Food Chemistry 139 (2013) 597–603

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Tenderization effect of soy sauce on beef *M. biceps femoris*

Hyun-Wook Kim [a], Yun-Sang Choi [a], Ji-Hun Choi [a], Hack-Youn Kim [b], Mi-Ai Lee [a], Ko-Eun Hwang [a], Dong-Heon Song [a], Yun-Bin Lim [a], Cheon-Jei Kim [a,*]

[a] Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, South Korea
[b] Department of Animal Resources Science, College of Industrial Sciences, Kongju National University, Yesan 340–702, South Korea

ARTICLE INFO

Article history:
Received 16 November 2012
Received in revised form 13 January 2013
Accepted 15 January 2013
Available online 8 February 2013

Keywords:
Biceps femoris
Marination
Soy sauce
Tenderization

ABSTRACT

This study was conducted to evaluate the tenderization effect of soy sauce on beef *M. biceps femoris* (BF). Five marinades were prepared with 4% (w/v) sodium chloride and 25% (w/v) soy sauce solutions (4% salt concentration) and mixed with the ratios of 100:0 (S0, pH 6.52), 75:25 (S25, 5.40) 50:50 (S50, 5.24), 25:75 (S75, 5.05), and 0:100 (S100, 4.85), respectively. The BF samples which were obtained from Hanwoo cows at 48 h postmortem ($n = 24$) were marinated with five marinades for 72 h at 4 °C (1:4 w/w), and the effects of soy sauce on tenderness were evaluated. Soy sauce marination resulted in a decrease in the pH value of the BF sample. However, there were no significant differences in the water holding capacity ($P < 0.05$). The S100 treatment showed the significant ($P < 0.05$) increase in collagen solubility and myofibrillar fragmentation index, contributing to decreased shear force compared to S0 (control). Reduction in intensity of few myofibrillar protein bands were observed for S100 treatment compared to control using SDS–PAGE. Scanning electron microscopy revealed breakdown of connective tissue surrounding muscle fibers of the S100 treatment. The tenderization effect of soy sauce may attribute various mechanisms such as increased collagen solubility or proteolysis which depend on soy sauce level in marinade.

1. Introduction

Beef *M. biceps femoris* (BF), a collagen-rich muscle, is generally tough, resulting in decline of sensory tenderness ratings (Rhee, Wheeler, Shackelford, & Koohmaraie, 2004). Many researchers have been tried to improve tenderness of this muscle through various methods (Baublits, Pohlman, Brown, & Johnson, 2005; Hoffman, Muller, & Vermaak, 2008). Meat tenderness is one of the most important factors affecting palatability. Several factors influence meat tenderness such as ultimate pH (Silva, Patarata, & Martins, 1999), chilling temperature of carcasses (Yu & Lee, 1986), connective tissues content and solubility – especially of collagen (Torrescano, Sánchez-Escalante, Giménez, Roncalés, & Beltrán, 2003), as well as changes in muscle structure due to enzymatic proteolysis (Koohmaraie, 1996). Various enzymatic, mechanical, and chemical methods have been applied in an attempt to improve meat tenderness. Most of all, marination is considered as an effective method to enhance the tenderness and flavor of meat, as well as being an effective method of increasing the value of meat upon production (Aktaş, Aksu, & Kaya, 2003). Historically, a marinade is composed of oil, sugar, seasoning, and acidic materials, including vinegar, wine, or fruit juices (Oreskovich, Bechtel, McKeith, Novakofski, & Basgall, 1992). In previous studies to improve meat tenderness, additives used in marination mainly were organic acids (Aktaş et al., 2003) and various salts, including sodium chloride (Baublits et al., 2005), phosphates (Vote et al., 2000), calcium salts (Whipple & Koohmaraie, 1993), and ammonium hydroxide (Naveena et al., 2011).

Generally, the effects of marination on meat tenderness can be classed into three categories. Firstly, changes in pH value result in swelling of the muscle and connective tissue. This swelling is related to the solubility of muscle proteins (Rao, Gault, & Kennedy, 1989). Also, Aktaş et al. (2003) indicated that increased concentrations of organic acids and consequent reduction of the pH value resulted in improvement of tenderness. Secondly, the increase in activity of endogenous proteases and/or the addition of enzymes extracted from fruits contribute to an improvement in meat tenderness due to proteolytic weakening. Lastly, the addition of salt, including sodium chloride, calcium salts, and phosphates, also tenderizes meat due to an improvement of the water holding capacity (Baublits et al., 2005; Whipple & Koohmaraie, 1992).

Soy sauce, also known as Jiang-you, Shoyu, and Ganjang in China, Korea, and Japan, respectively, is a fermented sauce, originated from East Asia, which is extensively used worldwide (Fu & Kim, 2011). The fermentation procedure of soy sauce is similar among East Asia countries. Moreover, soy sauce is mainly prepared with defatted soybean flakes and roasted wheat as the prime ingredients. Even if the composition of soy sauce slightly differs by region, and depending on its specific formulation (ratio of soybean

* Corresponding author. Tel.: +82 2 450 3684; fax: +82 2 444 6695.
E-mail address: kimcj@konkuk.ac.kr (C.-J. Kim).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.01.050

to wheat, fermenting organisms used etc.), soy sauce is generally composed of a high amount of salt, free amino acids, peptides, carbohydrates, organic acids, and minerals (Lee, Seo, & Kim, 2006). Through the fermentation process, microorganisms within the soy sauce produce various breakdown products as they digest the prime ingredients. The low pH value (about pH 4–5), resulting from the formation of various organic acids including mainly acetic acid, lactic acid, succinic acid, and pyroglutamic acid, is one of the desired characteristics of soy sauce (Choi et al., 2000; Fu & Kim, 2011). Although the application of soy sauce is known to have a tenderization effect, there is little information available in literature related to the tenderization characteristics.

Therefore, the objectives of our study were (1) to verify the tenderization effect of soy sauce on beef BF sample and (2) to compare the tenderization effect of various soy sauce levels (see Fig. 2).

2. Materials and methods

2.1. Preparation of muscle sample and marinade

Twenty-four *biceps femoris* (BF) beef muscles from Hanwoo cows ($n = 24$, approximately 24–27 months of age), representing 1^{++}A (quality and yield grades) based on an official grader according to the Korean carcass grading procedure (National Livestock Cooperatives Federation), were purchased from a local processor at 48 h postmortem (three replicates of eight muscles each). Subsequently, excessive fat and connective tissue on outside were removed from the muscles, and each muscle was cut into four steaks of approximately 2.54 cm in thickness. Three to four rectangular cubes ($3.0 \times 3.0 \times 2.54$ cm) of approximately 20 g each were cut from the center of each steak. Total 100 cubes were assigned randomly to five treatments (20 cubes per each treatment).

Commercial soy sauce within 3 months from pack date (Fermented and heat sterilized soybean sauce, Sempio Foods Co., Seoul, Korea) was purchased from the local market. The salt concentration and pH value of soy sauce were 16.1% and 4.83, respectively. Five marinades were prepared with 4% (w/v) sodium chloride and 25% (w/v) soy sauce solutions (4% salt concentration) and mixed with the ratios of 100:0 (S0, pH 6.52), 75:25 (S25, 5.40) 50:50 (S50, 5.24), 25:75 (S75, 5.05), and 0:100 (S100, 4.85), respectively. Salt concentration of all marinades was fixed as 4% (w/v).

2.2. Sample marination

Each meat sample was weighed and dipped in each marinade solution at a ratio of 1:4 (meat:marinade, w/w) in polyethylene bags. The samples were marinated at 4 °C refrigerator for 72 h. After marination, the marinated sample were weighed to determination of weight gain, and stored at 4 °C refrigerator during analysis.

2.3. Analysis of marinated sample

Twenty cubes of each treatments were used for study of pH value and water holding capacity ($n = 4$), collagen content and solubility ($n = 3$), myofibrillar fragmentation index ($n = 3$), sodium dodecyl sulfate–polyacrylamide gel electrophoresis ($n = 1$), and scanning electron microscopy ($n = 1$). Warner–Bratzler shear force ($n = 8$) was determined after determination of cooking loss ($n = 8$). This experiment was carried out in three replicates.

2.3.1. pH measurement

The pH values of 5 g raw samples mixed with 50 ml distilled water for 60 s in a homogenizer (Ultra-Turrax T25, Janke & Kunkel, Staufen, Germany) at 8000 rpm speed was determined with a pH meter (Model 340, Mettler-Toledo GmbH, Schwerzenbach, Switzerland).

2.3.2. Weight gain and cooking loss

The marinated meat was removed from polyethylene bags, and excessive water on the surface of meat was removed with paper towels (Aktaş et al., 2003). The meat was weighed, the weight gain was calculated as follows: weight gain (%) = [the weight after marination (g)/the weight before marination (g)] × 100. Each marinated meat was sealed with polyethylene bags and cooked in a 75 °C water bath to reach 71 °C of central temperature of sample for 15 min. After cooking, the cooked meat was cooled at room temperature for 6 h and weighed. The cooking loss was calculated as follows: cooking loss (%) = [the weight before cooking (g) − the weight after cooking (g)/the weight before cooking] × 100.

2.3.3. Water holding capacity (WHC)

WHC was determined in triplicate by filter paper pressed method (Grau & Hamm, 1953). Sample of 300 mg was weighed onto a Whatman No. 2 filter paper and pressed between two plexiglass plates under 36 kg/cm^2 using a carver laboratory press for 3 min. The areas of pressed water and sample were measured using planimeter (Koizumi, Type KP-21, Japan). WHC was calculated as follows: WHC (%) = [area of pressed sample/area of pressed water] × 100.

2.3.4. Warner–Bratzler shear force (WBSF)

The samples of 1.25 cm core were obtained from the center of cooked sample which is used for measurement of cooking loss. The WBSF of the cores was measured using Warner–Bratzler shear attachment (V-type blade set) on a texture analyzer (TA-XT2*i*, Stable Micro Systems Ltd., Surrey, England). Test speeds were set at 2 mm/s. Data were collected and analyzed from the shear force values to obtain the maximum force required to shear through each sample.

2.3.5. Collagen content and solubility

Collagen content was determined from the hydroxyproline content according to the method of Nueman and Logan (1950) modified by Naveena and Mendiratta (2001), and the collagen content was expressed as mg/g tissue by multiplying hydroxyproline content with 7.14 (Naveena et al., 2011). Also, collagen solubility was determined by the method of Mahendrakar, Dani, Ramesh, and Amla (1989) described by Naveena et al. (2011).

2.3.6. Myofibrillar fragmentation index (MFI)

Myofibrils was obtained according to the method of Olson and Parrish (1976) using MFI buffer (20 mM K_2HPO_4/KH_2PO_4, pH 7.0, 100 mM KCl, 1 mM EDTA, 1 mM NaN_3). The myofibrils were suspended in MFI buffer. An aliquot of myofibril suspension was diluted with the MFI buffer to 0.5 mg/ml protein concentration using Biuret method (Gornall, Bardawill, & David, 1949) and the absorbance of this suspension measured at 540 nm. MFI values were recorded as absorbance units per 0.5 mg/ml myofibril protein concentration multiplied by 200.

2.3.7. Separation of the myofibrillar proteins

Myofibrillar protein fraction was separated by using a modification of the method of Busch, Stromer, Goll, and Suzuki (1972) described by Sikes, Tornberg, and Tume (2010). A portion of each muscle was knife-minced and 4 g was homogenized with 40 ml of extraction buffer (50 mM Tris–HCl, pH 7.0, 100 mM KCl, and