Effect of Different Temperature and Time Combinations on Quality Characteristics of Sous-vide Cooked Goat Gluteus Medius and Biceps Femoris

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Abstract
The combination of proper temperature and time duration in sous-vide cooking could provide good water-holding capacity, color parameters, and tender cooked meat. In this study, goat muscles gluteus medius (GM) and biceps femoris (BF) treated with single-stage sous-vide (cooked at 60 °C, 65 °C, 70 °C) and two-stage sous-vide (cooked at 45 and 60 °C, 45 and 65 °C, 45 and 70 °C) methods for 6 h and 12 h were compared. Cooking loss decreased by 5–10% for GM and 10–13% for BF after 6 h of heat treatment with two-stage sous-vide likely due to high sarcoplasmic solubility. Cooking time and temperature combination in two-stage sous-vide contributed to better a* values for both GM and BF, with higher values recorded for 6 h at 45 and 60 °C. Significant reduction of toughness was successfully achieved using stepped cooking temperatures compared with sous-vide cooking at a single temperature. The lowest shear force values were achieved at a combined temperature of 45 and 60 °C with only 6 h of cooking duration (GM 28 N; BF 40 N) likely from desmin degradation. However, the tenderness effect of single-stage sous-vide was seen after collagen solubility was maximized in prolonged cooking at 70 °C, but other quality features such as redness values and water content had recorded the lowest values.

Keywords Sous-vide · Low temperature–long time · Tenderness · Goat meat · Collagen solubility

Introduction
Low temperature–long time sous-vide cooking using vacuum-sealed heat-stable pouches is a good technique that gives better control of doneness, texture, and color than traditional cooking methods. The sous-vide cooking method allows heat transferred efficiently and evenly from water bath to meat, thereby providing moist, tender, and flavorful meat for almost any cut (Baldwin 2010). Identifying the precise time and controlling the right temperature for different meat cuts are the challenges in low temperature–long time of sous-vide cooking. Baldwin (2012) has reported that for tough meat cuts such as beef chuck and pork shoulder to become fork-tender, it takes 10–12 h at 80 °C or 24–48 h at 55–60 °C. However, for intermediate meat cuts such as beef sirloin, it only takes 6–8 h at 55–60 °C. Other effects of sous-vide cooking on color parameters and water retention have also been reported (García-Segovia et al. 2007; Sanchez Del Pulgar et al. 2012; Roldan et al. 2013). Obviously, not only temperature but also cooking duration can have a large effect on physico-chemical properties of slow-cooking regime meat (Christensen et al. 2011).

Biceps femoris is a single large muscle on the lateral surface of mammalian hindlimb (more than 7% total muscle mass) while gluteus medius is a major muscle of mammalian pelvic girdle (approximately 3.8% total muscle weight) (Swatland 2000). It is worthy to study these muscles because they are categorized as less tender meat (Calkins and Sullivan 2007). Nevertheless, biceps femoris is tougher than gluteus medius due to variation in collagen characteristics (Lawrie 2006). In many cuts, connective tissues are known as the “background toughness.” Collagen in these connective tissues is usually denatured in slow-heating regime at 55–60 °C (Purslow 2001).
In sous-vide cooked meat, collagen solubility plays an important role in the tenderness. Several investigations have linked soluble collagen in a cook loss with tenderness, including in young bulls and cows at 53–63 °C (Christensen et al. 2013), in pork longissimus dorsi at 48–63 °C (Christensen et al. 2011), and in beef brisket at 60–70 °C (Alahakoon et al. 2018). However, few studies have focused on the effect of myofibrillar components and related this component with shear strength effect because myofibril is also linked to tough meat when meat is cooked at above 60 °C (Christensen et al. 2000). Structural changes in meat particularly in muscle fibers due to heat can lead to water loss owing to shrinkage of fibrous proteins such as actin (Zielbauer et al. 2016), thereby reducing water retention and contributing to the discoloration of meat due to alteration or destruction of meat pigments (Lawrie 2006).

Many studies have also focused on traditional sous-vide cooking with a combination of single thermal treatment and prolonged heating time for beef (Vaudagna et al. 2002; Rinaldi et al. 2014), pork (Christensen et al. 2011), lamb loins (Roldan et al. 2013), and cows/bulls (Christensen et al. 2013). Temperature and time range used in those sous-vide cooking was 53–100 °C for 2 to 48 h. Some previous studies have also aged meat for several days in a chiller prior to sous-vide cooking to obtain maximum tenderization by proteolysis (Christensen et al. 2013; Botinestean et al. 2016). However, extending cooking time and additional pre-treatment in sous-vide cooking will increase processing cost and meat price. Besides, its application in a restaurant or home is not practical. In our previous study, we have found that semitendinosus beef with sous-vide heat treatment at 45 °C cooked for at least 3 h could produce lower shear strength than that at 65 °C for 3 h. Also, the combination of two-stage temperature at 45 °C and 65 °C resulted in no difference of redness values even if it was cooked for an extended period of 6 h compared with single thermal sous-vide meat at 65 °C for 3 h or conventional cooking at 75 °C cooked for 30 min (Ismail et al. 2019). Other previous authors have also found the same tendency of the tenderness of meat at 45 °C and 49 °C cooked just for 4 h (Lawrie 2006; Myhrvold et al. 2011).

With such background, putting together temperature used in the previous study (at 45 °C) and current study temperature at 60 °C, 65 °C, and 70 °C, the two-stage sous-vide cooking method was developed and objectively assessed. This two-stage temperature method might affect both the muscle fiber (myofibril) and connective tissues, thus improving quality properties of cooked meat. To the best of our knowledge, no studies have reported heat treatment with a two-stage temperature during sous-vide cooking. The effect of two-stage sous-vide cooking on physical properties of meat has not been fully explored either. Although comprehensive investigations have been made on various muscles and animals, no studies have investigated the effect of sous-vide cooking on goat biceps femoris, gluteus medius, or goat meat. Thus, such a study can be used for future reference. Therefore, the aim of this study was to study the effects of sous-vide cooking using a single-stage temperature (60 °C, 65 °C, and 70 °C) and a two-stage temperature (45 + 60 °C, 45 + 65 °C, and 45 + 70 °C) on quality characteristics of goat muscles gluteus medius (GM) and biceps femoris (BF).

Materials and Methods

Raw Materials

At 24 h postmortem, both gluteus medius (GM) and biceps femoris (BF) from 36 goat carcasses were purchased (yielding 72 GM and BF in total from left and right muscles; GM pH24 < 6.13 and BF pH24 < 6.27). Uncastrated male goat (Korean native black goat, Capra hircus coreanae) averaging 16–18 months of age with a body weight of 48.3 ± 13.0 kg were used in this study. The muscles were immediately vacuum packaged, placed on ice, and transported to the Laboratory of Meat Science at Gyeongsang National University. On the same day, pH of raw muscles was measured (Mettler Toledo, MP230, Switzerland, calibrated to pH 7, 4.01, and 9.21), and samples were stored at 4 °C. All heat treatments were carried out on the next day at 36 h postmortem.

Sampling and Heat Treatment

All muscles were cut into steaks 35 mm in thickness, weighed (GM 151–155 g; BF 134–137 g), packaged in a vacuum plastic bag (food grade materials), and cooked at different combinations of temperature and time in water baths (Travellortech precision cooker immersion, USA) using single-stage sous-vide at 60 °C, 65 °C, and 70 °C for 6 h and 12 h or two-stage sous-vide at 45 °C for the first temperature and 60 °C, 65 °C, and 70 °C for the second temperature for 6 h (3 h for the first temperature + 3 h for the second temperature) and 12 h (3 h for the first temperature + 9 h for the second temperature). These combinations of temperature and time are shown in Table 1. Left and right muscles were assigned to have 6 h and 12 h of cooking time, respectively (n = 6 for each treatment). Once the cooking process was finished, bags were removed from the water bath and submerged in icy cold water (1 °C) for 1 h. Subsequently, packaged GM and BF were kept in a refrigerator (4 °C) overnight prior to analysis.

The day after the cooking process, the samples were weighed to measure the percentage of cooking loss (GM 90–122 g; BF 84–110 g). The cooking loss fluid was then collected, centrifuged at 3000g at 4 °C for 15 min, and subsequently frozen at −80 °C for the measurement of collagen solubility as described by Christensen et al. (2011). Water content, color parameters, and the Warner-Bratzler shear force
features were also measured. In addition, the samples were subjected to sarcoplasmic and myofibrillar solubility analysis. The rest of the samples was kept in a freezer (−80 °C) until analysis. SDS-PAGE analysis and collagen solubility were performed after finishing meat quality measurement.

### Meat Quality Measurement

Water content was determined by drying the samples (4 g) at 105 °C until the weight was stable (AOAC 2000). Water-holding capacity was measured by the weight difference of Whatman no. 1 after compressing 3-g meat with 2.5-kg load (Joo et al. 2018). The water-holding capacity was recorded as the percentage of the water loss.

Color determination of the samples was carried out using a Konica Minolta Colorimeter (Chroma meter, CR-300, Japan) equipped with a standard illuminant D65 using a 2° position of the standard observer with a pulse xenon lamp and 8 mm of reading surface area. \( L^* \) (lightness), \( a^* \) (redness), and \( b^* \) (yellowness) values were recorded three times at three different locations. CIE \( a^* b^* \) values were used to calculate the saturation index \([(a^*+b^*)^{1/2}] \) and hue angle \([\tan^{-1}(b^*/a^*)] \) (AMSA 2012). Before each series of measurements, the instrument was calibrated using a white ceramic plate (\( X=93.5, Y=0.3132, \gamma=0.3198 \)).

The Warner-Bratzler shear force was determined for the cooked sample based on the AMSA guideline (AMSA 1995). After overnight chilling at 4 °C, three sample cores (about 1 cm in diameter) were removed parallel to the myofiber. These sample cores were randomly collected from three different locations with less or no presence of connective tissue. They were sheared three times perpendicular to myofibers orientation using an Instron tensile testing system (Instron 4443, USA). Peak force was obtained using a100-N load cell tension applied at a crosshead speed of 250 mm/min. The maximum peak force was reported as the shear force.

### Protein Solubility

Protein solubility was determined according to the method described by Joo et al. (1999). Briefly, sarcoplasmic proteins were extracted from 1 g of muscle using 20 ml of ice-cold 0.025M potassium phosphate buffer (pH 7.2). The samples were minced and homogenized with a high-speed homogenizer (IKA, model T25D, Germany) at the lowest speed (11,000 rpm/min). These homogenized samples were kept under refrigeration condition for 20 h at 4 °C and then centrifuged at 3000g for 15 min (4 °C). The supernatant was decanted and the protein concentration was measured using the Biuret method with bovine serum albumin as standard. The total protein solubility was determined in 1.1M KI, 0.1M potassium phosphate buffer (pH 7.2). Myofibrillar protein solubility was calculated by the difference in solubility of total and sarcoplasmic proteins.

### Collagen Content and Collagen Solubility

Collagen contents of raw and cooked samples were determined according to ISO-3496 (1994). Briefly, 4 g of meat was hydrolyzed with 30 ml of 3.5M H_2SO_4 for 16 h at 105 °C. The hydrolysate was filtered using a Whatman no. 1 after compressing 3-g meat with 2.5-kg load (Joo et al. 1995). After overnight chilling at 4 °C, three sample cores (about 1 cm in diameter) were removed parallel to the myofiber. These sample cores were randomly collected from three different locations with less or no presence of connective tissue. They were sheared three times perpendicular to myofibers orientation using an Instron tensile testing system (Instron 4443, USA). Peak force was obtained using a100-N load cell tension applied at a crosshead speed of 250 mm/min. The maximum peak force was reported as the shear force.
To determine the soluble collagen in cooking loss, the frozen cooking loss fluid was thawed overnight at 4 °C and centrifuged at 3000g for 30 min at 4 °C. Then, 5 ml of supernatant was hydrolyzed in 30 ml of 3.5M H2SO4 for 16 h at 105 °C. The day after, hydrolysate was filtered and diluted with distilled water to a volume of 100 ml followed by a 10-fold dilution (5 ml to a volume of 50 ml). The addition of oxidation solution and color reagent for the measurement of hydroxypropylation concentration was similar to that described for collagen content. Collagen solubility was expressed as the percentage of soluble collagen/collagen content of raw meat.

Desmin Degradation

Cooked samples (gluteus medius and biceps femoris) for 1D SDS-PAGE were taken (approximately 300 mg) and homogenized with Laemmli (1970) sample buffer containing 125mM Tris-HCl (pH 6.8), 200mM dithiothreitol (DTT), 4% SDS, 20% glycerol, and 0.02% bromophenol blue. The sample was centrifuged at 3000g for 15 min at 4 °C to remove traces of insoluble components. Supernatants were heated at 95 °C for 5 min and subsequently cooled in cold water. Protein concentrations of supernatants were determined before running the gel.

Denatured samples (20 μg protein/lane) were separated onto 12.5% polyacrylamide separating gel of 1.5 mm in thickness (12.5 ml of acrylamide solution containing 30% acrylamide and 0.8% bisacrylamide, 7.5 ml of 1.5M Tris-HCl pH 8.8, 0.3 ml of 10% SDS, 9.6 ml ddH2O, 150 μl of 10% ammonium persulphate, 10 μl TEMED) and stacking gel (0.88 ml of acrylamide solution, 1.66 ml of 1.5 M Tris-HCl pH 8.8, 66 μl of 10% SDS, 4.06 ml of ddH2O, 33.4 μl of 10% ammonium persulfate, 3.3 μl TEMED). Broad-range molecular weight standards (5 to 250 kDa) were run on each gel to determine protein molecular weights and account for gel-to-gel variations. The gels were run at a constant voltage (20 mA for 240 min) using a mini-vertical gel electrophoresis unit SE 260 (Amersham Biosciences, USA).

The gels were stained with Coomassie brilliant blue R-250 staining solution (0.025% Coomassie brilliant blue R-250, 40% methanol, 7% acetic acid). These gels were then destained with the destaining solution I (40% methanol and 7% acetic acid) for 30 min and destaining solution II (7% acetic acid and 5% methanol) until a clear background was obtained. Images of gels were captured on a Hoefer UV-Vis Light Transilluminator (Hoefer® MacroVue UVis-20, Holliston, MA, USA). The appearance of desmin (~ 57 kDa) was analyzed using the ImageJ software (Version 1.52a, National Institute of Health, Bethesda, MD, USA).

Statistical Analysis

All statistical analyses were carried out with SPSS v23.0. Effects of cooking time and cooking temperatures were analyzed by the analysis of variance (2 cooking time × 6 cooking temperatures) together with their interactions using the general linear model (GLM) procedure separately for GM and BF muscles. The comparison between muscle means was analyzed by the one-way analysis of variance. Duncan’s test was used at 5% level to make comparisons between the sample means. The mean values from three repetitions of each treatment were performed with six muscles were assigned for each treatment.

Results and Discussion

Water Content, Water Loss, and Cooking Loss

Table 2 shows the results of water content (%), water loss (%), and cooking loss (%) of GM and BF from goats. Water contents and water loss of both GM and BF muscles were significantly affected by temperature (p < 0.001) and time (p < 0.001) (Table 3). Both muscles cooked at a high temperature, and prolonged heating (12 h) showed significantly lower water content and higher water losses regardless of the treatment used. The result was consistent with the results of Roldan et al. (2013) in lamb and Garcia-Segovia et al. (2007) in beef. The relationship between protein solubility of myofibrillar and sarcoplasmic to water retention is frequently used to evaluate protein denaturation and its effect on water-holding capacity (Mudalal et al. 2014). Increased protein solubility, particularly sarcoplasmic in the present study, was seen at a lower temperature (60 °C). However, protein solubility was markedly decreased once the temperature was increased to 70 °C (p < 0.001) (Fig. 1). Therefore, at a lower cooked temperature (60 °C), sous-vide samples (GM and BF) showed higher protein solubility and greater water retention, resulting in the lowest cooking loss. Joo et al. (1999) have observed a correlation between water-holding capacity and the solubility of the sarcoplasmic protein in pork. Such correlation was higher than the correlation between the water-holding capacity and the solubility of myofibrillar proteins.

Taking the above results into account, protein denaturation at different temperatures is worthy of discussion. The two-stage sous-vide treatment for the first 3 h at 45 °C gave a benefit at the upward temperature (60 °C, 65 °C, and 70 °C). At 45 °C, transverse shrinking of myofibril can occur, leading to larger gaps between muscle fiber bundles owing to inter-myofibrillar water that can be squeezed out easily (Offer et al. 1984). The cooking loss released into...
the vacuum pack that was cooked at the first 3 h (at 45 °C) seemed to be reabsorbed into the meat structure before reaching the final temperature at 60 °C, 65 °C, and/or 70 °C. Zielbauer et al. (2016) have found that at some temperature points between 45 °C and 60 °C, cooking loss again is decreased even lower when the temperature reaches 51 °C during a long time of cooking in the vacuum bag. This could be a sign of protein denaturation process, leading to gel formation and improved water binding in the meat system.

Table 3  Significant main effects ($p < 0.05$) of temperature, time, and their interaction (temp × time) on moisture, water loss, cooking loss, color parameters, shear force, sarcoplasmic solubility, myofibrillar solubility, collagen solubility, and collagen content of *gluteus medius* (GM) and *biceps femoris* (BF) from goats

| Parameter                     | GM            | BF            |
|-------------------------------|---------------|---------------|
|                               | Temp | Time | Temp × time | Temp | Time | Temp × time |
| Moisture content              | ***  | ***  | ***         | ***  | ***  | NS           |
| Water loss                    | ***  | ***  | ***         | ***  | ***  | NS           |
| Cooking loss                  | ***  | ***  | ***         | ***  | ***  | ***           |
| Color                         |      |      |             |      |      |              |
| $L^*$                          | ***  | ***  | ***         | ***  | ***  | ***           |
| $a^*$                          | ***  | ***  | ***         | ***  | ***  | ***           |
| $b^*$                          | ***  | ***  | ***         | ***  | ***  | ***           |
| $C$                            | ***  | ***  | ***         | ***  | ***  | ***           |
| $h^*$                          | ***  | ***  | ***         | ***  | ***  | ***           |
| Shear force                   | ***  | ***  | ***         | ***  | ***  | ***           |
| Sarcoplasmic solubility       | ***  | ***  | ***         | ***  | ***  | NS           |
| Myofibrillar solubility       | ***  | ***  | ***         | ***  | ***  | ***           |
| Collagen solubility           | ***  | ***  | ***         | ***  | ***  | ***           |
| Collagen content              | ***  | ***  | ***         | ***  | ***  | ***           |

NS not significant

$*** p < 0.001$

$** p < 0.01$

$* p < 0.05$
Table 4 shows obtained instrumental color parameters of lightness (L*), redness (a*), yellowness (b*), chroma (C); saturation index/indication of color intensity), and hue angle (h°; indication of color discoloration) in goat GM and BF samples after sous-vide cooking under different experimental conditions. L* values appeared to be distinct between treatments. However, no crucial point was found for either GM or BF muscles, although a significant interaction was observed between temperature and time (p < 0.001) as shown in Table 3. Similarly, although there were significant differences in temperature and time (p < 0.001) for b* values of GM and BF muscles, the numerical difference between temperature/time treatments was very marginal (around 1–2 units). It would not be practically important for further discussion. However, the significant effect of temperature (p < 0.001) and time (p < 0.001) on a*, C, and h° regardless of L* or b* values of

Table 4  Means of color measurements L*, a*, b*, C, and h° values in low temperature–long time treated gluteus medius (GM) and biceps femoris (BF) from goats with different combination temperatures and time duration

| Time | 6 h | 12 h | SEM |
|------|-----|------|-----|
| 60   | 65  | 70   | 65  | 65  | 70   | 65  | 65  | 70   |
| **GM** | | | | | | | | |
| L*  | 57.14b | 57.69h-X | 55.29c-Y | 54.88c | 55.27c | 51.57d-Y | 57.82h,X | 57.13b | 57.86h-X | 55.73c | 57.71h-X | 59.06h-X | 0.32 |
| a*  | 20.00h-X | 12.34c-Y | 11.59h-x | 23.80a | 15.36a | 12.09c-X | 17.20h-Y | 11.46h-X | 10.20h-X | 19.95b | 12.08c-Y | 10.33c-Y | 0.17 |
| b*  | 13.04h-X | 12.27b-Y | 12.16d-X | 13.43a | 12.08d-a | 10.83f | 11.63h-Y | 11.98d-X | 11.97d-X | 11.95c-d-X | 12.25d-X | 11.81d-c-X | 0.12 |
| C   | 23.87h,X | 17.40c-Y | 16.79d-h,X | 27.32a | 19.54a-F | 16.23h-X | 20.76h-X | 16.57h-X | 15.78c-X | 23.26c | 17.20h-b,Y | 15.70h | 0.19 |
| h°  | 33.03c-X | 44.87c-Y | 46.47h-y | 29.37i | 38.13a-X | 41.80e-Y | 34.03c-Y | 46.37h-b | 49.37h-a | 30.90h | 45.50c-Y | 48.80h-a | 0.24 |

| **BF** | | | | | | | | |
| L*  | 56.55d | 52.61c-Y | 61.65b-X | 55.29d | 53.65f | 63.49b-X | 52.73c-Y | 58.82c | 55.60h-y | 55.42d | 55.07b-c-Y | 53.18c-Y | 0.49 |
| a*  | 18.87b-c,Y | 14.90c-X | 9.77c-Y | 23.51a | 17.04c-X | 10.24b-h,Y | 18.62h-c-X | 10.50b-h-Y | 8.94c | 19.44b | 12.16e | 11.24d-c-X | 0.22 |
| b*  | 10.94d-c-Y | 11.73c-Y | 11.15c-d | 12.92a | 11.50b-c | 11.21b-c | 10.87c-e-Y | 11.24b-Y | 10.18c | 11.35b-c,Y | 10.52b-c-Y | 10.56d-c-Y | 0.20 |
| C   | 21.81b-c,Y | 19.29e-X | 14.82d-Y | 26.82a | 20.55c-X | 15.18e-Y | 21.56c-X | 15.37e-Y | 13.55h-b | 22.51b | 16.07c-Y | 15.42h-b | 0.25 |
| h°  | 30.03c-Y | 38.13c-Y | 48.83a-X | 28.73h | 33.97c-Y | 47.67h-X | 30.23c-Y | 47.03h-b | 48.77h-n | 30.23g | 40.83c-Y | 43.13c-Y | 0.31 |

Different superscript letters (a–i) within the same row mean significant differences between treatments (p < 0.05)  
Different superscript letters (X–Y) within the same column mean significant differences between muscles (p < 0.05)  
Values within a column lacking any superscript letter are not significantly different

SEM: standard error of mean (n = 6)  
60, 65, and 70: single-stage sous-vide cooked at 60 °C, 65 °C, and 70 °C for 6 h and 12 h  
45 + 60 + 65, and 45 + 70: two-stage sous-vide cooked at 45 and 60 °C, 45 and 65 °C, 45 and 70 °C for 6 h (3 + 3 h) and 12 h (3 + 9 h)
sous-vide cooked meat was interesting. With increasing temperature and time, $a^*$ and $C$ values were decreased, resulting in high $h^o$ values for both muscles.

As shown in Fig. 2, there was a difference in the color of cooked GM and BF muscles as a consequence of applying sous-vide at different temperatures and time. Sous-vide applied at a temperature above 60 °C resulted in reddish-pink to apparent changes in visual appearance and colorimetric readings. Apparent changes in meat color of GM and BF muscles by cooking temperature were observed for samples cooked at 65 °C and 70 °C with a slight pink to grayish-dull brown color. These changes were more intense with prolonged heating. The denaturation temperature for difference redox forms of myoglobin is inconsistent (AMSA 2012). Thus, there were variations in the relative brown color of cooked meat interiors. As shown in Table 4, goat muscles (GM and BF) cooked at 60 °C were redder than those cooked at 65 °C or 70 °C under both cooking conditions. The same tendency has been reported previously for sous-vide cooked beef (Vaudagna et al. 2008) and pork (Sanchez Del Pulgar et al. 2012). According to Lawrie (2006), denaturation of myoglobin below 65 °C might arise from enzymic action or co-precipitation rather than from the temperature. Also, changes in redness values due to the degree of myoglobin denaturation are highly dependent on the end-point temperature of cooking (Lawrie 2006). Denaturation of myoglobin starts at a temperature between 55 and 65 °C. This process is the most extreme when the temperature rises between 75 and 80 °C (Hunt et al. 1999). Besides, two-stage sous-vide samples cooked for 6 and 12 h showed higher color saturation than those cooked with the single-stage sous-vide method. This might be due to the fact that the cooking duration of two-stage sous-vide cooked meats exposed to the second temperature (60 °C, 65 °C, and 70 °C) was shorter (3 and 9 h) than that of single-stage sous-vide cooked meats (6 and 12 h) (Table 1).

Hue angle ($h^\circ$) is affected by the chemical state of myoglobin (Hunt et al. 1999). The $h^\circ = 0^\circ$ is fixed at the horizontal axis with $a^*$ (redness). Rotating it counterclockwise will lead to $h^\circ = 90^\circ$ (yellow), $h^\circ = 180^\circ$ (green), and $h^\circ = 270^\circ$ (blue) (Ramos et al. 2016). In the present study, our data showed that $h^\circ$ values were in the range of 29.37 to 49.37° for GM and 28.73 to 48.83° for BF. These obtained $h^\circ$ values between 0 and 90° were characterized to have red to yellow quality. Interestingly, among all treatments, two-stage sous-vide treatment at 45 + 60 for 6 h (both GM and BF) had the lowest discoloration of $h^\circ$ value (Table 4).

**Warner-Bratzler Shear Force, Desmin Degradation, Soluble Collagen, and Collagen Content**

Table 5 shows the shear force values (N), collagen solubility (%), and collagen content (mg/g) of GM and BF muscles of a goat. GM muscles were significantly tenderer than BF muscles throughout the investigation period. The shear force values of GM and BF from slaughter goats were affected by
both temperature and time ($p < 0.001$). These observations suggest that two-stage sous-vide cooking is more effective in lowering shear force values of goat GM and BF than single-stage sous-vide treatments. The combination of temperature at 45 °C and 60 °C in two-stage sous-vide only takes 6 h to reach a lower value of shear force while single-stage sous-vide requires a higher temperature and prolonged cooking time to obtain better tenderness values.

In the present study, soluble collagen and collagen contents had less/no role in tenderizing sous-vide goat muscles with 6 h of the cooking period. However, they showed an effect after 12 h of extended heating (Table 5). With 6 h of cooking, it seems that myofibrillar component is more dominant in contributing to higher shear force values at a temperature above 60 °C. Single-stage sous-vide cooked BF muscles recorded significantly higher shear force values at 65 °C and 70 °C while GM muscle at 70 °C. The cooking temperature above 60 °C mainly shows shear strength of myofibrillar components (Christensen et al. 2000) while collagen in intramuscular connective tissue is less dominant (Purslow 2018). On the contrary, the samples treated with two-stage sous-vide presented obvious lower shear force values for both muscles. This can be elucidated by degradation of desmin in myofibrillar component known to be an indicator of the extent of meat tenderization through proteolysis (Zhang et al. 2006). Ertbjerg et al. (2012) have reported that desmin degradation occurs at different temperatures and time durations. They found that desmin degradation was more rapid at 40 °C within 3 h while an only minor amount of desmin was degraded at 55 °C and 70 °C after 24 h of heat treatment. This finding is in line with our results (Fig. 3) that the two-stage sous-vide cooking method with a temperature of 45 °C (45 + 60, 45 + 65, and 45 + 70) significantly resulted in higher desmin degradation (lower intensity) than the single-stage sous-vide cooking method, resulting in lower toughness of these BF and GM samples. Lower desmin degradation in single-stage sous-vide cooked samples at 60 °C, 65 °C, and 70 °C suggests that proteolytic enzymes specific for desmin such as calpain-1 and calpain-2 as well as cathepsin B are thermally unstable at these temperatures (Ertbjerg et al. 2012). The temperature at 45 °C used in the two-stage sous-vide cooking method might have catalyzed enzymes’ activity because Davey and Gilbert (1976) have found that the proteolytic activity is increased exponentially up to 40 °C. It then rose more slowly to a maximum at 60 °C typically due to an enzymatic reaction. This finding was also supported by results of Lawrie (2006) and Myhrvold et al. (2011), showing that cooking at 45 and 49 °C for 4 h could significantly improve the tenderness of the meat.

Additionally, increasing in shear force values for both GM and BF treated with sous-vide at 70 °C for 6 h of cooking although contain higher collagen solubility can be attributed to a higher loss in cooking thereby modified its physical state. The similar finding was also reported by Alahakoon et al. (2018) in beef brisket sous-vide cooked at 70 °C. According to Roldan et al. (2013), water loss after cooking could contribute to tough meat. This toughness results from alteration in

### Table 5

| Time | 6 h | 12 h |
|------|-----|------|
| **Temperature** | | |
| 60 | 65 | 70 | 45 + 60 | 45 + 65 | 45 + 70 | 60 | 65 | 70 | 45 + 60 | 45 + 65 | 45 + 70 |
| **Shear force (N)** | | | | | | | | | | | | | |
| GM | 55.72 aX | 47.68 bY | 57.29 aY | 27.76 bY | 38.55 dY | 42.87 cY | 46.50 bY | 39.73 dY | 31.49 fX | 34.34 eY | 29.72 gY | 25.51 hY | 9.06 iY |
| BF | 51.31 aX | 77.30 cX | 74.50 bX | 39.83 aX | 46.21 aX | 68.18 aX | 56.11 bX | 62.29 aX | 57.88 aX | 61.21 aX | 57.88 aX | 58.05 aX | 0.07 |
| **Collagen solubility (%)** | | | | | | | | | | | | | |
| GM | 5.10 bV | 11.09 cV | 21.93 dV | 5.15 fV | 7.16 j | 27.59 eV | 7.03 hV | 24.00 dV | 39.18 eV | 6.78 h | 11.11 g | 36.49 bV |
| BF | 6.31 cX | 7.29 dX | 14.64 eX | 5.50 fX | 7.13 j | 10.70 dX | 8.97 cX | 13.49 dX | 26.22 eX | 6.84 f | 10.01 e | 20.84 bX |
| **Collagen content (mg/g)** | | | | | | | | | | | | | |
| GM (raw 6.47 f) | 4.64 cX | 4.4 cX | 3.3 hX | 4.02 hX | 4.60 ab | 3.80 bX | 2.36 d | 2.17 d | 5.86 eX | 3.03 dX | 4.49 hX | 4.45 bX |
| BF (raw 8.54 f) | 3.27 cX | 2.8 gX | 3.4 hX | 2.17 fX | 3.98 ab | 2.37 fX | 1.58 g | 1.85 f | 4.49 eX | 1.49 eX | 1.93 fX | 2.59 dX |

Different superscript letters (a–j) within the same row mean significant differences between treatments ($p < 0.05$)

Different superscript letters (X–Y) within the same column mean significant differences between muscles ($p < 0.05$)

Values within a column lacking any superscript letter are not significantly different

**SEM** standard error of mean ($n = 6$)

60, 65, and 70: single-stage sous-vide cooked at 60 °C, 65 °C, and 70 °C for 6 h and 12 h
45 + 60, 45 + 65, and 45 + 70: two-stage sous-vide cooked at 45 and 60 °C, 45 and 65 °C, 45 and 70 °C for 6 h (3 + 3 h) and 12 h (3 + 9 h)

† All raw materials within each muscle was pooled.
modulus state (viscoelastic to a less elastic) when the cooking temperature shifts from 50 °C to 80 °C (Tornberg 2005; Rabeler and Feyissa 2018). Significant regressions ($p < 0.001$) between shear force and water loss for both GM and BF muscles were observed, providing evidence that they could interact with each other in 6 h of cooking time (GM $R^2 = 0.37$, $p < 0.001$; BF $R^2 = 0.001$, $p > 0.05$) except for extended cooking time (GM $R^2 = 0.13$, $p = 0.03$; BF $R^2 = 0.001$, $p > 0.05$). For a cooking period of 12 h, soluble collagen seemed to play an important role in shear force of sous-vide cooked meat. We empirically observed that for single-stage sous-vide with longer cooking time, collagen solubility contributed significantly to the reduction of toughness at 70 °C. At this temperature, both GM and BF shear forces were significantly lower than they were at 60 °C and 65 °C, and both samples were also similar in tenderness after 12 h of cooking. However, it was not pronounced for BF after prolonged cooking with the two-stage sous-vide treatment. This indicated that differences in collagen solubility contributions were influenced by the time this temperature was persistently cooked (12 h vs 9 h for single-stage and two-stage treatment, respectively), with longer time produced higher solubility of collagen, in agreement with the observations of Christensen et al. (2013) and Purslow (2018). On the other hand, collagen content of cooked GM and BF showed a slight fluctuating overall, but coincidently, collagen content showed significantly higher at 70 °C for 12 h of cooking suchlike in collagen solubility, which might be the result of gelatin formation. Previous literature relates gelatin formation with the collagen part shrinks, swells, and softens at a temperature above 65 °C or on further heating (Tornberg 2005; Lawrie 2006). However, there is currently no information available to evidence this idea of gelatin formation in sous-vide cooked meat.

Conclusion

Prolonged sous-vide cooking at high temperature could improve the tenderness of goat GM and BF. However, the meat became discolored and less moist due to higher losses. The two-stage sous-vide treatment can provide a good alternative as it strongly reduces shear force values while preserving the water-holding capacity, redness, and color saturation of goat GM and BF muscles with only 6 h at 45 and 60 °C (45 + 60). However, such quality features could not be seen in single-stage sous-vide after 12 h of cooking at 70 °C, although lower shear force values were obtained.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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