INTRODUCTION

In Korea, hens are used until 75 to 100 wk of age for egg production, and then these birds become spent-hen meat for further processed products. In general, spent-hen meat is a potential source for low-price minced meat products. The spent-hen meat becomes very tough, and this attribute makes it unsuitable for use in whole meat products, reducing its market value (Nurmahmudi and Sams, 1997). Spent-hen myofibril gel was weaker than broiler myofibril gel under similar gelation conditions (Xiong and Brekke, 1991). This indicates that myofibrillar protein extract from spent-hen meat may be used as an ingredient to improve the functional properties of whole-muscle processed meat products (Li, 2006). Nowsad et al. (2000) reported that the gel strength was higher in spent-hen surimi compared with that of broiler surimi under similar gelation conditions.

Many attempts have been made to tenderize spent-hen meat (Naveena and Mendiratta, 2001; Bhaskar et al., 2006). Meat tenderness is one of the most important attributes for consumer acceptability (Savell et al., 1989). Several studies have been performed to improve the tenderness of meat by using phosphates, inorganic salts, enzymes, electrolytes, and pressure treatment (DeVitre and Cunningham, 1985; Lyon and Hamm, 1986; Sheard et al., 1999). Plant proteolytic enzymes, such as papain, bromelin, and ficin, have been widely used for the tenderization of meat (Kang and Rice, 1970; Bawa et al., 1981; Gerelt et al., 2000). Also, well-known proteinases from Aspergillus sojae and Aspergillus oryzae that are conventionally used for soy sauce production (Gerelt et al., 2000) were used for tenderization. Gerelt et al. (2000) suggested that treatment after osmotic dehydration using papain and proteinases from Aspergillus was effective in meat tenderizing.

Our purpose was to perform a study to determine if lower-cost materials for the tenderization of spent-hen breast meat are effective. Aspergillus proteinases are typical enzymes that have been investigated for practical purposes, such as fermentation of food products (Malathi and Chakraborty, 1991). The Flammulina velutipes was derived from Aspergillus containing cellulose; this enzyme is effective for fiber degradation.

Effect of Flammulina velutipes on spent-hen breast meat tenderization


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ABSTRACT An experiment was carried out to investigate the effects of powdered vegetable dip sauces to improve the tenderness of spent-hen breast meat. Our overall purpose was to find lower-priced materials for the tenderization of spent-hen breast meat. The spent-hen breast meat was dipped into vegetable powder for 24 h at 4°C, and then the samples were analyzed. In the results for vegetable-powder treated samples, those treated with papain and pineapple had higher (P ≤ 0.05) myofibrillar fragmentation indices compared with those of the other samples. The kiwi-, pineapple-, and Flammulina velutipes-powder (winter mushroom) treated samples had new peptides of about 32 kDa and degradation to 30 kDa. Also, the Flammulina velutipes-powder treated samples showed new peptides of 15 kDa. These data imply that Flammulina velutipes is superior for common use than papain or pineapple for the tenderization of spent-hen meat.

Key words: Flammulina velutipes, meat tenderization, spent-hen breast meat, vegetable

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Therefore, the objective of this study was to investigate the effects of powdered-vegetable dip sauces to improve the tenderization of spent-hen breast meat.

**MATERIALS AND METHODS**

**Sample Preparation**

In total, 10,000 spent hens (~75–78 wk in age) were slaughtered from a local commercial meat plant. The breast meat was automatically cold-boned postmortem immediately, and then it was frozen at −20°C. The weight of each batch was 10 kg, and 3 batches were randomly selected. The packaged breast meat was stored at 4°C overnight and used to manufacture seasoned meat. The materials for meat tenderization were dried pear, kiwi, pineapple, and *Flammulina velutipes* powder and papain powder (Sigma-Aldrich, St. Louis, MO).

The samples were directly dipped into Korean vegetable powder for 24 h at 4°C (Table 1) and then analyzed. Seasoned meats from 3 batches were manufactured in a polypropylene box with a cap (HPL883A; 295 × 230 × 138 mm; Lock & Lock Co. Ltd., Asan-si, Korea).

**Warner-Bratzler Shear Force.** The meat samples were cooked until they reached a final internal temperature of 70°C in a constant water bath at 90°C. After cooking, samples were placed in zipper bags to be cooled in cold water to room temperature before coring. At least three 0.5-inch-diameter cores from each sample were removed parallel to the longitudinal orientation of the muscle fibers. The cores were sheared perpendicular to the muscle fiber’s orientation using an Instron (Instron Co., Norwood, MA) with a Warner-Bratzler shear device and crosshead speed set at 200 mm/min (AMSA, 1995). The considered parameter was expressed as the maximum shear force (N).

**Myofibrillar Fragmentation Index.** The myofibrillar fragmentation index was measured according to Olson et al. (1976). The homogenization was performed in duplicate on 2.5-g muscle samples in 30 mL of cold buffer (20 mM K$_2$HPO$_4$/KH$_2$PO$_4$, pH 7, 100 mM KCl, 1 mM EDTA, 1 mM Na$_3$N) at 15,000 rpm using an Ultra-Turrax T25D equipped with an S25N-18 G dispersing element (Ika Laboratory, Staufen, Germany). The homogenate was centrifuged for 15 min at 2,000 × g at 4°C. The supernatant was then discarded and the myofibrils were suspended in 25 mL of buffer and centrifuged for 15 min at 2,000 × g at 4°C. After 3 centrifugation procedures, the supernatant was discarded and the myofibrils were suspended in 15 mL of buffer and subsequently filtered through a 400-μm nylon mesh to remove the connective tissue. The absorption of an aliquot of myofibril suspension was measured at 540 nm using a spectrophotometer (Genesyst 5, Spectronic Instruments Inc., Irvine, CA) to have 0.5 mg/mL of protein sample. The protein concentration was determined by the Biuret method (Gornall et al., 1949).

**Myofibril Isolation.** Samples for SDS-PAGE were obtained from the seasoned meat. Myofibrils were isolated according to the procedure by Greaser et al. (1969). All of the steps were performed at 4°C. The samples (2 g) were added to 20 mL of rigor buffer containing 75 mM KCl, 10 mM KH$_2$PO$_4$, 2 mM MgCl$_2$, and 2 mM EGTA (pH 7.0) and homogenized with an Ultra-Turrax T25D equipped with an S25N-18 G dispersing element (Ika Laboratory). The homogenate was centrifuged at 10,000 × g at 4°C for 20 min; the supernatant was discarded and the pellet was collected. Fresh rigor buffer (20 mL) was added to the pellet and the entire

<table>
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<tr>
<th>Ingredient (%)</th>
<th>Control</th>
<th>Papain</th>
<th>Pear</th>
<th>Kiwi fruit</th>
<th>Pineapple</th>
<th><em>Flammulina velutipes</em></th>
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1All treatments performed in 3 replicates.
homogenization process was repeated 3 times. The final pellets were diluted with rigor buffer.

**Electrophoresis Conditions.** The SDS-PAGE was performed according to the method of Laemmli (1970). The diluted protein solutions were mixed at a 1:1 (vol/vol) ratio with the SDS-PAGE sample treatment buffer [0.125 M Tris-HCl (pH 6.8), 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, and 1% bromophenol blue] and heated at 100°C for 1 min in a heating block (Digi-Block 5402, Electrothermal, Essex, UK). Each sample (1 mg/mL) was loaded in the gel made of 4% stacking [0.5 M Tris-HCl (pH 6.8)] and 12% separating gels [1.5 M Tris-HCl (pH 8.8)]. Electrophoresis performed at a constant current of approximately 10 to 20 mA per gel using a mini-gel unit (Might Small SE 245, Hoefer Scientific Instruments, East Lyme, CT). The gels were stained with 0.1% brilliant blue R (B0149, Sigma-Aldrich, St. Louis, MO) in 40% methanol and 7% acetic acid and destained with 40% methanol and 7% acetic acid. The molecular protein band weight was calculated using a standard marker (062K9280, Sigma-Aldrich). All electrophoreses were performed at room temperature.

**Sensorial Evaluation.** Meat tenderness was evaluated by a 15-member trained test panel. The samples were prepared by cutting the meat into 1- × 2- × 1-cm rectangles. The panelist indicated the degree of tenderness on a 10-point scale (0 = very tough; 5 = moderate; and 10 = very tender).

**Statistical Analysis**

All of the seasoned meats were manufactured from 3 batches and then measured in triplicate per batch. Data were collected for the determination of shear force values, myofibrillar fragmentation index, and sensorial tenderness from each batch. The shear force values and myofibrillar fragmentation of raw meat were obtained without any sauce treatment. Statistical analysis was determined by a one-way ANOVA using the SAS package (SAS Institute Inc., Cary, NC), followed by a Duncan’s multiple range test to determine the significance between the treatments (P ≤ 0.05). Data are expressed as mean ± SD.

**RESULTS AND DISCUSSION**

**Powdered-Vegetable Dip Sauces and Meat Tenderization**

The shear force values of the meat seasoned with vegetable powder were significantly (P ≤ 0.05) lower than those of the nontreated samples (Figure 1). In addition, shear force values were not significantly different among the samples treated with vegetable powder. The samples treated with papain and pineapple had significantly (P ≤ 0.05) higher myofibrillar fragmentation indices than the other samples (Figure 2). These results were identical to the results of the sensorial evaluations (Figure 3). However, for improvements in tenderness, the effects from pineapple were poorer compared with those of the papain treatment.

Traditionally, it is known that proteinases from papain, soy sauce, pear, and *Aspergillus* are effective for meat tenderization (Zapelenka et al., 1999; Gerelt et al., 2000). Enzymatic tenderization of meat has been used for many years, most commonly using enzymes of plant origin, such as papain, bromelain, and ficin. However, many tenderizing enzymes require substrate specificity, and without careful control over application and action, overtenderization may result in an undesirable mushy, pasty texture (Ashie et al., 2002; Saiga et al., 2003). Li (2006) suggested that myofibrillar protein extracts from spent-hen meat can be used to improve the functional properties of whole-muscle processed meats.

Therefore, these data imply that dried *Flammulina velutipes* is better for common use than papain or pine-
apple. Consequently, the results suggest that effective meat tenderization occurred by the disruption of muscle fibers due to Flammulina velutipes treatment.

**Myofibrillar Protein Degradation and Meat Tenderization**

Figure 4 shows the degradation of myofibrillar proteins according to electrophoresis. Myosin degradation only occurred by myofibril disruption when the meat was treated with papain. The samples treated with vegetable powder showed degradation of myofibrillar proteins and the appearance of new peptides in low-molecular weight proteins. In particular, the kiwi-, pineapple-, and Flammulina velutipes-treated samples had new peptides of about 32 kDa and degradation to 30 kDa. Also, the Flammulina velutipes-treated samples showed new peptides of 15 kDa.

The pineapple-treated sample had significantly (P ≤ 0.05) higher myofibrillar fragmentation (Figure 2) with increasing degrees of degradation (Figure 4) compared with that of the other samples. In addition, the pineapple-treated sample had sensorial tenderness and was softer than the other samples (Figure 3). Therefore, the results suggest that the Flammulina velutipes-powder treatment affected degradation patterns of the myofibrillar proteins, but its effectiveness for meat tenderization was less than that of the pineapple treatment. On the other hand, the tenderness by shear force was lower (P ≤ 0.05) compared with that of the nontreated samples, whereas no significant differences were found among the powder-treated samples (Figure 1). Changes in low-molecular weight proteins of about 15 kDa indicated more disruption with Flammulina velutipes compared with the other samples (Figure 4). The intensity of myosin is related to myofibrillar fragmentation, and the change to approximately 15 kDa affected sensorial tenderness more than myofibrillar fragmentation of the muscle fiber structure (Figure 2). This result suggests that changes in low-molecular weight proteins of about 15 kDa are related to the appearance of new peptides or some degradation products derived from high-molecular weight proteins.

It is believed that the calpain proteolytic system has more than one mechanism of activation depending on the cellular condition, and that autolysis is perhaps one of the mechanisms of calpain activation (Suzuki et al., 1981; Saido et al., 1993; Molinari et al., 1994). The breakdown of troponin-T into ~30-kDa and ~28-kDa products is commonly accepted as an indicator of post-mortem proteolysis (Cheng and Parrish, 1977; MacBride and Parrish, 1977; Huff-Lonergan et al., 1996).

Therefore, the Flammulina velutipes-powder treatment affected the degradation patterns of myofibrillar proteins, and its effectiveness for meat tenderization was more than that by the control. Finally, our purpose was to perform an investigation to determine lower-priced materials for the tenderization of spent-hen breast meat. Overall, the data imply that Flammulina velutipes powder is superior in terms of common use for this purpose than papain or pineapple.

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