

Texture Softening of Beef and Chicken by Enzyme Injection Process

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Abstract

This research focuses on a new softening technology for use with chicken breast and eye of round beef in order to assist elderly individuals who have difficulty with eating due to changes in their ability to chew (masticatory function) or swallow. We investigated the hardness of chicken breast and eye of round beef through use of a texture analyzer after injection of a commercial enzyme. Among 7 commercial enzymes, bromelain and collupulin exhibited a marked softening effect on the tested chicken breast and eye of round beef given a 1.00% enzyme concentration. The hardness of bromelain-treated chicken breast reached 1.4×10^4 N/m², of collupulin-treated chicken breast reached 3.0×10^4 N/m², and of bromelain-treated eye of round beef reached 3.2×10^4 N/m², respectively, while their original shapes did not change. To find the level of tissue degradation with specific enzyme concentrations, enzyme injections at 0.1%, 0.25%, 0.50%, and 1.00% concentration of bromelain and papain were also evaluated. The results of this research could be useful for softening chicken breast and eye of round beef and will contribute to the development of foods that can be more easily eaten as part of a balanced diet for elderly adults.

Keywords: bromelain, chicken breast, collupulin, enzyme injection, eye of round beef, hardness

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Introduction

The rapid movement towards an aging society is unprecedented among the countries in the Organization for Economic Cooperation and Development (OECD) (Kim, 2000). According to the Korea National Statistical Office, 15.7% of the Korean population was 65 years and older (elderly ratio) in 2010. The ratio is expected to continue to rise.

The aging process is associated with gradual and progressive loss of muscle mass along with lowered strength and physical endurance (Deutz *et al.*, 2014). In elderly people, age-related deterioration of the masticatory or swallowing functions is common and affects their perceptions and choices of food and their ability to eat. Swallowing disorders are particularly common and may cause dehydration, weight loss, aspiration pneumonia, and airway obstruction (Palmer *et al.*, 2000; Rothenberg *et al.*, 2007; van der Bilt, 2002). Accordingly, the need exists for soft foods as “care food” that can be used in nursing homes and assisted living facilities as well as during reha-

bilitation in a hospital setting. However, boiled, minced, and paste foods are served at hospitals and nursing homes in spite of the fact that they are less palatable in appearance and flavor than are normally cooked foods.

Among various food energy sources, protein is an important macronutrient used by the body for building, repairing, and maintaining muscle function. Consuming an adequate amount of protein can help adults sustain muscle strength and function (Deutz *et al.*, 2014; Hoffman and Falvo, 2004). Although dietary data for the elderly are limited, it has been reported that the optimal protein intake for older adults is greater than their current recommended dietary allowance (RDA) and that increasing protein intake to approximately 1.5 g protein/kg/day may be ideal (Wolfe *et al.*, 2008). Concerns about the potential detrimental effects of increased protein intake on bone health, renal function, neurological function, and cardiovascular function are generally unfounded. Among the different types of protein-containing foods, chicken breast and eye of round beef are considered to provide high-quality protein and to be good sources of minerals such as iron, niacin, selenium, phosphorus, choline, iron magnesium, vitamin B₆, and vitamin B₁₂ (Keen and Zidenberg-Cherr, 1994; Williams, 2007). In addition, both chicken breast and eye of round beef contain more protein and less

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fat in comparison to many other potential protein sources.

This research was undertaken to verify and evaluate the effects of a protease enzyme injection on chicken breast and eye of round beef on tenderness. The goal of this research is to help elderly Koreans significantly improve their protein intake and their general health with palatable foods that can be eaten without excessive difficulty.

Material and Methods

Eye of round beef

For this study, the round was divided into cuts including the eye of round, bottom round, and top round. Eye of round was obtained from Dongah Food Co., LTD. (Korea), which imported the beef from Swift® Beef Company (Greeley, USA). This boneless beef consists only of the semitendinosus muscle. The meat was cut into four equal parts and stored at -20°C in the freezer. The frozen eye of round was thawed slowly in a refrigerator at 4°C overnight, sliced into 2 cm-thick chops perpendicular to the fiber direction, and cut into 8 cm × 8 cm pieces.

Chicken breast meat

Frozen boneless chicken breasts were purchased from a commercial chicken company (Harim Co., Ltd., Korea) and kept at 20°C in the freezer. Frozen chicken breast was thawed slowly in a refrigerator at 4°C overnight and sliced into 2 cm-thick chops perpendicular with both end pieces except for smaller pieces/quantities.

Enzyme solution for softening samples

The following softening enzymes were used: Alcalase 2.4L FG (Alc; Novozymes, Denmark), Neutrase 0.8L (Neu; Novozymes), Flavourzyme 500MG (Fla; Novozymes), Protamex (Pro; Novozymes), Collupulin MG (Coll; DSM Food Specialties, Netherlands), Alphasase NP (Alp; DuPont Danisco, USA), and Bromelain 1200 GDU (Bro; Great Food [Biochem] Co. Ltd., Thailand). Conditions of the enzyme solutions were as follows: 1.0% (v/v) Alc, 1.0% (v/v) Alp, 1.0% (w/v) Bro, 1.0% (w/v) Fla, 1.0% (w/v) Coll, 1.0% (v/v) Neu, and 1.0% (v/v) Pro. All enzyme solutions were made using 0.1 M phosphate buffered saline (PBS) buffer (Biosesang Inc., Korea) at pH 7.0.

Determination of protease activity

The protease activity of all the tested enzymes was determined as the substrate following the method of Cupp-Enyard (2008) with slight modification. One unit of protease (Alc, Alp, Bro, Coll, Fla, Neu, and Pro) was defined

as the amount of enzyme required to produce 1 mg of tyrosine from casein in 10 min. For each enzyme, 1 mL (10 mg of enzyme powder or 10 µL of enzyme solution in 50 mL of potassium phosphate buffer, pH 7.5) was added to 5 mL of 0.65% (w/v) casein (Sigma Chemical Co., USA) substrate solution; enzyme was not added to one of the substrate solutions (leaving a “blank” unmodified substrate). After enzyme incubation for 10 min at 37°C, the reaction was stopped by the addition of 5 mL of 0.11 M trichloroacetic acid (Sigma Chemical Co.) and was kept at 37°C for 30 min. Then, the precipitate was finally filtered off using a 0.45 µm PVDF syringe filter (Whatman, USA) and the amount of soluble digested peptides in the filtrate was estimated. 2 mL of filtrate was taken and added to 5 mL of 0.5 M sodium carbonate (Junsei Co., Japan) and 1 mL of 0.5 M Folin and Ciocalteu's reagent (Sigma Chemical Co.) since the released tyrosine developed a blue coloration (Bartkiene *et al.*, 2013). The reaction mixture was incubated at 37°C for 30 min and the absorbance of the colored solution at 660 nm was measured using a Spectra Max M3 spectrophotometer (Molecular Devices, USA). Tyrosine (Sigma Chemical Co.) was used as the standard and the enzyme activity was calculated in U/mL or U/mg.

Injection and enzyme reaction

To infuse the enzyme into the intercellular spaces, a thawed sample was injected with the 10% enzyme solution (based on the weight of the meat) perpendicular to the direction of the muscle fiber at approximately 1.0-cm intervals using a syringe. The ratio of enzyme volume to the pieces of eye of round and chicken breast was 1:10 (based on the weight of meat) (Liu *et al.*, 2011). Injected and non-injected samples were stored for 24 h at 4°C for equilibrium to inactivate enzymes (Harris *et al.*, 2001). The samples were exposed to steam treatment for 10 minutes in order to inactivate the enzyme (Nakatsu *et al.*, 2012). After steaming, the samples were immediately cooled in a water bath using ice. To select the optimal enzyme, further injections were performed with enzyme concentrations of 0.10%, 0.25%, 0.50%, and 1.00% prepared in a PBS buffer.

Mechanical properties

The texture of protein-based foods was measured with a TA.HD Plus Texture Analyser (Stable Micro Systems Ltd., U.K.) equipped with a 50-kg load cell. All textural analyses were carried out using Texture Exponent software version 6.1.5.0. (Stable Micro Systems Ltd.) by a

slight modification of a previously described method (Sakamoto *et al.*, 2006). The preparation procedure (duration of cooking; time period between cooking and testing) was the same for all tested samples. The hardness of the steamed sample was measured for each enzyme using an 10-mm cylinder probe with the following instrument settings: measured force in compression, pre-test speed: 1.0 mm/s; test speed: 2.0 mm/s; post-test speed: 10.0 mm/s; strain: 70%; trigger force: 10 g. The maximum force correlates to the hardness of the sample. The tests were performed on 10 replicates per sample. The sample was positioned centrally under the probe during testing.

Statistical analysis

All experiments were repeated in triplicate; statistical significance was confirmed using Duncan's Multiple Range Test. Statistical analysis was performed using IBM SPSS Statistics version 20.0 for Windows (IBM/SPSS Inc., USA).

Results and Discussion

Selection of the softening enzymes for use in chicken breast

Seven commercial proteases selected for their softening ability were examined to determine their softening effect in the chicken breast. These seven enzymes have been classified as 'Generally Recognized as Safe' and come from varying plant, bacterial, and fungal sources. The enzymes are also used to treat cuts of meat and enhance tenderness. It has been shown that different enzymes react differently to the myofibrillar and connective tissue portions of the meat (Calkins and Sullivan, 2007). The characteristics and test conditions of each enzyme are described in Table 1.

According to Goto *et al.* (2008), we also tried to apply a vacuum pressure impregnation technique for the chic-

ken breast. With this technique, the chicken breast was immersed in a 1% protease solution at room temperature, and subjected to a decompression treatment for 20 min under a reduced pressure of 20,000 Pa using the volume deairation equipment (Datsukikun VP-2; Thermal Chemical Industrial Co., Ltd., Japan). However, the enzyme was decreased in the central region of the tissues using this vacuum pressure technique. Thus, we sought the enzyme injection that would have a sufficient amount of the enzyme present the central region of chicken breast (data not shown).

To select the enzymes that would be most appropriate for softening chicken breast, the effect of different commercial enzymes on the hardness of chicken breast was compared to control conditions using by TA.HD Plus Texture Analyser. Fig. 1 shows the different hardness values for chicken breast treated with different enzymes when compared to the control without the enzyme addition. The hardness value for the chicken breast before the treatment was 3.4×10^5 N/m². Of the 7 enzymes tested, 6 enzymes (Alc, Alp, Bro, Coll, Neu, and Pro) softened the chicken breast to 1.7×10^5 N/m² or less; Fla was the exception. Bro and Coll were shown to have a particularly pronounced softening effect on the chicken breast. Bro and Coll softened the chicken breast to 1.5×10^4 N/m² and 3.0×10^4 N/m², respectively. Although Bro and Coll designated by the same letter are not statistically different at the 0.05 level, Brom was a little superior to Coll in softening chicken breast, thereby demonstrating the potential effect of selective enzymes on softness. Therefore, Brom and Pap were used for treating the chicken breast in a dose-dependent manner.

Influence of enzyme concentration on the hardness of the chicken breast

To investigate the optimal enzyme to soften the chicken breast, the influence of the concentrations of the two

Table 1. Optimal softening conditions of various enzymes

Enzyme	Optimum conditions		Buffer used	Enzyme composition	Protease activity	Company
	pH	Temp.				
Alcalase 2.4L FG	7.0	50°C	0.1 M PB ^a	endoprotease	235.04±6.85 units/mL	Novozymes
Alphalase NP	6.5-6.8	60°C	0.1 M PB	protease	239.26±6.72 units/mL	Danisco-DuPont
Bromelain 1200 GDU	7.0	50°C	0.1 M PB	proteolytic enzyme	183.58±4.02 units/mg	Great Food (Biochem) Co. Ltd.
Collupulin MG	7.0	65°C	0.1 M PB	cysteine protease	179.07±8.56 units/mg	DSM food specialties
Flavourzyme 500MG	7.0	50°C	0.1 M PB	aminopeptidase, exopeptidase	225.42±7.41 units/mg	Novozymes
Neutrase 0.8L	7.0	50°C	0.1 M PB	endoprotease	225.32±7.15 units/mL	Novozymes
Protamex	7.0	50°C	0.1 M PB	protease serine, endoprotease	233.65±6.21 units/mg	Novozymes

^aPB, phosphate buffer.

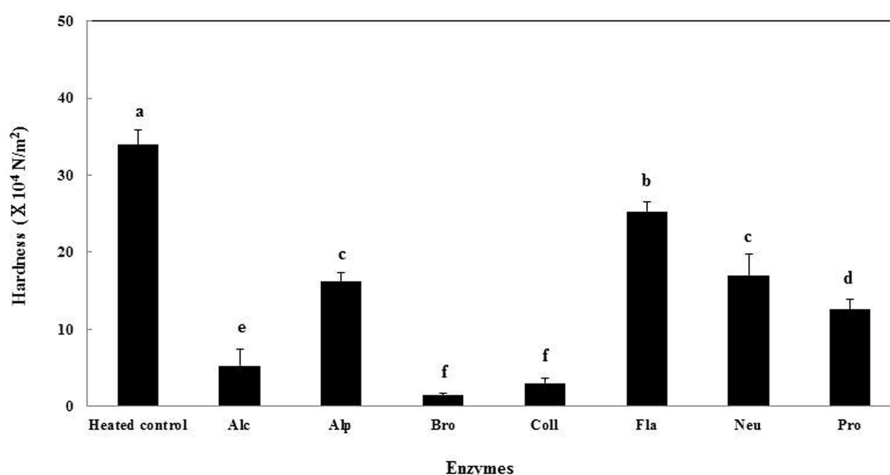


Fig. 1. Hardness of chicken breast processed using the enzyme injection method. Reactions were carried out at 4°C for 24 h. Different letters indicate statistically significant difference ($p < 0.05$). Alc, alcalase 2.4L FG; Alp, alphasase NP; Bro, bromelain 1200 GDU; Coll, collupulin MG; Fla, flavourzyme 500MG; Neu, neutrase 0.8L; Pro, protamax.

selected enzymes on the hardness were investigated. The results are shown in Fig. 2. Chicken breast treated with injection of Brom gave a hardness of 3.8×10^4 N/m² at an enzyme concentration of 0.25%, 2.1×10^4 N/m² at 0.50%, and 1.5×10^4 N/m² at 1.00%, showing a great degree of softening (Fig. 2(a)). The sample shape at concentrations of 0.25%, 0.50%, and 1.00% was only retained in a state in which the slightest stress caused the disintegration and coming apart of the softened chicken breast. In contrast, these samples were softened a little at concentrations of 0.100%.

With the Coll enzyme, the hardness of the chicken breast was measured at 2.6×10^4 N/m² at an enzyme concentration of 1.0% and 3.8×10^4 N/m² at 0.50%, showing a great

degree of softening. As with Brom, the slightest stress led to the disintegration of the sample (Fig 2(b)). In contrast, the samples were softened a little at decreased concentrations of 0.25% and less at decreased concentrations of 0.10%. At 0.50% and 1.00% concentration, the chicken breast was softened but also completely retained its shape.

The Japan Care Food Conference has established the Universal Design Foods (UDF) concept, in which food is classified into 4 categories based on hardness and viscosity (Japan Care Food Conference, 2011). The UDF concept has provided consumers with the standard at the time of choosing goods (Evaluation of Food Texture; Goto *et al.*, 2008). A hardness of category 1 is below 5×10^5 N/m² and reflects softened meat that is easy to chew. Category

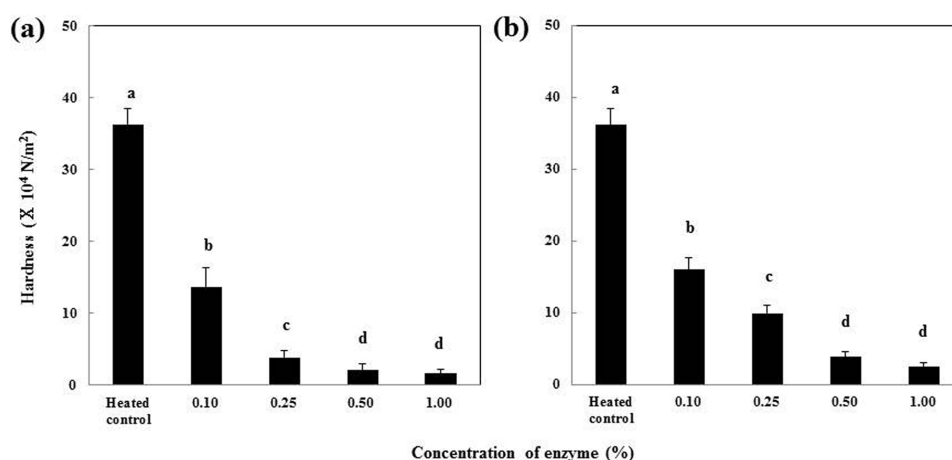


Fig. 2. Effect of enzyme concentrations on hardness of chicken breast processed using the enzyme injection method. Enzyme reactions were carried out at 4°C for 24 h. Different letters indicate statistically significant difference ($p < 0.05$). (a) bromelain, (b) collupulin.

2 meat is 5×10^4 N/m² and can be broken up using the gums; category 3 is 1.0×10^4 N/m² (the case of the sol) and can be broken up by the tongue, and category 4 is 3.0×10^3 N/m² (the case of the sol) and does not need chewing.

Softened food can be produced so that its hardness can be adjusted using enzyme treatments (Chizu *et al.*, 2013). The present research shows that chicken breast treated with injections of Brom or Coll can be used for the diet of elderly individuals who have challenges with mastication and swallowing; when prepared as described, chicken breast falls under the category of UDF 2. Chicken breast treated with Bro at 0.25%, 0.50%, and 1.00% can be broken up using the gums; also recognized as UDF 2 is chicken breast treated with Coll at concentrations of 0.50% and 1.00%.

Selection of the softening enzymes in the eye of round beef

Since eye of round beef is usually used for beef jerky, or marinated grilled beef in Korea, it is one of the familiar dishes to elderly Koreans (Kwon *et al.*, 2013). In this study, we investigated the effect a protease enzyme can have when used to soften eye of round beef. However, protease enzymes do not penetrate eye of the round; the method of enzyme injection was evaluated as being important with respect to ensuring uniform distribution in the meat. Since only limited knowledge has been obtained regarding the application of enzymes to soften eye of round beef, we have tried to examine the effect of seven exogenous enzymes (Table 1). According to Calkins and Sullivan (2007), these enzymes have also been added to meat to enhance tenderness and react differently to the myofibrillar and

connective tissue portions of the meat.

Likewise, we evaluated the softening effects of commercial enzymes on chicken breast to select the most appropriate enzymes in comparison to the predefined control conditions. Fig. 3 shows the different hardness values for eye of round beef treated with different enzymes when compared to the control group (which did not have added enzymes). The hardness values for eye of round beef before treatment were 6.7×10^5 N/m². Among seven enzymes tested, five enzymes (Alc, Bro, Coll, Neu, and Pro) softened eye of round beef to 2.8×10^5 N/m² or less (Alp and Fla did not). Bro softened eye of round beef to 3.2×10^4 N/m² in five enzymes, thereby demonstrating the efficacy of selective enzymes in softening eye of round beef. Takei *et al.* (2015) reported that the enzyme solution containing 0.007% w/w papain and 0.001% w/w bromelain was injected into raw Australian beef top round cuts through 16 needles (four needles/cm²) until the weight of the meat increased by 50%. Although the enzyme concentrations are about 4 times higher than those in this study, the firmness value of top round was calculated at 3.3×10^4 N/m². Therefore, further investigations are needed to elucidate the optimal condition among multiple potential enzyme concentrations to treat eye of round beef.

Influence of enzyme concentration on the hardness of eye of round beef

To find the optimal enzyme concentration to soften eye of round beef, Brom was used in a dose-dependent manner. Fig. 4 shows the influence of two enzymes and their concentration on the hardness of the eye of round beef. The beef treated with an injection of Brom gave a hard-

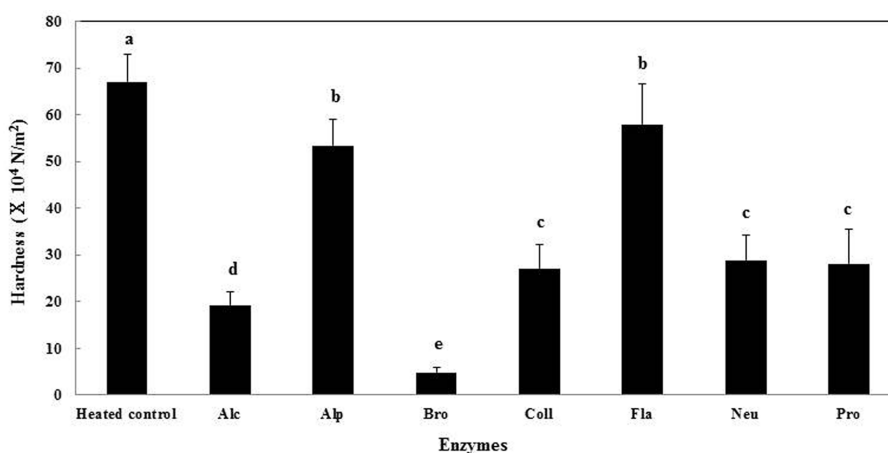


Fig. 3. Hardness of the eye of round beef processed using the enzyme injection method. Reactions were carried out at 4°C for 24 h. Different letters indicate statistically significant difference ($p < 0.05$). Alc, alcalase 2.4L FG; Alp, alphasalve NP; Bro, bromelain 1200 GDU; Coll, collupulin MG; Fla, flavourzyme 500MG; Neu, neutrase 0.8L; Pro, protamax.

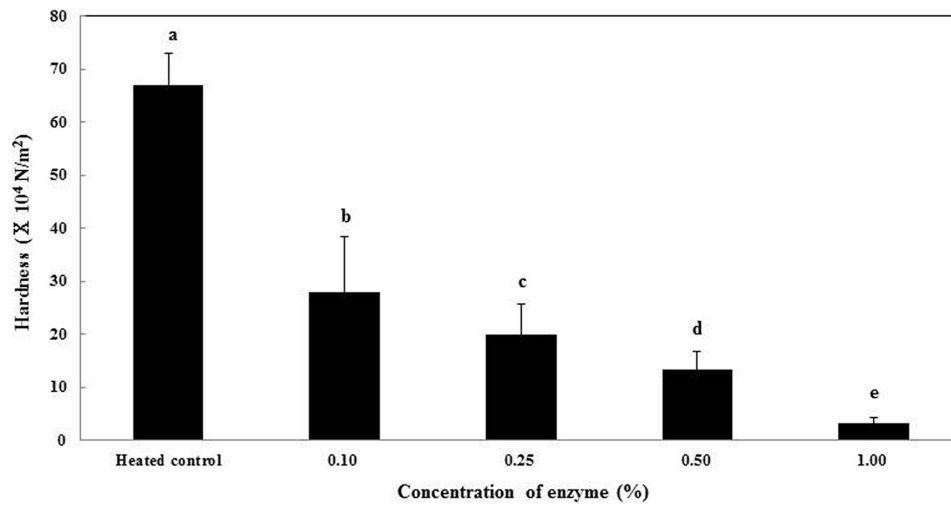


Fig. 4. Effect of bromelain enzyme concentrations on hardness of eye of round beef processed using the enzyme injection method. Enzyme reactions were carried out at 4°C for 24 h. Different letters indicate significant difference ($p < 0.05$).

ness of $3.2 \times 10^4 \text{ N/m}^2$ at an enzyme concentration of 1.00% (Fig. 4), showing a large degree of softening. The sample shape for the eye of round beef at concentrations of 1.00% was only retained until anything more than slight stress was applied; the sample shape for the eye of round beef disintegrated. In contrast, the samples were softened to some degree at decreased concentrations of 0.25% and 0.50%. The hardness of eye of round was $2.8 \times 10^5 \text{ N/m}^2$ at an enzyme concentration of 0.10%, $2.0 \times 10^5 \text{ N/m}^2$ at 0.25%, and $1.3 \times 10^4 \text{ N/m}^2$ at 0.50%. Therefore, eye of round beef treated with Bro at 1.00% can be recognized as fitting the criteria of UDF 2 (that is, it can be broken up by the gums). Eye of round beef treated with Brom

can be prepared for individuals who have mastication and swallowing problems. Above all things, it is necessary when cooking to use these enzymes at an optimum concentration.

In conclusion, several methods are used to soften meat, including immersing the meat in a marinade, injecting and tumbling with a marinade, or a combination of injecting and tumbling (Ergezer and Gocke, 2011). Softening is the most important feature in food texture and has the greatest influence on the perception of a given food among the elderly. The findings in this study show that “care food” products for patients admitted to the hospital and elderly patients with mastication or swallowing disorders

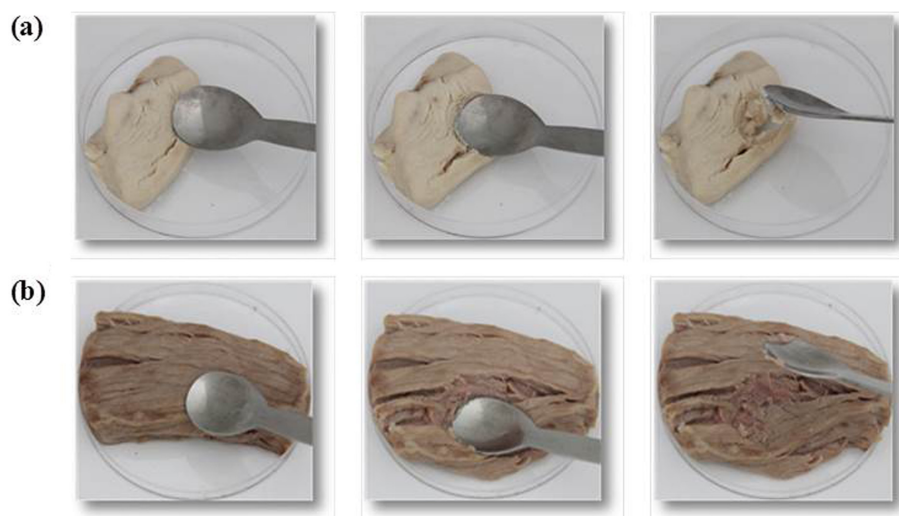


Fig. 5. Appearance of chicken breast (a) and eye of round beef (b) softened by 1% bromelain injection method with a spoon by hand.

or disruptions can be obtained by controlling enzyme reaction times and enzyme concentrations with enzyme injections. Although limited information exists concerning the softening of food products for the elderly in Korea, there are a variety of care foods, including frozen foods, retort foods, and dysphagia thickening agents, for people in Japan with reduced ability to masticate according to the UDF concept established by the Japan Care Food Conference (Sasada *et al.*, 2011). The UDF standard has four categories (I to IV) of care foods. The texture of these foods is provided with consideration of masticatory/swallowing ability and oral sensation in dysphagic patients (Yokoyama *et al.*, 2014).

Moreover, 30% of people over the age of 65 years are at risk of protein intake below the Recommended Dietary Allowance based on the survey by the Ministry of Health and Welfare of Korea (2011). Therefore, Korea also needs to establish standards to provide Korean care food that adheres to a set of appropriate guidelines. Collectively, chicken breast and eye of round beef treated with an enzyme injection can be easily mashed between the alveolar arches without teeth (Fig. 5(a), 5(b)) and therefore fit UDF category II (Nakatsu *et al.*, 2012). This research revealed that the degree of softening in chicken breast and eye of round beef with the injection of protease enzymes can be altered by selective enzymes Bro and Coll. Further study will be required to provide commercialized Korean care food products in accordance with a new type of enzyme invention for injection and with the classification of processing technologies. We hope the results of the present investigation contribute to the development of Korean care food.

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