, has a short production time and is applicable in the meat industry is needed. This study aims to determine the protein and enzyme content in the water extract of *Agaricus bisporus* and to explore its use as a meat tenderizer for culling hens. Meat tenderization application experimented with a completely randomized design by the soaking method. The completely randomized design used 6 treatments, each repeated 5 times. The treatments consisted of 4 concentrations of water extract of *A. bisporus* mushroom, namely, 2.5, 5.0, 7.5 and 10%, one positive control treatment using 0.2% papain and one negative control treatment using distilled water. The study's results showed that the bodies of *A. bisporus* mushrooms and other parts had different protein content, protease, and collagenase activities. Water extract of *A. bisporus* mushroom at a concentration of 5% significantly increased tenderness. Furthermore, the water extract did not impact the pH, WHC, cooking loss, water, protein and fat content, color, and aroma but increased preference for the toughness and taste of culling hens' meat. It concluded that *A. bisporus* mushroom water extract has great potential to be utilized as a meat tenderizer with a recommended dose of 5%.

Keywords: *Agaricus bisporus*; Culling hens; Protease; Meat; Tenderizer; Toughness and taste of culling hens' meat

Introduction

Meat plays an important role not only as a source of food nutrition but in economies and cultures worldwide. Consumers are very concerned about meat's sensory quality and tenderness. Meat quality is strongly influenced by several factors: muscle composition, collagen content, intramuscular connective tissue structure, and post-slaughter myofibrillar protein degradation. Meat tenderness also depends on the type of muscle, age, factors before and after slaughter, pH and postmortem temperature (Anderson *et al.* 2012). Different physical and chemical methods are used to tenderize meat. However, using exogenous protease enzymes as meat tenderizer is a relatively progressive method and is considered efficient for improving meat quality (Rawdkuen *et al*. 2012).

Meat from old animals becomes rigid due to the formation of cross-links between collagen molecules (Ionescu *et al*. 2008). Protease enzymes can reduce the amount of connective tissue, degrade collagen and elastin in connective tissue to reduce meat hardness (Ryder *et al.* 2015). Papain and bromelain are the most widely used protease enzymes of plant origin for meat tenderization (Liu *et al*. 2008). Papain can tenderize meat by hydrolyzing meat fibers' peptide bonds (Clark 2016), including collagen and myofibrilla, by turning the collagen suspension into a compact gel (Ionescu *et al.* 2008). The papain enzyme shows optimum activity at a pH range of 4–9 and a temperature of 40–80°C (Azmi *et al*. 2023). Meanwhile, bromelain has a softening effect by degrading myosin, troponin T chains without affecting actinin and resulting in the generation of protein fragments with smaller sizes (Feng *et al*. 2017). The bromelain enzyme shows optimum activity in the pH range of 5–8.5 and a temperature of 50–80°C (Azmi *et al*. 2023). Bromelain enzyme activity and performance are affected by pH and temperature. Under acidic conditions, bromelain enzyme activity is optimum at a temperature range of 10–20°C, whereas under alkaline conditions, bromelain enzyme activity is optimum at 30–40°C. In addition, at neutral pH values, a temperature of 40–60°C is required to achieve optimum activity (Manzoor *et al*. 2016).

However, the utilization of papain as a meat tenderizer is limited because it can reduce juiciness and cause a bitter taste in meat derived from bitter peptides resulting from proteolytic degradation (Gerelt *et al*. 2000). Meanwhile, using bromelain can result in excessive tenderness and a mushy texture (Ha *et al*. 2014; Nam *et al*. 2016).

The invention of cheap protease enzymes, a short production process, easy to obtain, and can be applied in industry is still being carried out, including from edible mushrooms such as *Pleurotus ostreatus* (Chung and An 2012), *Sarcodon aspratus*, *Agaricus bisporus* and *Lentinula edodes* (Lee *et al*. 2017). Microbial-derived proteases are preferred over plant and animal proteases because of their various characteristics, which are more suitable for biotechnological applications, such as activity over a wide temperature, pH range, thermal stability, and high catalytic activity (Savitha *et al*. 2022). Most of the proteases from Basidiomycetes fungi are neutral to slightly acidic (Sumantha *et al*. 2006; Sabotič *et al*. 2007). White button mushrooms (*Agaricus bisporus*) are cultivated worldwide and contribute 35–45% of total mushroom production (Research and Markets 2020). Studies on meat tenderizers using *A. bisporus* have been conducted on beef *longissimus dorsi* (Lee *et al*. 2017).

However, studies on poultry meat, primarily culling hens, have yet to be published. Culling hens' meat comes from rejected laying hens at about 96 weeks due to low egg production, around 20–25%. Culling hens' meat can be used as a source of animal protein. However, the number of culling hens' meat consumption is low because of the rough, challenging, juicy texture and fishy aroma, which is limited to the use of chicken soup. Depending on the animal's age and specific muscle type, the chemical composition, structure, and amount of connective tissue also affects meat tenderness (Bolumar *et al*. 2013). Tenderization technology must be applied to increase the tenderness and quality of culling hens' meat. This study examines the content of protease and collagenase enzymes in the fruiting bodies of *A. bisporus* mushrooms and their principal parts. This study also aims to explore water extract from *A. bisporus* mushroom as a tenderizer in culling hens' meat.

Materials and Methods

Slaughtering of chicken

Slaughtering is done by professionals at the Wadung Asri Chicken Slaughterhouse in Sidoarjo, East Java – Indonesia, following Islamic procedures. In brief, healthy culled laying hens from a local farm in Sidoarjo were placed in a holding cage for 12 h, did not receive feed but were still given water. Slaughter was performed with a sharp knife at the base of the chicken's neck to sever the respiratory tract, digestive tract, 2 jugular veins, and arteries with one incision without severing the neck. The slaughtered chicken was hung on a hook with its legs upside down for 3 min. Scalding was done in warm water at 55–60°C for 90 s, then soaked in cold water. Immediately after scalding, de-feathering was done by an automatic chicken feather removal machine. Slices did from the cloaca to the thoracic post, then the crop, trachea, and internal organs were removed and separated from the carcass. The carcass was washed 2 times, examined thighs, drumsticks, breasts, backs, and wings and then separated in the cut-up room. The skin and bones from the carcass parts were removed and then stored in the refrigerator at 4°C.

Preparation of mushroom extract

This current study used water as a button mushroom enzyme extractor because it is abundantly available, cheap, and environmentally friendly. A total of 12.5 kg of whole fresh *A. bisporus* mushrooms were cleaned from impurities, then air-dried, sliced into small pieces, crushed with a blender, and macerated in 125 L distilled water, stirred in a shaker for 24 h at 150 rpm. After maceration, the mixture was filtered, took supernatant part and then evaporated in a rotary evaporator at 60°C for 2 h until concentrated. The concentrated A. bisporus mushroom water extract was dried in a rack dryer at 60°C to constant weight. Furthermore, the concentration of *A. bisporus* mushroom water extract consisting of 2.5, 5, 7.5 and 10% extract of *A. bisporus* mushroom in distilled water was made to be used as a meat tenderizer.

Soaking of meat of culling hens

The skinless and boneless culling hens' breast meat was purchased from a commercial chicken slaughterhouse in Wadung Asri, Sidoarjo, East Java, Indonesia, originating from the Lohmann strain from the same farm, slaughtered and processed at the same time. The culling meat soaking treatment used the method of Lee *et al.* (2017). About 30 min after postmortem, a total of 60 pieces of breast culling hens' meat were divided into 6 parts of 10 pieces each, and the first 4 parts were each soaked in 2.5% (CMWE 2.5%), 5% (CMWE 5.0%), 7.5% (CMWE 7.5%), and 10% (CMWE 10.0%) white button mushroom water extract. One part was immersed in 0.2% papain and used as a positive control. The remaining part was soaked in distilled water (CMWE 0%) and used as a negative control. The meat of culling hens that were soaked at 4°C for 48 h, then observed for tenderness, water holding capacity, cooking loss, pH value, biochemical composition (water, protein, and fat content), and sensory properties of color, taste, toughness, and aroma.

Determination of protein content

The protein content in the water extract of *A. bisporus* mushroom was determined by the titrimetric method according to AOAC (2005). Fresh *A. bisporus* was obtained from Malang, East Java, Indonesia mushroom farmers. Fresh *A. bisporus* mushrooms were cleaned of adhering dirt, then air-dried, and the main components of mushroom fruiting bodies were separated. Sliced separately whole *A. bisporus* mushrooms (250 g), pileus parts (250 g), stipe parts (250 g), and volva parts (250 g), then each mixed with

2.5 L distilled water, crushed in a blender and then put into a mixer constant in 150 rpm at 25°C for 24 h. The pulverized was filtered using Whatman No.1 filter paper in a vacuum. The obtained filtrate and then concentrated in a rotary evaporator at 60°C for 2 h to produce concentrated *A. bisporus* water extract. Dried extract in a rack dryer at 60ºC to constant weight. The 1 g sample contains an extract of whole *A. bisporus* mushrooms, pileus, stipe, and volva wrapped in parchment paper. Weighed and put into a 300 mL Kjeldahl flask, plus 1 g of a mixture of selenium (7 g potassium sulfate and 0.8 g cupric sulfate) and 12 mL concentrated sulfuric acid and then digested at 420°C for 1 h. After being cooled to room temperature, the Kjeldahl flask containing the digested sample was added to 50 mL of distilled water and 50 mL of 40% sodium hydroxide. The distillate was accommodated in a 250 mL Erlenmeyer flask containing 25 mL of 4% boric acid. The distillation process was stopped when the color of the distillate in the holding Erlenmeyer flask changed from red to green. Then, the distillate was titrated with 0.2 N hydrochloric acid solution until the color changed from green to red.

Determination of protease activity

The protease enzyme activity from the water extract of *A. bisporus* mushroom was determined based on Cupp-Enyard (2008) method. Briefly, 1 mL of water extract of *A. bisporus* mushroom was added with 0.65% casein substrate (0.65 g casein in 100 mL of 0.05 *M* K-phosphate buffer solution at pH 7.5) and incubated mixture at 37°C for 10 min. Incubation and reaction were stopped by adding 5 mL of 110 m*M* trichloroacetic acid (TCA) reagent and incubating at 37°C for 30 min. The mixture was centrifuged at 10000 rpm for 10 min and 2 mL, then took the supernatant. Furthermore, 5 mL of sodium carbonate and 1 mL of Folin Ciocalteau reagent were added to the supernatant filtrate and incubated at 37°C for 30 min. The absorbance of the mixture was measured using a spectrophotometer at a wavelength of 660 nm. One unit of enzyme activity was defined as the amount of enzyme required to release 1 mmole of tyrosine on a casein substrate per minute.

Determination of collagenase activity

The collagenase enzyme activity of water extract from *A. bisporus* mushroom was experienced according to Moore and Stein (1954) method described by Park *et al.* (2002). Briefly, 5 mg collagen was added into 1 mL 50 mM Tris-HCl (pH 7.5) containing 5 mM calcium chloride and 0.1 mL water extract of *A. bisporus* mushroom. Then homogenized and incubated the mixture at 37°C for 1 h. Incubation and reaction were stopped by adding 0.2 mL of 50% trichloroacetic acid (TCA). After standing for 10 min at room temperature, centrifuge the 1800 g mixture for 20 min. A total of 0.2 mL of the supernatant was taken and mixed with 1.0 mL of dilute ninhydrin solution, incubated at 100°C for 20 min, then cooled at room temperature. Next, the mixture was diluted with 5 mL 50% 1-propanol, and a spectrometer measured the absorbance at a wavelength of 570 nm. A buffer solution (50 m*M* Tris-HCl, pH 7.5) containing 5 mM calcium chloride was used instead of the control enzyme solution. The amino acid tyrosine solution was used as the standard curve. One unit (U) of enzyme activity was defined as the amount of enzyme required to hydrolyze 1 mole of collagen substrate per h.

Determination of meat tenderness

Meat tenderness was measured according to the method of Hinnergardt and Tuomy using a penetrometer described by Wardah *et al.* (2023). Briefly, 10 g samples of breast culling hens' pieces from each treatment were placed on a penetrometer mat weighing 100 g. The pointer sat in contact with the sample surface, and the scale showed zero. The penetrometer level was pressed for 10 s and then released tenderness measurement results in mm 100 g^{-1} 10 s^{-1}.

Determination of cooking loss

Measurement of cooking loss was done by using the Lee *et al*. (2017) method. A total of 10 g of meat samples from each treatment were steamed at 80°C for 15 min. Cool the meat and it dries with tissue paper without pressing. The weight of the meat before and after boiling was weighed. The calculation of the cooking loss value is as follows:

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

Determination of water holding capacity

Measurement of water holding capacity (WHC) experienced according to the method of Honikel and Hamm described by Wardah *et al.* (2023). Briefly, 2 g of meat samples from each treatment were crushed, and the pulverized product was placed into a 20 mL centrifuge tube containing 10 mL of distilled water at 3000 rpm for 20 min. Then, took the supernatant portion and the volume was measured following WHC calculation:

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

Determination of pH value

The pH of tenderized meat products was measured using a pH meter previously calibrated using a phosphate buffer solution of 4.0 and 7.0 at 25°C. Meat samples from each treatment were taken as much as 10 g, crushed, and added with 10 mL of distilled water. The minced meat and water were homogenized and measured with a pH meter.

Biochemical composition

The biochemical composition includes water, protein, and fat content of culling meat from each soaking treatment. The meat's water, protein, and fat content were determined using the oven, titrimetric and the Babcock methods (AOAC 2005).

Sensory characteristics

The sensory characteristics evaluation of chicken meat was done following the guidelines of Sow and Grongnet (2010), which were assessed by 40 untrained panelists consisting of students and lecturers from the faculty of science and technology, Universitas PGRI Adi Buana, Surabaya. All panelists have a taste threshold of 10% sugar in tea water, do not smoke, and are not color blind. Researchers were asked to rate the preference for meat from each immersion treatment coded and cooked at 71°C. The meat samples were cut into cubes of 2 x 2 cm. Panelists were asked to state their preference for the color, aroma, toughness, and taste of culling hens' meat tenderization products based on a 5-point Likert scale, namely, 1 = very disliked, 2 = disliked, 3 = neutral, 4 = like, and 5 = very liked.

Statistical analysis

Data from observations of protein content, enzyme activity, tenderness, pH value, cooking loss, water holding capacity, and biochemical composition of culling hens' meat tenderization products were analyzed by one-way analysis of variance at a significance level of 0.05. Tukey's test at a significance level of 0.05 was used to see differences between treatments. The data from the observation of sensory characteristics before the analysis of variance was transformed into log + 0.5 numbers. Statistical analysis was performed using the Statistical Program for Social Science (SPSS) version 22 software.

Results

Protein content and enzyme activity of *A. bisporus* water extract

The measurement results (Fig. 1) showed that there was a significant difference ($P < 0.05$) in protein content between the water extract of the main fruiting body of the *A. bisporus* mushroom. The protein content of the water extract of the volva part (19.87 ± 1.13%) was significantly ($P < 0.05$) higher than pileus (18.13 ± 1.12%) and stipe (8.67 ± 1.11%), but significantly ($P < 0.05$) lower than the whole fruiting bodies (25.51 ± 1.35%). Meanwhile, the protein content of the pileus was significantly ($P < 0.05$) higher than the stipe part.

Fig. 1 also shows a significant difference ($P < 0.05$) in the activity of the protease and collagenase enzymes between the water extracts of the main parts of the fruiting bodies of the *A. bisporus* mushrooms. The protease enzyme activity of the volva part (74.63 ± 1.63 U mL^{-1}) was significantly ($P < 0.05$) higher than pileus (66.34 ± 1.52 U mL^{-1}) and stipe (51.66 ± 1.26 U mL^{-1}), but not significantly different ($P > 0.05$) compared to whole fruiting bodies (74.41 ± 1.43 U mL^{-1}). Collagenase enzyme activity in the volva part (9.14 ± 1.53 U mL^{-1}) was significantly ($P < 0.05$) higher than pileus (4.01 ± 1.19 U mL^{-1}) and stipe (2.57 ± 1.18 U mL^{-1}), but not significantly different ($P > 0$ 0.05) compared with whole fruiting bodies (8.29 ± 2.74 U mL^{-1}). Meanwhile, the collagenase enzyme activity of pileus was significantly ($P < 0.05$) higher than stipe.

Tenderness and pH value of meat tenderization products

The results of the tenderness measurement (Fig. 2) showed that soaking culling hen's meat in the water extract of the *A. bisporus* mushroom has a significant effect ($P < 0.05$) on the tenderness. The tenderness of culling hens meat at CMWE 10% (102.75 ± 2.23 mm 100 g^{-1} 10 s^{-1}) was significantly higher ($P < 0.05$) compared to CMWE 7.5% (84.25 ± 2.44 mm 100 g^{-1} 10 s^{-1}), CMWE 5% (78.75 ± 2.72 mm 100 g^{-1} 10 s^{-1}), CMWE 2.5% (68.25 ± 1.33 mm 100 g^{-1} 10 s^{-1}) and CMWE 0% (62.75 ± 2.22 mm 100 g^{-1} 10 s^{-1}), but not significantly different ($P > 0.05$) compared to 0.2% papain (99.25 ± 3.64 mm 100 g^{-1} 10 s^{-1}). Culling hens' meat tenderness at CMWE 7.5% was significantly ($P < 0.05$) higher than CMWE 5%, CMWE 2.5% and CMWE 0%. The tenderness of culling hen's meat at CMWE 5% was significantly ($P < 0.05$) higher than CMWE 2.5% and CMWE 0%. The tenderness of the meat in the CMWE 2.5% was significantly ($P < 0.05$) higher than in CMWE 0%.

Fig. 2 also shows that soaking culling hens' meat in water extract of *A. bisporus* mushroom has no significant effect ($P > 0.05$) on the pH of the meat. There was no significant difference ($P > 0.05$) between the pH of culling hens meat on papain 0.2% (6.20 ± 0.09 mm 100 g^{-1} 10 s^{-1}), CMWE 0.0% (6.15 ± 0.06 mm 100 g^{-1} 10 s^{-1}), CMWE 2.5% (6.15 ± 0.07 mm 100 g^{-1} 10 s^{-1}), CMWE 5.0% (6.13 ± 0.07 mm 100 g^{-1} 10 s^{-1}), CMWE 7.5% (6.12 ± 0.11 mm 100 g^{-1} 10 s^{-1}) and CMWE 10% (5.95 ± 0.19 mm 100 g^{-1} 10 s^{-1}).

Water holding capacity and cooking loss of meat tenderization products

The results of the measurement of water holding capacity (Fig. 3) showed that soaking of culling hens' meat in the water extract of *A. bisporus* mushroom had a significant effect ($P < 0.05$) on the water holding capacity (WHC). WHC of culling hen's meat at CMWE 0.0% (32.90 ± 0.74%) was significantly ($P < 0.05$) lower than WHC at papain 0.2% (43.68 ± 1.79%), CMWE 7.5% (37.878 ± 1.59%) and CMWE 10% (39.04 ± 2.265%) but not significantly different ($P > 0.05$) compared to CMWE 2.5%

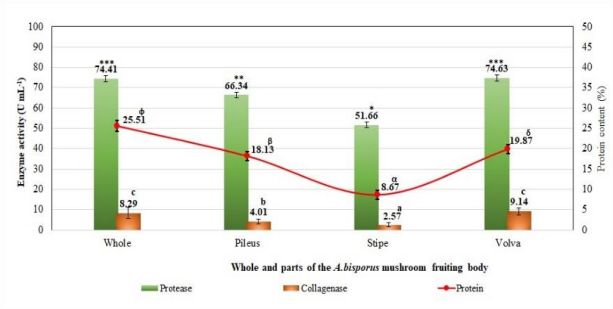

Fig. 1: Protein content and activity of protease and collagenase enzymes in the fruiting body of *A. bisporus* mushroom and its parts. The mean value with standard deviation (error bar) and given a different notation showed a significant difference (*P < 0.05*)

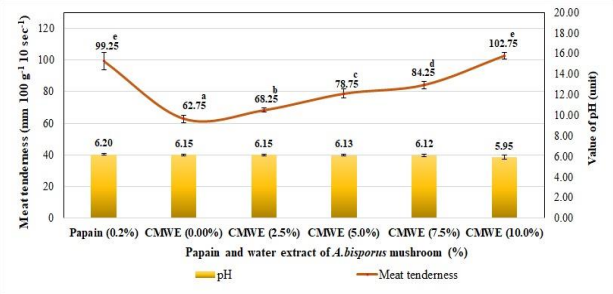

Fig. 2: Tenderness and pH of culling hens' meat soaked in papain and several concentrations of water extract of *A. bisporus* mushroom. The mean and standard deviation values (error bars) which were given different notations showed significant differences (*P < 0.05*)

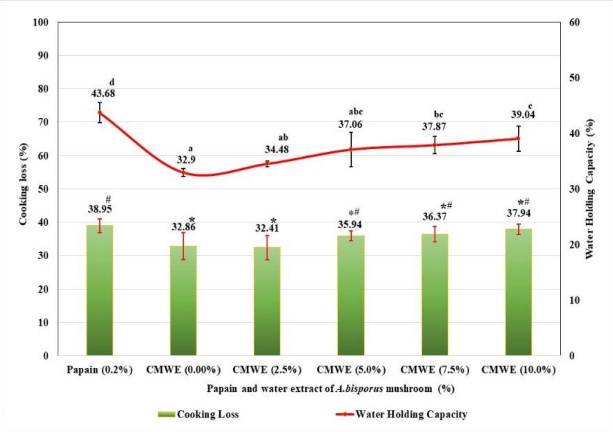

Fig. 3: Water holding capacity and cooking loss of culling hens' meat soaked in papain and some concentrations of *A. bisporus* mushroom water extract. The mean and standard deviation values (error bars) which were given different notations showed significant differences (*P < 0.05*)

(34.48 ± 0.49%) and CMWE 5% (37.06 ± 3.05%). WHC of culling hens' meat at CMWE 2.5% was significantly (*P < 0.05*) lower than WHC at papain 0.2% and CMWE 10%, but not significantly different (*P > 0.05*) with CMWE 5% and CMWE 7.5%. WHC of culling hens' meat at CMWE 5% and CMWE 7.5% was not significantly different (*P > 0.05*) with CMWE 10%, but CMWE 10% was significantly (*P < 0.05*) higher than papain 0.02%.

Fig. 3 also shows that soaking culling hens' meat in water extract of *A. bisporus* mushroom has a significant (*P < 0.05*) effect on cooking loss. Cooking loss culling hens at CMWE 0.0% (32.86 ± 3.99%) was not significantly different (*P > 0.05*) with CMWE 2.5% (32.41 ± 3.61%), CMWE 5% (35.94 ± 1.45%), CMWE 7.5% (36.37 ± 2.34 %), and CMWE 10% (37.94 ± 1.53%), but significantly (*P < 0.05*) lower than papain 0.2% (38.95 ± 2.04%). There was no significant difference (*P > 0.05*) between cooking loss in papain 0.2% and CMWE 5, CMWE 7.5 and CMWE 10%.

Biochemical composition of meat tenderization products

The results of the measurement of water content (Fig. 4) showed that soaking culling hens' meat in the water extract of *A. bisporus* mushroom has no significant effect (*P > 0.05*) on the water content. There was no significant difference (*P > 0.05*) between the water content of culling hens' meat soaked in water extract of *A. bisporus* mushroom and 0.2% papain.

Fig. 4 shows that the protein content of culling hens meat in papain 0.2% (21.41 ± 0.75%), CMWE 0.0% (22.53 ± 0.65%) and CMWE 2.5% (21.65 ± 0.61%) was significantly (*P < 0.05*) higher than the content of culling hens meat protein in CMWE 10% (19.67 ± 0.86%), but not significantly different (*P > 0.05*) compared to CMWE 5% (20.47 ± 0.54%) and CMWE 7.5% (19.86 ± 0.73%). There was no significant difference (*P > 0.05*) in culling hens meat protein content in CMWE 5.0, CMWE 7.5 and CMWE 10%.

Fig. 4 also shows that the fat content of culling hen's meat was significantly (*P < 0.05*) affected by the soaking of the water extract of *A. bisporus* mushroom. The fat content of culling hen's meat in CMWE 0.0% (1.53 ± 0.24%) was not significantly different (*P > 0.05*) compared to papain 0.2% (1.45 ± 0.29%), CMWE 2.5% (1.67 ± 0.19%), CMWE 5% (1.72 ± 0.15%), and CMWE 7.5% (1.89 ± 0.39%), but significantly (*P < 0.05*) lower than CMWE 10% (1.97 ± 0.26%). There was no significant difference (*P > 0.05*) in fat content of culling hen's meat between CMWE 2.5%, CMWE 5%, CMWE 7.5%, CMWE 10%, and papain 0.2%.

Sensory characteristics of meat tenderization products

The results of the panelists assessment (Fig. 5) showed that soaking culling hen's meat in the water extract of *A. bisporus* mushroom has no significant effect (*P > 0.05*) on the sensory characteristics of color and aroma. The color and aroma of culling hen's meat soaked in water extract of *A. bisporus* mushroom were included in the category favored by the panelists (panelists score more than 4).

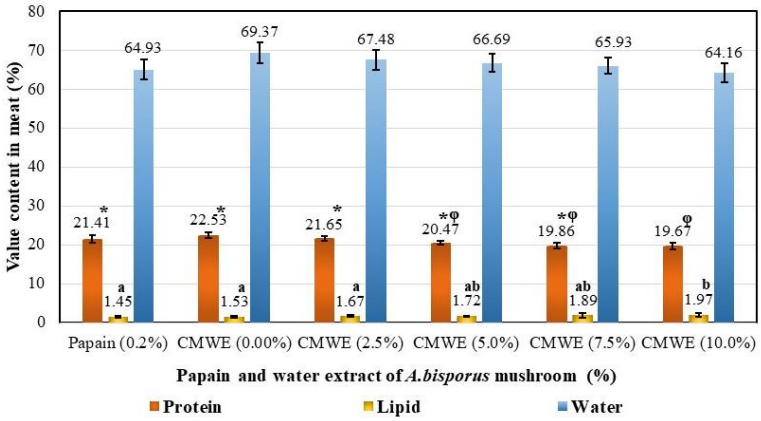

Fig. 4: Biochemical composition of culling hen's meat soaked in papain and some concentrations of water extract of *A. bisporus* mushroom. The mean and standard deviation values (error bars) which were given different notations showed significant differences ($P < 0.05$)

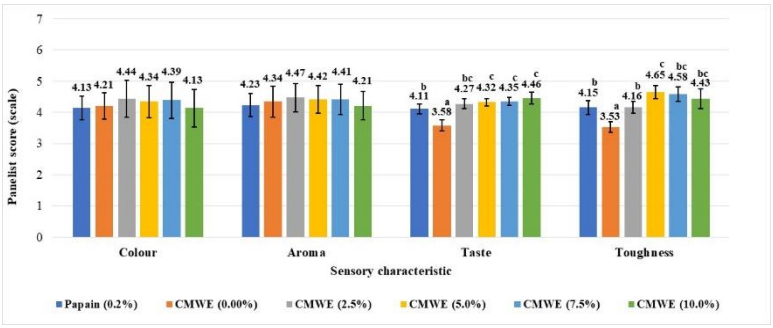

Fig. 5: The assessment of panelists on the sensory characteristics of color, aroma, taste, and toughness of culling hens' meat soaked in papain and some concentrations of *A. bisporus* mushroom water extract. The mean and standard deviation values (error bars) which were given different notations showed significant differences ($P < 0.05$)

However, the results of this study showed that soaking culling hen's meat in water extract of *A. bisporus* mushroom has a significant effect ($P < 0.05$) on the taste and toughness of the meat. The taste and toughness of the meat that was not soaked in papain or water extract of *A. bisporus* mushroom were included in the neutral category according to the panelists' assessment.

The taste of culling hen's meat at CMWE 0% was significantly ($P < 0.05$) lower than that of papain 0.2, CMWE 2.5, CMWE 5, CMWE 7.5 and CMWE 10%. The taste of culling hen's meat in papain 0.2% was significantly ($P < 0.05$) lower than CMWE 5, CMWE 7.5 and CMWE 10%, but not significantly different ($P > 0.05$) compared to CMWE 2.5%. There was no significant difference ($P > 0.05$) between the taste of culling hen's meat at 5 CMWE, 7.5 CMWE and 10% CMWE.

The study also showed that soaking culling hen's meat in the water extract of *A. bisporus* mushroom has a significant effect ($P < 0.05$) on the toughness of the meat. The toughness of culling hen's meat at CMWE 0% was significantly ($P < 0.05$) lower than papain 0.2%, CMWE 2.5, CMWE 5, CMWE 7.5 and CMWE 10%. The toughness of culling hen's meat in CMWE 2.5% was significantly ($P < 0.05$) lower than CMWE 5% but not significantly different ($P > 0.05$) with papain 0.2, CMWE 7.5 and CMWE 10%. The toughness of culling hen's meat at 5% CMWE was significantly ($P < 0.05$) higher than that of papain 0.2%, but not significantly different ($P > 0.05$) compared to CMWE at 7.5% and CMWE at 10%.

Discussion

The present study proves that the fruiting body of the *A. bisporus* mushroom and its parts contain protein. The protein content of the water extract of *A. bisporus* mushroom in this study was included in the range of protein content of *A. bisporus* that several researchers had reported. Usman *et al*. (2021) have reported that the crude protein content of *A. bisporus* is around 19–38% based on the dry weight. Several researchers have also reported variations in the protein content of *A. bisporus*. Mohiuddin *et al*. (2015) have reported the protein content of the mushroom *A. bisporus* around 17.7–24.7% and Ahlavat *et al*. (2016) have

reported 29.1%. Protein content variations of *A. bisporus* were suspected due to differences or variations in the nutrient composition of the substrate. Sebaaly *et al*. (2019) have reported that the nutritional composition of *A. bisporus* mushroom is influenced by the type of substrate.

The present study indicates that the fruiting bodies of *A. bisporus* mushrooms contain protease and collagenase enzymes. Similarly, Inăcio *et al*. (2015) have reported that the mushroom *A. bisporus* can produce protease enzymes. The protease activity of the water extract of *A. bisporus* mushroom in this study was higher than that reported by Lee *et al*. (2017) which is 0.160 U mL^{-1}. The difference in level of activity in protease enzyme is thought to be caused by differences in the nutrient composition of the substrate. Several researchers have reported that the protease enzyme activity of *A. bisporus* (Savoie 1998), *Schizophyllum commune* (Johnston *et al*. 2000) and *Pleurotus sapidus* (Zorn *et al*. 2005) is affected by the availability of nitrogen in the substrate.

This current research indicated significant differences in protein, protease content, and collagenase activity between water extracts of the main fruiting body of *A. bisporus* mushrooms. The highest protein content, protease, and collagenase activity were found in the volva part, followed by the pileus, and the lowest in the stipe part. Differences in value of protein content, activity of protease, and collagenase enzymes in each part of *A. bisporus* mushrooms are suspected due to physiological functions between fruiting body parts for growth and reproduction. Zied *et al*. (2017) reported that differences in the mycochemical composition of the morphological parts of mushrooms were related to the physiological stage of maturity. Sakinah *et al*. (2019) stated that mushroom volva functions in the absorption and storage of nutrients. Zhou *et al*. (2019) suggested that the stipe is a stem or stalk that supports mushroom pileus, which consists of sterile hyphae tissue and is considered an intermediary for spore dispersal. Inăcio *et al*. (2015) stated that proteases are essential in physiological processes such as germination and sporulation. Several researchers have reported the protein content between the fruiting parts of the *A. bisporus* mushroom, but the protein content in the volva part has not been reported. The present study is in line with Nasiri *et al*. (2013) who reported that the protein content of the pileus part of the *A. bisporus* (33.65%) was higher than that of the stipe part (19.01%). Valchev (2020) reported that the total content of amino acids and essential amino acids in the pileus part of the *A. bisporus* was 139.22 mg kg^{-1} and 54.59 mg kg^{-1}, respectively, higher than the stipe part, which was 47.80 mg kg^{-1} and 39.49 mg kg^{-1}, respectively. Golak-Siwulska *et al*. (2018) reported that the chitin content in the pileus part (6.68%) was lower than in the stipe part (7.25%). Zhuang and Sun (2011) reported that the quality and protein content of the volva part of the mushroom *Dictyophora rubrovolvata* (26.74%) was higher than that of the pileus (15.55%).

The present study indicated that *A. bisporus* water extract in a minimum concentration (2.5%) could tenderize culling hens' meat. The tenderness of culling hens' meat increased with *A. bisporus* mushroom of water extract concentration. However, achieving the same tenderness as 0.2% papain requires 10% concentration. The present study is in line with Lee *et al*. (2017), who reported that using water extract of *A. bisporus* with a concentration of 5% reduced the hardness of beef *longissimus dorsi*. The ability of the water extract of *A. bisporus* mushroom to tenderize meat is thought to be due to the activity of protease and collagenase enzymes. Those enzymes in the water extract of *A. bisporus* mushroom can degrade protein and collagen. The data on the tenderness of culling hens' meat soaked in water extract of *A. bisporus* mushroom confirmed that water extract of *A. bisporus* mushroom produced protease and collagenase enzymes. Some researchers reported the effect of using mushroom water extract on tenderness and tissue degradation in meat. Kim *et al*. (2015) reported that a water extract of *Sarcodon aspratus* can tenderize meat. Due to the degradation of many myosin chains in myofibrillar proteins in beef, *longissimus dorsi*. The protease enzyme can hydrolyze peptide bonds, and collagenase degrades collagen fibers in meat (Pal and Suresh 2016; Sprangers and Everts 2019). Kemp and Parr (2012) suggested that meat tenderness generally depends on connective tissue, sarcomere length, and the degree of muscle proteolytic degradation. Gelse *et al*. (2003) reported that in old cattle, the formation of more substantial and more complex collagen crosslinks in connective tissue was increased; proteolytic enzymes can degrade the connective tissue, which consists of 80% collagen, to soften the meat. Santos *et al*. (2020) reported that proteolytic enzymes could degrade myofibrillar protein and collagen in meat and cause an increase in tenderness.

Several researchers have also reported the effect of using mushroom body parts on meat tenderness. Chung and An (2012) reported that proteases in powder and extracts of whole and pileus of oyster mushrooms (*Pleurotus ostreatus*) with a concentration of 20–30% could reduce hardness and increase the amino nitrogen content of beef and pork. Barido and Lee (2021) reported that soaking meat in the extract of the mushroom *Cordyceps militaris* increased the protein solubility and myofibrillar fragmentation index of chicken breast.

The present study indicated that using a water extract of *A. bisporus* mushroom could tenderize meat without causing changes in pH. However, there was a tendency to decrease the pH of culling hens' meat and increase the concentration of *A. bisporus* mushroom water extract to 10% extract concentration. The pH of culling hens' meat soaked in *A. bisporus* mushroom water extract was not different from meat without soaking in water extract *A. bisporus*. Protein hydrolysis by protease enzymes from water extract of *A. bisporus* mushroom into small molecular units such as amino acids was not high enough to lower the pH of meat. The present study is in line with Lee *et al*.

(2017), who reported that using water extract of *A. bisporus* as a meat tenderizer very little affected the pH of beef *longissimus dorsi*.

The present study showed that soaking culling hens' meat in water extract of *A. bisporus* mushroom at concentrations of 2.5 and 5% had no effect on meat WHC, but at high concentrations (7.5 and 10%) increased meat WHC. The present study is in line with Lee *et al*. (2017), who reported that using of water extract of *A. bisporus* mushroom with a concentration of 5% did not affect the WHC of beef *longissimus dorsi*. The same thing was also reported by Kim *et al*. (2015) who reported that the water extract of *S. aspratus* mushroom with a concentration of 2% did not affect WHC *longissimus dorsi*.

Controversial research results regarding the effect of using plant enzymes as meat tenderizers on WHC have been reported. Some researchers report that meat tenderizing enzymes can reduce WHC, some have no effect, and others can increase WHC. Maqsood *et al*. (2018) reported that using enzymes bromelain, ficin, or papain reduced the WHC of camel meat. Gokoglu *et al*. (2017) reported that using bromelain and papain solutions did not increase the WHC of squid (*Loligo vulgaris*). Doneva *et al*. (2018) reported that soaking rabbit meat in papain, ginger extract, and kiwifruit extract for 48 h increased WHC. Naveena *et al*. (2004) reported that ginger homogenate for 48 h increased the WHC of buffalo meat. Variations in the effect of using meat tenderizing enzymes on WHC are thought to be caused by several factors. The factors are differences in muscle structure and enzyme type, duration, and method of tenderization. The results of our study support the hypothesis of Doneva *et al*. (2018) which reported that an increase in WHC meat could occur due to an increase in reactive protein groups that can bind water after partial enzymatic hydrolysis.

The present study showed that soaking culling hens' meat in water extract of *A. bisporus* mushroom up to a concentration of 10% did not affect cooking loss. The present study is in line with Lee *et al*. (2017) who reported that the water extract of *A. bisporus* with a concentration of 5% did not affect the cooking loss of beef *longissimus dorsi*. As in WHC, several researchers have reported the controversial effects of meat tenderizing enzymes on cooking loss. Kadıoğlu *et al*. (2019) reported that soaking a layer of chicken meat in pineapple juice increased cooking loss. Nadzirah *et al*. (2016) reported that using bromelain powder from pineapple increased the cooking loss of steak round cuts. However, Woinue *et al*. (2021) reported that the bromelain enzyme from pineapple peel extract did not affect the cooking loss of mutton thigh meat.

The present study showed that soaking culling hen's meat in water extract of *A. bisporus* mushroom did not affect water content, decreased protein, and increased meat fat content. Using a water extract of *A. bisporus* mushroom as a meat tenderizer with a concentration of 10% can reduce the protein content of culling hens' meat by about 2.86%. This decrease in protein content is thought to occur due to protein hydrolysis by protease enzymes to produce amino acids or low molecular weight compounds. Degradation of connective tissue, collagen and elastin by proteinase enzymes can reduce the ability of meat to bind protein and increase cooking losses as shown in this study on the effect of soaking meat in button mushroom water extract on cooking loss at a concentration of 10%. Ketnawa *et al*. (2012) reported that the decrease in the protein content of meat using protease enzymes as tenderizers occurred due to the degradation of protein or collagen structures into hydroxyproline and resulted in protein fragments with shorter peptide chains. The decrease in protein content increased along with the increase in proteolytic activity. Several researchers have reported decreased protein content due to meat tenderizing enzymes. Barido and Lee (2021) reported that proteases from extracts of *C. militaris* at high concentrations increased protein solubility and reduced the total protein content of chicken breast.

The present study showed that using water extract from *A. bisporus* mushroom as a meat tenderizer with a concentration of 10% increased the fat content of culling hens' meat by about 0.44%. The increase in fat content is thought to be due to fat emulsion, the formation of new fatty acid compositions, and free fatty acids from the work of protease and collagenase enzymes contained in the water extract of *A. bisporus* mushroom. In addition, it is suspected that the fat derived from the water extract of the *A. bisporus* mushroom was absorbed in the meat. Wang *et al*. (2019) reported that hydrolysis by protease enzymes will decompose the combination of protein and fat and release fat.

The present study indicated that the color and aroma of culling hens' meat soaked in water extract of *A. bisporus* mushroom was included in the preferred category at all concentrations used. This study also indicated that the taste of culling hen's meat at a concentration of 5–10% water extract of *A. bisporus* mushroom was preferable to a concentration of 2.5% water extract of *A. bisporus* mushroom and 0.2% papain. The toughness of culling hens' meat at a concentration of 5% water extract of *A. bisporus* mushroom was preferred compared to that without soaking in *A. bisporus* mushroom water extract and 0.2% papain. The increase in taste preference and toughness of culling hens' meat soaked in 5% water extract of *A. bisporus* mushroom was thought to be due to changes in the protein and fat content of the meat, the formation of new components, or the addition of flavor components derived from the *A. bisporus* mushroom. Barido and Lee (2021) reported that the use of *C. militaris* mushroom extract as a meat tenderizer was due to an increase in inosinic acid content. Lee *et al*. (2017) reported that using water extract of *A. bisporus* mushroom with a concentration of 5% increased the preference for flavor but did not affect the color and juiciness of beef *longissimus dorsi*.

Conclusion

The present study concluded that the fruiting body of the mushroom *A. bisporus* and its parts could produce protease and collagenase enzymes. The activity of the two enzymes had different activities in each part of the fruiting body, and the highest activity was found in the volva. *A. bisporus* mushroom has excellent potential to be used as a meat tenderizer. The effect of using mushroom water extract as a meat tenderizer is influenced by concentration. The minimum concentration of *A. bisporus* mushroom water extract required to tenderize culling hens' meat is 2.5% and an optimum of 5% without affecting pH, WHC, cooking loss, protein, fat content, and preferred sensory characteristics. We believe that *A. bisporus* mushroom water extract can be used as a meat tenderizer which is cheap, easy to obtain and environmentally friendly in the future. However, further research is needed including the mechanism of action, activity, and performance of protease enzymes from *A. bisporus* mushroom water extract as a meat tenderizer in various tenderizing methods, pH, and temperature conditions.

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Author Contributions

W and TS planned, coordinated the research, collected data, prepared and made up the articles. RRS, DPA and PJTI collected data, statistically analyzed the data and made illustrations.

Conflicts of Interest

All authors declare no conflict of interest in writing articles, financing, and personal beliefs.

Data Availability

All research data is available and can be requested from the correspondent authors.

Ethics Approval

The ethical clearance of this research has been approved by the Health Research Ethical Clearance Commission, Faculty of Dental Medicine, Universitas Airlangga, Indonesia.

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