Applications of Proteolytic Enzymes from Kiwifruit on Quality Improvements of Meat Foods in Foodservice

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Abstract
The purpose of this study was to evaluate kiwifruit as a tenderizer by its effects in improving the quality of various beef parts. Basic data are provided for development of standard recipes for convenient cooking in foodservice. The optimum temperature of the crude proteolytic enzymes in the kiwifruit was determined to be 65°C. The substrate specificity of the enzymes was higher in beef than in pork, chicken, or duck. The enzymes had their greatest effects on chuck and rib parts, and had lower effects on loin, breast, and round parts, in that order. As the amount of kiwifruit extract increased, the moisture content of the cooked beef also increased. The addition of 10% kiwifruit improved the sensory quality of the cooked beef. In particular, the texture scores of cooked beef samples treated with 10% and 15% kiwifruit extract were significantly higher than the other samples (p<0.001), and juiciness and overall acceptability scores were also highest. In summary, 10% kiwifruit extract is deemed an appropriate addition to improve.

Key words: kiwifruit, proteolytic enzyme, beef, tenderness, foodservice

Introduction
School foodservice provides nutritionally balanced meals, enables students to have appropriate eating habits, and resolves unbalanced diets in students. Therefore, students can be healthy, aiding increases in academic efficiency and an inspired community spirit (Kwak et al., 2007). In 2009, among all elementary, middle, high, and special schools in Korea, 99.9%, or 11,303 of them, had school lunch programs, and 98.5% of all students (7.3 million) were therefore affected (Ministry of Education, Science and Technology, 2010).

With increases in national income and dietary lifestyle changes from grains to dairy products, demands for meat and meat products are increasing in foodservice. According to Kim et al. (2006) who performed research on preferences toward school lunch program menus in high school students in Daegu, the students preferred meat foods prepared by steaming, braising, stir frying, grilling, and deep frying. In addition, boys seemed to prefer meat more than girls. According to Park and Kim (2008), in middle school foodservice, meat was the most liked ingredient by 39.8%. But what students wanted school authorities to do most was to improve the taste of school foodservice menu items (Lee and Park, 2010). Girls tended not to eat meat dishes because they didn’t like their taste (Oh et al., 2006). Thus, improvements in ingredient quality and cooking technology are urgently needed.

People tend to prefer meat more than any other food (Kim et al., 2006; Kim, 2008; Yoon and Woo, 1999), but preferences differ according to the kind of meat (Jang and Kim, 2009; Kim et al., 2009; Lee et al., 2009) and cooking method (Kim et al., 2009; Park and Park, 2001). Lawrie and Leward (2006) proposed that appearance, juiciness, tenderness, and flavor are important factors in judging the quality of beef. Calkins and Hodgens (2007) mentioned that fat content affects juiciness and flavor in a good way. Tenderness is the most important factor in overall acceptability (Destefanis et al., 2008), and tenderness, juiciness, and flavor affect overall acceptability in the respective order, (Serra et al., 2008). The Gyeonggi Provincial government (2010) is making efforts to raise student satisfaction of foodservice by providing first-rate Hanwoo. However, students prefer soft meat parts such as the sirloin, tenderloin, and ribs over tougher parts such as the chuck, round, foreshank, and brisket. In order to utilize the tougher parts and provide preferred meat menus, it is very important to develop food preparation tech-
niques appropriate for mass cooking that can enhance tenderness and water-holding capacity.

Koreans have used pear (Choe and Park, 1996; Han and Chin, 2004), ginger (Kim and Lee, 1995), paper mulberry (Yun and Jang, 1997), and bark (Park and Kwon, 1998; Kwon et al. 1998) to tenderize meat. It was reported that the proteolytic activity of pears is much lower than that of tropical fruits such as pineapple, kiwifruit, and papaya (Bai and Roh, 2000). Thus, much research (Cho et al., 1994; Kim et al., 2003; Kim et al., 2009; Lee et al., 2009; Oh et al., 2002) has been performed on using proteolytic enzymes from tropical fruits like pineapple and kiwifruit as meat tenderizers. However, at home or in foodservice, food preparation using fruit extracts is preferred (Bai and Roh, 2000). In order to prevent excessive decomposition due to the strong activity of proteolytic enzymes, the development of standard recipes is necessary.

The purpose of this study was to evaluate kiwifruit as a tenderizer in various beef part and to provide basic data for the development of standard recipes for convenient cooking in foodservice.

Materials and Methods

Samples preparation
Domestic kiwifruit (*Actinidia chinensis*), Hanwoo (Korean beef; chuck, loin, breast, rib, and round), domestic port (ham), and domestic chicken and duck (breast) were purchased from a local supermarket.

Preparation of the crude proteolytic enzymes
One-hundred grams of eatable kiwifruit parts were taken, squeezed, and centrifuged at 15,000 g, for 20 min at 4°C. The supernatant was used as the crude enzymes.

Assay of proteolytic enzymes
The activity of the proteolytic enzymes was measured by the following method. First, 0.5 mL of acetate buffer (pH 5.0) and 0.05 mL of crude enzymes were added to 0.5 g of beef chuck. Next, the meat was incubated at 37°C for 30 min. After incubation, 0.5 mL of TCA was added followed by centrifugation at 1,000 rpm for 5 min. The supernatant was used in measurements. The products of enzymatic proteolysis were measured by Lowry's method (Low et al., 1951), and absorbance was measured by a spectrophotometer at 660 nm. One unit of enzyme activity was defined as the activity of enzymes per 1.0 mL to increase absorbance 0.001 per min.

Moisture content
The moisture contents of the cooked meat samples were measured by AOAC (1995) methods with dehydration at 105°C.

Sensory properties
The round muscle of the beef was sliced to 2.0 cm wide, 2.5 cm thick, and 0.3 cm long, and then it was seasoned. The seasoning was composed (w/w) of water (10%), soy sauce (5%), and kiwifruit extract (5%, 10%, 15%). After seasoning for 2 h, the meat was broiled in an oven at 120°C for 3 min. The control was seasoned with water and soy sauce only.

The sensory qualities of the broiled beef samples were evaluated by an experienced panel of 10 judges who were majors in food and nutrition. The samples of broiled beef were served on identical dishes to each panelist and all of them looked the same. Sensory profile analysis was used to describe taste, appearance, color, texture, juiciness, flavor, and overall acceptability. Sensory property evaluations were carried out using ratings from 1.0 (very weak) to 5.0 (very strong). The panelists tested each set of three sample three times.

Statistical analysis
The data were analyzed by ANOVA using the SAS statistical program, and significant differences among various treatments were compared using Duncan's multiple range tests (SAS Institute, Inc., Cary, NC, USA, 1996).

Results and Discussion

Properties of proteolytic enzymes
The effects of temperature on enzyme activity are shown in Fig. 1. As temperature increased, crude enzyme activity increased sharply until reaching 65°C and then it decreased rapidly. The enzyme activity at 65°C was 433.7 mLM/mL, which was referred to 100% relative activity. The enzyme activities at various temperatures were described as relative activity (%) compared to 65°C for easier comparison between temperature effect. Therefore, the optimum temperature of the crude proteolytic enzymes in the kiwifruit was determined as 65°C. This result disagrees with reports by Bai and Roh (2000) and Cho et al. (1994), which claimed that the optimum temperature was 40°C. However, our data were similar to results found by Yamaguchi et al. (1982), which indicated an optimum temperature of 58-62°C. These differences in optimal temperature might be due to differences in the substrate
used. The results of this study indicate that proteolytic enzymes from kiwifruit have tenderizing effects during heat processing.

**Substrate specificity**

The effects of the proteolytic enzymes on various kinds of meat foods (beef, pork, chicken, duck) are shown in Fig. 2. The enzyme activity with beef as a substrate was 228.5 unit/mL at 37°C, which was referred to 100% relative activity, while relative enzyme activities with various meats were described compared to enzyme activity with beef as a substrate. The enzymes had their greatest effects on beef, while they had lower effects on pork, chicken, and duck, but at similar levels. Therefore, the substrate specificity of the enzyme was relatively high in the beef. These results disagree with a report by Suh et al. (1998) where proteases from vegetables had greater effects on pork than on beef.

The effects of the proteolytic enzymes on various beef parts are shown in Fig. 3. The enzyme activity with beef chuck was referred to 100%, while enzyme activities with various beef parts were described as relative activity (%)

![Fig. 1. Effects of temperature on the activity of proteolytic enzyme from kiwifruit.](image1)

![Fig. 2. Proteolysis of various meat foods by kiwifruit.](image2)

![Fig. 3. Proteolysis of various beef parts by kiwifruit.](image3)

![Fig. 4. Moisture contents of cooked beef samples made with different amounts of kiwifruit.](image4)
compared to enzyme activity with beef chuck for better comparison. The enzymes had their greatest effect on the beef chuck, followed by rib, and had lower effects on the loin, breast, and round, in the respective order.

### Moisture contents

The moisture contents of the cooked beef samples prepared using different amounts of kiwifruit extract are shown in Fig. 4. As the amount of kiwifruit increased, the moisture content of the broiled beef also increased. This is similar to data reported by Lee et al. (2001). Proteolytic enzymes from kiwifruit hydrolyze beef proteins leading to increases in water-soluble substances. Such enzymes are thought to have caused the above results. However, Kim et al. (2003) reported that the water-holding capacity of low-grade ribs seasoned with kiwi powder decreased compared to a control as the amount of kiwi powder increased. The excessive activity of actinidin in kiwifruit causes excessive protein decomposition and increases drip loss, which is thought to cause the above results. These data indicate that appropriate amounts of proteolytic enzymes lead to increases in juiciness, but excessive amounts decrease juiciness.

### Sensory evaluation

The sensory characteristics of the cooked beef samples made with different amounts of kiwifruit extract are shown in Table 1.

The sensory scores of the cooked beef samples made with 10% kiwifruit extract were highest for taste, appearance, texture, juiciness, flavor, and overall acceptability. Particularly for texture, the score of the cooked beef made with 10% kiwi (3.7) was significantly higher than that of the control (1.9) as well as the samples made with 5% (2.3) and 15% (2.9) extract ($p<0.001$). For juiciness, the score of the cooked beef made with 10% kiwi (3.3) was significantly higher compared to beef samples made with 5% (2.3) and 15% (2.2) ($p<0.05$). The cooked beef prepared with 15% kiwi extract received low scores for texture (2.9) and juiciness (2.2); therefore, overall acceptability was also lower. These results agree with a report by Kim et al. (2003) where natural tenderizers such as kiwi and pear powder had tenderizing effects on low-grade seasoned ribs, and overall acceptability was closely connected with tenderness and juiciness. To summarize, in order to improve the quality of broiled beef, 10% kiwifruit extract is deemed appropriate.

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