TITLE PAGE
- Food Science of Animal Resources -
Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title	Effects of phosphate and two-stage sous-vide cooking on textural properties of the beef semitendinosus
Running Title (within 10 words)	Phosphate brining in sous-vide meat
Author	Nurul Nazirah Ruslan[1], John Yew Huat Tang[1], Nurul Huda[2], Mohammad Rashedi Ismail-Fitry[3], and Ismail Ishamri[1*]
Affiliation	[1]Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia [2]Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan 90509, Sabah, Malaysia [3]Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
Special remarks – if authors have additional information to inform the editorial office	
ORCID (All authors must have ORCID) https://orcid.org	Nurul Nazirah Ruslan https://orcid.org/0000-0003-2107-4343 John Yew Huat Tang https://orcid.org/0000-0003-4608-9840 Nurul Huda https://orcid.org/0000-0001-9867-6401 Mohammad Rashedi Ismail-Fitry https://orcid.org/0000-0002-2280-5075 Ismail Ishamri https://orcid.org/0000-0003-4820-8292
Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This work was supported by research grant UniSZA/2021/GOT/01 (Project No. R0343) from the Universiti Sultan Zainal Abidin.
Author contributions (This field may be published.)	Conceptualization: Ishamri I, Tang JYH. Data curation: Ishamri I, Ruslan NN. Formal analysis: Ruslan NN. Methodology: Ishamri I, Ruslan NN. Software: Ishamri I, Tang JYH. Validation: Ismail-Fitry MR, Huda N, Tang JYH. Investigation: Ishamri I, Ruslan NN. Writing – original draft: Ishamri I, Ruslan NN. Writing – review & editing: Ishamri I, Ruslan NN, Tang JYH, Ismail-Fitry MR, Huda N.
Ethics approval (IRB/IACUC) (This field may be published.)	This article does not require IRB/IACUC approval because there are no human and animal participants.

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Ismail Ishamri

Email address – this is where your proofs will be sent	ishamriismail@unisza.edu.my
Secondary Email address	
Postal address	School of Food Industry, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia
Cell phone number	+60-17-370-0704
Office phone number	+09-6993129
Fax number	+609-6993100

7

8

Abstract

Comparing the effects of STPP (sodium tripolyphosphate) concentrations of 0.2% and 0.4% on beef semitendinosus is the objective of the current investigation. The samples were cooked at varied temperatures (45+60℃ and 45+70℃) and times (1.5+1.5 h and 3+3 h) using staged cooking. The colour properties, cooking loss, water retention, shear force, water-holding capacity, sarcoplasmic, and myofibrillar solubility, and total collagen were investigated. The cooking time and temperature affected the water-holding capacity, cooking loss, lightness, redness, yellowness, myofibrillar, and sarcoplasmic solubility, with lower temperature and short time having the lower detrimental effect. However, the significant effect can be intensified after the addition of STPP with higher water-holding capacity and tender meat obtained with 0.4% phosphate concentration at any cooking conditions. The STPP lowered the collagen content and increased the protein solubility of myofibrillar and sarcoplasmic, which this degradation is used as a good indicator of tenderness.

Keywords: Sous-vide, Phosphate, Beef, Collagen, Protein Solubility

Introduction

Among the several heat treatment methods for meat, the sous-vide approach is gaining popularity. This method has been utilised in the food sector since it was discovered to be effective for extending the shelf life of pasteurised meals and vacuum-packed goods in the 1960s. In the mid-1970s, French chef George Pralus developed the technique (Przybylski et al., 2021). Sous-vide cooking involves cooking meat at low temperatures (50–80℃) for a long time, however the method depends on the type of meat (Sanchez Del Pulgar et al., 2012). Studies conducted in the past have demonstrated that sous vide cooking lowers shear force, volatile flavour loss, and moisture loss, conserving sensory qualities linked to quality (Trbovich et al., 2017).

Semitendinosus is a large muscle located on the hind leg of mammals and it is categorized as tough meat (Ismail et al., 2019c). The tough cuts of semitendinosus are optimized by mild temperatures or effectively by staged cooking as proposed by Ismail et al. (2022). This optimization minimizes the shear toughness from connective tissue and myofibrillar proteins. Connective tissues are the leading cause of toughness in many cuts of meat which denaturation occurs between 53 and 63°C, while contraction occurs around 65℃ (Ismail et al., 2019b). Meanwhile, myofibrillar proteins are likely contributing to the subsequent rise in meat toughness (Baldwin, 2012) which denaturation of myosin occurs at 60℃ while actin at 66-73℃ (Ismail et al., 2019b).

Phosphates in meat products have a variety of functions and can affect pH, chelation, ionic strength, and antibacterial activity. Also, researchers enhanced the tenderness and juiciness of beef by adding salts and phosphate to the meat (Hoffman et al., 2008). Monophosphates are excellent buffers, yet they have little effect on muscle proteins. Tri- and polyphosphates are helpful in activating meat proteins because they partially chelate protein-bound magnesium and calcium ions. This results in increased myosin and actin solubilization and

depolymerization of thick and thin filaments (Glorieux et al., 2017). Roldán et al. (2014) tested how phosphate brining affected the physical, chemical, and sensory qualities of lamb cooked in a vacuum bag. They found that phosphate solution improved sensory textural features and reduced the toughness of lamb loins. Thus, the goal of this investigation was to ascertain how varying phosphate concentrations affected the textural characteristics of beef semitendinosus subjected with two-stage sous vide cooking.

Materials and Methods

Sampling and thermal treatment

At 24 hours postmortem, the semitendinosus muscle (from native cow) was procured from the local market. All of the muscles were chosen at random on the day of collection (30-32 months old). The collection of paired semitendinosus muscles was carried out and repeated every week until week 8. The fresh cuts were immediately placed in an ice box and brought to the muscle laboratory of University Sultan Zainal Abidin. Muscles were refrigerated to 36 h postmortem at 4℃.

The muscle was cut to 1.5 cm thickness (90-105 g) and divided according to the treatment in Table 1. Prior to vacuum packaging, each steak was injected with sodium tripolyphosphate (STPP) at different concentrations (Table 1) and subsequently was tumbled intermittently for 1 h at 8 rpm and at 4℃ to ensure the STPP spread evenly within the muscle. The steaks were tumbled, vacuum sealed, then cooked in water baths on an Anova Precision stove (Anova, San Francisco, CA, USA). For a temperature of 45+60℃, steaks were heated to 45℃ at first and 60℃ at second. The same applies to 45+70℃. Both treatments were cooked for 3 and 6 h (Table 1). Each block of samples was cooked and then cooled at 4℃ for 15 h before analysis.

Cooking loss, water-holding capacity, and water content

After removing the steaks from the vacuum bag and wiping away excess moisture on the meat surface with a clean wipe, the cooking loss was measured. The proportion of cooking loss was determined by comparing the steaks' pre- and post-cooking weights (Ismail et al., 2019c). Based on AOAC (2002), the water content was carried out by drying a 4 g sample in an oven for 16 hours at 105℃. Water-holding capacity was conducted based on Joo (2018) using the filter paper press method of a 3 g sample with a 2.5 kg load.

Colour properties

A Colorimeter (Chroma metre, CR-300, Japan) outfitted with a pulse xenon lamp, 8 mm of reading surface area, a standard observer in 2° position, and standard illuminant D65 was used to test colour attributes at different locations on the steak cut surface. The instrument was first calibrated to y = 0.3198, X = 0.3132, Y = 93.5, using a white ceramic plate. The L* (lightness), a* (redness), and b* (yellowness) values of steak samples were recorded.

Shear force

A double arm texture analyser was used to analyse the shear force (Stable Micro System, London, England). From each sample, beef cubes of the same size and shape were cut perpendicular to the myofiber, measuring about 1 cm in diameter. Only muscle with no discernible fat and connective tissue were used for this study. A 3-mm thick steel blade (HDP/BSW) with a 73°V cut into its lower edge was used for the test, which was fitted through a 4-mm wide slot in the platform. The sample was tested and placed onto a platform under the blade and cut perpendicular to muscle fibre with constant speed (parameter: test speed, 2.00 mm/sec; post-test speed, 10.00 mm/sec; travel distance, 50 mm). The highest peak force that was measured throughout the test was shear force. The Warner-Bratzler shear force (WBSF)

value in N was calculated using the average of five measurements for each sample (Newtons).

Total collagen

Total collagen was measured according to Ismail et al. (2019c). In 30 mL of 3.5 M H_2SO_4, 4 g of beef were hydrolysed at 105℃ for 16 h. The hydrolysate was filtered and diluted with distilled water to a volume of 500 mL. A graduated cylinder with a volume of 100 mL was filled with distilled water after pipetting the diluent (1 mL) into it. The oxidation solution (100 mL of buffer solutions with a pH of 6 and 1.4 g of the chloramines-T reagent) was then mixed to the final diluent, which had a volume of 2 mL. The buffer solution was made by combining 29 mL of 1-propanol with 1.5 g of sodium hydroxide, 9 g of sodium acetate trihydrate, and 3 g of citric acid monohydrate. From there, it was diluted to 100 mL with distilled water. A freshly prepared 1 mL of colour reagent (produced by dissolving 10 g of 4-(dimethylamino) benzaldehyde in 65 mL of 2-propanol and 35 mL of HCl) was then added to the oxidised sample and vortexed. The test tube was sealed, placed in a water bath set at 60 ℃ for 15 min, and then swiftly cooled with running water. A UV-Vis spectrophotometer operating at 560 nm was used to measure the absorbance of solutions after cooling. Hydroxyproline at concentrations of 0, 1.2, 2.4, 3.6, and 4.8 g hydroxyproline/mL was used to generate a standard calibration curve. Using coefficient factor 8, the amount of collagen was calculated from the amount of hydroxyproline.

Actomyosin preparation

The process of extraction of actomyosin was described Donald and Lanier (1994). A homogeniser (Model IKA® T25, Staufen, Germany) was used to homogenise 1 g of beef sample for 4 min in 10 mL of cold (4 ℃) 0.6 M KCl, pH 7.0. The sample beaker was placed in an ice bath, and every 20 s of blending was followed by a 20 s rest period to prevent overheating during the extraction process. At 0℃, the extract was centrifuged for 30 min at

4000 rpm using a Sigma 3-18K centrifuge. Three volumes of cold, deionized water were added, followed by a 2-min vortex, to precipitate the actomyosin. The water-soluble sarcoplasmic protein was obtained by 20 min of centrifugation at 0℃ and 4000 rpm. In an equal volume of cold, 1.2 M KCl, pH 7.0, the pellet was dissolved overnight in an incubator shaker at 120 rpm at 1℃ to obtain actomyosin. Any undissolved material was removed from the preparation by centrifugation for 20 min at 0℃ at 4000 rpm.

Protein solubility

A 1 ml sample of soluble myofibrillar and sarcoplasmic was pipette into a Kjeldahl tube to measure protein solubility. The AOAC official method 981.10 was used for digestion, distillation, and titration (AOAC, 2000). The solubility of proteins was determined using a conversion factor of 6.25.

Statistical analysis

SPSS v23.0 was used to conduct all statistical analyses. The interaction between cooking times (3 and 6 h), cooking temperatures (45+60 and 45+70℃) and STPP concentrations (0, 0.2, and 0.4%) were analysed using the general linear model (GLM) and Duncan test for multiple mean comparisons with level of significance at 0.05.

Results and Discussion

Cooking loss, water content, and water-holding capacity

Table 2 illustrates the influence of different times, temperatures, and STPP concentrations on cooking loss, water content, and water-holding capacity (WHC). Cooking loss of beef semitendinosus was affected by time ($p = 0.002$), temperature ($p < 0.001$), STPP concentration

150 ($p < 0.001$), and interaction between cooking temperature * time ($p = 0.022$). Lower cooking
151 temperatures and phosphate added resulted in greater water content values. STPP concentration
152 ($p = 0.007$) and temperature ($p < 0.001$) both had an impact on these values. Similarly, for
153 WHC of sous-vide cooked beef was affected by STPP ($p < 0.001$), time ($p = 0.009$), and
154 temperature ($p = 0.002$). Despite being cooked for a longer period of time and at a higher
155 temperature, the STPP inclusion in the current study successfully increased the water retention
156 of sous-vide beef. According to changes in lamb loins cooked sous-vide by Roldán et al. (2014),
157 adding phosphate had an effect on the moisture content and cooking loss but not the WHC.
158 The incorporation of phosphate was thought to positively affect meat pH as described by
159 Roldán et al. (2014). This is an unarguable finding as adding alkaline phosphate (such as
160 pyrophosphate or tripolyphosphate) to manufactured meat products will increase the pH. As a
161 result, the meat proteins' electrostatic repulsion with one another or inside the meat causes a
162 rise in WHC (Glorieux et al., 2017). In addition, the change in the ionic strength will also be
163 related to an increase in WHC. Phosphate addition enhances actomyosin solubility by forming
164 polyelectrolytes in water, causing the protein filaments to swell more (Glorieux et al., 2017;
165 Roldán et al., 2014). However, our results did not support the first evidence because phosphate
166 has no significant effect on pH ($p = 0.170$, data not shown). The present study was related to
167 the second evidence as shown in Table 4. Proteins solubility of myofibrillar were significantly
168 shown an effect ($p < 0.001$) and a strong correlation to WHC ($r = 0.817$) with the STPP
169 concentration. Meanwhile, the trend of cooking loss was consistent with that found in the water
170 content.
171 Sous-vide cooked for a shorter period showed a lower cooking loss than prolonged cooking
172 time. Meanwhile, a higher temperature of two-stage sous-vide (45+70℃) contributed to a
173 higher cooking loss as compared to a mild temperature (45+60℃), this was consistent with our
174 previous finding on the Korean beef semitendinosus (Hanwoo steers) and Korean native black

goat biceps femoris and gluteus medius (*Capra hircus coreanae*), regardless of phosphate addition (Ismail et al., 2019b; Ismail et al., 2019c).

Colour analysis

Table 3 lists the mean values of the meat's L* (lightness), a* (redness), and b* (yellowness) for various temperatures, cooking periods, and STPP concentrations. The L*, a*, and b* values of sous-vide meat were significantly affected by the time ($p < 0.001$), temperature ($p < 0.001$) and STPP concentration ($p = 0.007$) as well as their interaction ($p < 0.001$). The L* values in the present study were in line with Roldán et al. (2014). As shown in Table 3, the sous-vide treatment at 45+60℃ resulted in lower L* values after brining with STPP. Nevertheless, sous-vide beef at 45+70℃ for both cooking durations (3 and 6 h) demonstrated a small increase in lightness with STPP concentration. We empirically observed that the effect of pH in the present study is not the primary concern to relate with the lower L* values, because as mentioned above phosphate addition has no significant effect on pH. Contrary to the findings of Ayub and Ahmad (2019) and Roldán et al. (2014), they found that lower L* values were due to the phosphates that increase the pH thereby lowering the lightness of meat. According to Roldán et al. (2014), the addition of phosphate alters the pH and ionic strength and causes the myofibrillar proteins to swell. Lower L* values result from the enlarged proteins' deeper light penetration into the tissue. Nevertheless, this argumentation is much related to the actomyosin complex dissociation in which phosphate promotes the depolymerization of myosin and actin filaments into separate fibres (Glorieux et al., 2017; Tan et al., 2018). This is demonstrated by Table 4's data, which shows that myofibrillar solubility is higher at mild temperatures (45+60℃) than at high temperatures (45+70℃). Therefore, this finding strongly supports the lower L* values in sous-vide cooked beef due to the higher protein solubility. Nevertheless, larger loss caused by increased protein denaturation and shortened sarcomere at a temperature

above 60℃ was the cause of the higher L* values for treatment at 45+70℃ (Ismail et al., 2019c). According to Christensen et al. (2011), this resulted in an increase in light scattering and higher L* values.

The thermal treatment of the cooked beef semitendinosus at various temperatures and times caused a change in the a* values, as indicated in Table 3. The sous-vide samples that were prepared at lower temperatures and in less time had the highest redness values. However, the effect of phosphate on a* values varied and even showed significance ($p = 0.007$). According to Lawrie (2006), the myoglobin denaturation and endpoint cooking temperature have a significant impact on the redness characteristics of cooked beef. However, as stated by Hunt et al. (1999), myoglobin denaturation started at 55℃ and continued until 80℃, occurring at no precise temperature threshold. Also, it depends on the cooking time and cooking temperature (Roldan et al., 2013), as evidenced by the significant effect of time ($p < 0.001$) and temperature ($p < 0.001$) in Table 3. However, there was inconsistent effect of STPP in sous-vide cooked meat on a* value. A similar effect can be seen on the b* values as the results show the variable. Although phosphate reduced the amount of oxidation in the restructured beef steaks studied by Lamkey et al. (2006) STPP considerably changed the values of raw beef L*, a*, and b* but had no effect on cooked beef (Bach et al., 2011). Higher b* values in Table 3 can be linked to a temperature increase and prolonged cooking time as a consequence of metmyoglobin formation. It seems that phosphate alone could not effectively modify the redox state of cooked meat, instead oxidation mechanism to metmyoglobin was more dominant. Similar results were also obtained by Roldán et al. (2014) and Ö nenç et al. (2004) who considered the effect of phosphate added was negligible to b* values.

Shear force, total collagen, myofibrillar and sarcoplasmic solubility

Shear strength, total collagen, myofibrillar, and sarcoplasmic solubility of sous-vide cooked beef samples at different durations, temperatures, and phosphate concentrations are shown in Table 4. Only the different STPP doses had a statistically significant impact on shear force ($p = 0.013$). The values of shear force in two-stage sous-vide without phosphate incorporation were significantly affected by cooking temperature and cooking duration ($p < 0.001$), contrary to what was previously reported in our work (Ismail et al., 2019c). Meaning that, STPP effectively speeds up the dissociation of actomyosin and reduces the toughness of beef semitendinosus. According to Table 4, regardless of cooking temperature or cooking duration, STPP at the highest concentration of 4% considerably reduces the shear force values of all treatments. In contrast, Roldán et al. (2014) found that adding phosphate to sous-vide lamb loins increases the hardness and shear force values. The reasons for these contrasts will be explained based on the total collagen content, myofibrillar, and sarcoplasmic solubility as described in Table 4 and all these parameters were significantly affected by the STPP concentration ($p < 0.001$).

The amount of total collagen is a good indicator of how tender or tough meat will be after being cooked sous vide. However, we discovered that collagen content and collagen solubility play a minor or nonexistent impact in tenderising red meat in our earlier investigation on the cow semitendinosus (Ismail et al., 2019c) and the goat gluteus medius and biceps femoris (Ismail et al., 2019b). Similarly, in the present study, we noticed that cooking temperature and time were less or not correlated to the total collagen (time: $r = -0.187$; temperature: $r= 0.125$) as well as their interaction ($p = 0.053$) detected no significant effect on collagen content. Interestingly, the addition of phosphate resulted in a significant interaction between the STPP concentration, time, and temperature in total collagen for sous-vide cooked beef (p time * p temperature * p STPP = 0.02). Nevertheless, the detected differences in Table 4 for total

collagen between treatments were not significant in a Duncan test. The fact that total collagen content decreased with the STPP ($p < 0.001$) and it clearly can be seen that sous-vide treated without phosphate resulted in the highest toughness. According to Chaosap et al. (2021), lower collagen content proportionally lowered the toughness of the meat. However, total collagen was not always used as an indicator to determine the toughness/tenderness, because previous literature has found the effect of myofibrillar components to be more dominant in causing toughness at certain temperatures (Christensen et al., 2000; Ismail et al., 2022; Ismail et al., 2019c; Purslow, 2018).

As mentioned by Christensen et al. (2000), the degree of toughness caused by collagen and myofibrillar proteins occurs through sequential and multi-steps non-proportional to the cooking conditions. Even though gradual cooking between 50 and 60°C causes collagen denaturation, once the temperature rises over 60°C, the influence of collagen strength is less noticeable and the myofibrillar components start to take over the strength (Purslow, 2018). The phosphate addition could affect both collagen and myofibrillar components ($p < 0.001$). Phosphate can make connective tissues more tender by increasing the solubility of collagen, decreasing the degree to which collagen in connective tissues is cross-linked, and dissociating the actomyosin complex (Shen et al., 2016; Shi et al., 2021). As shown in Table 4, the effect of temperature, time, and STPP concentration is significant on myofibrillar solubility ($p < 0.001$). The myofibrillar solubility decrease with the increased cooking temperature and cooking time and increase with STPP concentration. There are several effects of phosphate on myofibrillar proteins. Phosphates promote the ionic effect, which changes the pH and deviates the pH of proteins from the isoelectric point. Thus, the charges repulse each other and enlarge the space of myofibrils owing to entrapping more water (Shi et al., 2021). Next, due to the buffering effect of phosphate, the pH elevation may play a role in the increased activation of calpains (Shi et al., 2021). The activity of calpains was believed to dissociate the permanent actomyosin

bridge. All these mechanisms are thought to contribute to meat tenderness. According to Maqsood et al. (2018), increasing myofibrillar solubility reflects the higher myofibrillar degradation or myofibrillar proteolysis. Thus, lowering the toughness or shear force values (Table 4).

The sarcoplasmic solubility was affected by the time ($p < 0.001$), temperature ($p < 0.001$), STPP concentration ($p < 0.001$), and interaction between temperature and time ($p < 0.007$). The decreased sarcoplasmic proteins based on cooking temperature and time were approximately 15 – 42% and 6 – 36%, respectively. Increasing temperature and cooking time decreased the sarcoplasmic solubility, this was likely due to the denaturation of actomyosin that causes structural change and presses out the sarcoplasmic protein fluid from the myofibers (Li et al., 2013). However, the addition of phosphate effectively reduces the loss of sarcoplasmic protein as shown in Table 4. The relationship between lower shear force values and higher sarcoplasmic solubility ($r = -0.493$) can be explained by protein aggregation and water retention. According to Tornberg (2005), the sarcoplasmic proteins aggregated at a temperature between 40 to 60℃, which is the ideal temperature along with the myofibrillar proteins to provide consistency in the cooked meat. These proteins form a gel and minimize water loss from the meat proteins (Ismail et al., 2019a; Mudalal et al., 2014). According to Li et al. (2013), the higher water retention was substantially connected with the lower shear force value, and the addition of STPP amplifies this correlation (Table 4).

Conclusion

Two-stage cooking time and temperature, and the addition of STPP play a significant effect on the physicochemical properties of beef semitendinosus. Prolonged cooking at higher temperature (45+70℃ for 6 h) resulted in greater cooking loss and decreased water content

and WHC. Regardless of cooking temperatures and times, the effect of STPP at higher concentrations (0.4%) has been demonstrated by a reduction in cooking loss and an increase in water-holding capacity and water content. The effect of phosphate on colour properties was not apparent though it was significant. Cooking temperatures and times had little effect on the shear force values, but STPP made them tender. The shear force values were less connected with total collagen and more correlated with the solubility of the proteins (sarcoplasmic and myofibrillar). Nonetheless, the STPP concentration (0.4%) was effective in dissociating the collagen and producing tender sous-vide meat.

Conflicts of Interest

No potential conflicts of interest are disclosed by the authors.

Acknowledgements

This work was supported by research grant UniSZA/2021/GOT/01 (Project No. R0343) from the Universiti Sultan Zainal Abidin, Terengganu, Malaysia.

Author Contributions

Conceptualization Ishamri I, Tang JYH. Data curation: Ishamri I, Ruslan NN. Formal analysis: Ruslan NN. Methodology: Ishamri I, Ruslan NN. Software: Ishamri I, Tang JYH. Validation: Ismail-Fitry MR, Huda N, Tang JYH. Investigation: Ishamri I, Ruslan NN. Writing - original draft: Ishamri I, Ruslan NN. Writing - review & editing: Ishamri I, Huda N, Ruslan NN, Tang JYH, Ismail-Fitry MR.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

REFERENCES

1. AOAC. 2000. Official methods of analysis. Association of Official Analytical Chemists, Washington, DC.
2. AOAC. 2002. Official methods of analysis. 16th ed. Association of Official Analytical Chemists, Arlington.
3. Ayub H, Ahmad A. 2019. Physiochemical changes in sous-vide and conventionally cooked meat. Int J Gastron Food Sci. 17:100145.
4. Bach N, Long S, Gál R, Buňka F. 2011. Use of phosphates in meat products. Afr J Biotechnol. 10:19874-19882.
5. Baldwin DE. 2012. Sous vide cooking: A review. Int J Gastron Food Sci. 1:15-30.
6. Chaosap C, Chauychuwong N, Chauychuwong R, Sriprem C, Sivapirunthep P, Sazili AQ. 2021. Carcass composition, meat quality, calpain activity, fatty acid composition and ribonucleotide content in southern thai native goats and three-way crossbred goats. Foods. 10:1323.
7. Christensen L, Ertbjerg P, Aaslyng MD, Christensen M. 2011. Effect of prolonged heat treatment from 48°c to 63°c on toughness, cooking loss and color of pork. Meat Sci. 88:280-285.
8. Christensen M, Purslow PP, Larsen LM. 2000. The effect of cooking temperature on mechanical properties of whole meat, single muscle fibres and perimysial connective tissue. Meat Sci. 55:301-307.
9. Donald GM, Lanier TC. 1994. Actomyosin stabilization to freeze-thaw and heat denaturation by lactate salts. J Food Sci. 59:101-105.
10. Glorieux S, Goemaere O, Steen L, Fraeye I. 2017. Phosphate reduction in emulsified meat products: Impact of phosphate type and dosage on quality characteristics. Food Technol Biotechnol. 55:390-397.

11. Hoffman LC, Muller M, Vermaak A. 2008. Sensory and preference testing of selected beef muscles infused with a phosphate and lactate blend. Meat Sci. 80:1055-1060.
12. Hunt MC, Sørheim O, Slinde E. 1999. Color and heat denaturation of myoglobin forms in ground beef. J Food Sci. 64:847-851.
13. Ismail I, Hwang Y-H, Bakhsh A, Joo S-T. 2019a. The alternative approach of low temperature-long time cooking on bovine semitendinosus meat quality. Asian-Australas J Anim Sci. 32:282-289.
14. Ismail I, Hwang Y-H, Bakhsh A, Lee S-J, Lee E-Y, Kim C-J, Joo S-T. 2022. Control of sous-vide physicochemical, sensory, and microbial properties through the manipulation of cooking temperatures and times. Meat Sci. 188:108787.
15. Ismail I, Hwang Y-H, Joo S-T. 2019b. Effect of different temperature and time combinations on quality characteristics of sous-vide cooked goat gluteus medius and biceps femor*is*. Food Bioproc Tech. 12:1000-1009.
16. Ismail I, Hwang Y-H, Joo S-T. 2019c. Interventions of two-stage thermal sous-vide cooking on the toughness of beef semitendinosus. Meat Sci. 157:107882.
17. Joo ST. 2018. Determination of water-holding capacity of porcine musculature based on released water method using optimal load. Korean J Food Sci Anim. 38:823-828.
18. Lamkey J, Mandigo R, Calkins C. 2006. Effect of salt and phosphate on the texture and color stability of restructured beef steaks. J Food Sci. 51:873-875.
19. Lawrie RA. 2006. Lawrie's meat science. Seventh ed. Woodhead Publishing Limited.
20. Li C, Wang D, Xu W, Gao F, Zhou G. 2013. Effect of final cooked temperature on tenderness, protein solubility and microstructure of duck breast muscle. LWT - Food Sci and Technol. 51:266-274.
21. Maqsood S, Manheem K, Gani A, Abushelaibi A. 2018. Degradation of myofibrillar, sarcoplasmic and connective tissue proteins by plant proteolytic enzymes and their impact on camel meat tenderness. J Food Sci Technol. 55:3427-3438.
22. Mudalal S, Babini E, Cavani C, Petracci M. 2014. Quantity and functionality of protein fractions in chicken breast fillets affected by white striping. Poult Sci. 93:2108-2116.
23. Ö nenç A, Serdaroglu M, Abdraimov K. 2004. Effect of various additives to marinating baths on some properties of cattle meat. Eur Food Res Technol. 218:114-117.
24. Przybylski W, Jaworska D, Kajak-Siemaszko K, Sałek P, Pakuła K. 2021. Effect of heat treatment by the sous-vide method on the quality of poultry meat. Foods. 10:1610.
25. Purslow PP. 2018. Contribution of collagen and connective tissue to cooked meat toughness; some paradigms reviewed. Meat Sci. 144:127-134.

26. Roldan M, Antequera T, Martin A, Mayoral AI, Ruiz J. 2013. Effect of different temperature-time combinations on physicochemical, microbiological, textural and structural features of sous-vide cooked lamb loins. Meat Sci. 93:572-578.
27. Roldán M, Antequera T, Pérez-Palacios T, Ruiz J. 2014. Effect of added phosphate and type of cooking method on physico-chemical and sensory features of cooked lamb loins. Meat Sci. 97:69-75.
28. Sanchez Del Pulgar J, Gazquez A, Ruiz-Carrascal J. 2012. Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. Meat Sci. 90:828-835.
29. Shen QW, Swartz DR, Wang Z, Liu Y, Gao Y, Zhang D. 2016. Different actions of salt and pyrophosphate on protein extraction from myofibrils reveal the mechanism controlling myosin dissociation. J Sci Food Agric. 96:2033-2039.
30. Shi H, Shahidi F, Wang J, Huang Y, Zou Y, Xu W, Wang D. 2021. Techniques for postmortem tenderisation in meat processing: Effectiveness, application and possible mechanisms. Food Prod Process Nutr. 3:21.
31. Tan SM, De Kock R, Dykes GA, Coorey R, Buys E. 2018. Enhancement of poultry meat: Trends, nutritional profile, legislation and challenges. S Afr J Anim Sci. 48:199.
32. Tornberg E. 2005. Effects of heat on meat proteins - implications on structure and quality of meat products. Meat Sci. 70:493-508.
33. Trbovich V, Garza H, Wick M, England E, Garcia L. 2017. Investigating the effects of sous vide cooking on tenderness and protein concentration in young fed and cow semitendinosus muscles. Meat Muscl Bio. 1:34-34.

412 **Table 1** Two-stage sous-vide with and without addition of phosphate

*Treatment	Temperature 1 (℃)	Temperature 2 (℃)	Time 1 (h)	Time 2 (h)	STPP concentration (%)
45+60℃	45	60	1.5	1.5	0.0
					0.2
					0.4
	45	60	3.0	3.0	0.0
					0.2
					0.4
45+70℃	45	70	1.5	1.5	0.0
					0.2
					0.4
	45	70	3.0	3.0	0.0
					0.2
					0.4

413 *Beef was first cooked at 45℃ for 1.5 h or 3.0 h and then at 60℃ or 70℃ for 1.5 h or 3.0 h.

414 **Table 2.** Cooking loss, water content, and water holding capacity (WHC) of cooked sous-vide
415 at different time, temperature, and sodium tripolyphosphate (STPP) concentration

Time	Temperature (℃)	STPP Concentration (%)	Cooking loss (%)	Water content (%)	WHC (%)
3 h	45 + 60	0	33.11±0.85^{cd}	65.50±0.46^{abc}	85.90±0.81^{d}
		0.2	30.06±0.40^{de}	65.88±0.66^{abc}	92.38±0.91^{bc}
		0.4	28.53±0.42^{e}	67.82±0.39^{a}	96.64±0.21^{a}
	45 + 70	0	46.09±0.74^{a}	59.14±0.53^{e}	82.11±0.82^{ef}
		0.2	41.95±0.75^{b}	66.48±0.90^{abc}	90.99±0.13^{c}
		0.4	40.57±0.20^{b}	63.87±0.26^{bcd}	95.86±0.01^{a}
6 h	45 + 60	0	35.11±0.10^{c}	65.54±1.05^{abc}	82.73±0.69^{e}
		0.2	34.81±0.86^{c}	66.23±0.30^{abc}	92.70±0.86^{bc}
		0.4	33.43±0.10^{cd}	67.53±0.08^{ab}	94.80±0.85^{ab}
	45 + 70	0	48.20±0.29^{a}	59.61±0.84^{e}	79.63±1.02^{f}
		0.2	42.34±0.77^{b}	60.31±0.85^{de}	90.27±0.78^{c}
		0.4	40.78±0.90^{b}	62.64±0.15^{cde}	94.08±0.59^{ab}
P time			0.002	0.106	0.009
P temperature			<0.001	<0.001	0.002
P concentration			<0.001	0.007	<0.001
P time * P temp			0.022	0.097	0.928
P time * P temp * P conc.			0.406	0.096	0.250

416 Means value within different letters in same column referring to significant different ($p<0.05$).
417 Mean ± standard deviation.
418
419
420

421 **Table 3.** Means of colour properties (L*, a*, b*) of cooked sous-vide at different
422 temperature, time, and sodium tripolyphosphate (STPP) concentration

Time	Temperature (℃)	STPP concentration (%)	Lightness, L*	Redness, a*	Yellowness, b*
3 h	45 + 60	0	41.70±0.40^{d}	19.35±0.19^{a}	13.46±0.02^{g}
		0.2	38.56±0.01^{g}	19.41±0.33^{a}	13.64±0.05^{f}
		0.4	33.92±0.07^{h}	16.51±0.07^{b}	11.84±0.03^{i}
	45 + 70	0	38.67±0.02^{g}	13.71±0.14^{f}	14.12±0.03^{e}
		0.2	32.81±0.40^{i}	15.49±0.06^{cd}	14.06±0.11^{e}
		0.4	45.15±0.16^{b}	11.90±0.03^{g}	14.71±0.01^{b}
6 h	45 + 60	0	50.08±0.33^{a}	14.07±0.65^{ef}	14.32±0.16^{d}
		0.2	43.39±0.01^{c}	16.28±1.01^{bc}	14.52±0.10^{c}
		0.4	38.62±0.25^{g}	14.51±0.33^{ef}	13.75±0.08^{f}
	45 + 70	0	40.54±0.01^{e}	14.84±0.10^{de}	14.58±0.01^{b}
		0.2	39.41±0.30^{f}	15.89±0.01^{bc}	15.24±0.01^{a}
		0.4	43.25±0.05^{c}	16.11±0.06^{bc}	13.25±0.08^{h}
P time			< 0.001	<0.001	<0.001
P temperature			<0.001	<0.001	<0.001
P concentration			<0.001	0.007	0.007
P time * P temp			<0.001	<0.001	<0.001
P time * P temp * P conc.			<0.001	<0.001	<0.001

Means value within different letters in same column referring to significant different ($p<0.05$). Mean ± standard deviation.

423

424 **Table 4.** Shear force, total collagen, myofibrillar, and sarcoplasmic solubility of cooked sous-
425 vide at different time, temperature, and sodium tripolyphosphate (STPP) concentration

Time	Temperature (℃)	STPP concentration (%)	Shear force (kg)	Total collagen (%)	Myofibrillar solubility (%)	Sarcoplasmic solubility (%)
3 h	45 + 60	0	11.42±0.94^{b}	4.55±0.47^{b}	4.51±0.23^{d}	2.93±0.33^{def}
		0.2	10.34±1.32^{b}	3.82±0.72^{bc}	5.25±0.19^{c}	3.41±0.08^{bc}
		0.4	9.92±1.40^{b}	3.54±0.04^{bc}	6.21±0.32^{ab}	4.09±0.08^{a}
	45 + 70	0	18.24±1.71^{a}	7.53±1.50^{a}	4.18±0.08^{d}	2.50±0.01^{gh}
		0.2	13.14±1.68^{ab}	3.79±0.02^{bc}	4.51±0.08^{d}	2.83±0.71^{efg}
		0.4	9.52±1.19^{b}	2.98±0.16^{c}	5.85±0.19^{a}	3.18±0.14^{cde}
6 h	45 + 60	0	15.56±0.29^{ab}	4.44±0.03^{b}	4.17±0.09^{d}	2.75±0.04^{fg}
		0.2	13.45±1.64^{ab}	3.98±0.30^{bc}	4.70±0.04^{d}	3.22±0.08^{cd}
		0.4	9.96±1.27^{b}	3.38±0.19^{bc}	5.33±0.50^{bc}	3.75±0.18^{ab}
	45 + 70	0	14.15±1.70^{ab}	4.03±0.45^{bc}	3.45±0.32^{e}	1.60±0.28^{i}
		0.2	12.33±1.71^{ab}	3.65±0.42^{bc}	4.20±0.16^{d}	2.17±0.19^{h}
		0.4	12.05±1.09^{b}	3.62±0.18^{bc}	4.61±0.22^{d}	2.74±0.13^{fg}
P time			0.438	0.039	<0.001	<0.001
P temperature			0.812	0.187	<0.001	<0.001
P concentration			0.013	<0.001	<0.001	<0.001
P time * P temp			0.143	0.053	0.382	<0.007
P time * P temp * P conc.			0.507	0.02	0.189	0.487

Means value within different letters in same column referring to significant different ($p<0.05$).
Mean ± standard deviation.

426

427