

Physicochemical, Microstructural, and Sensory Attributes of Frozen Buffalo Loins Treated with Sodium Tripolyphosphate and Sous-Vide Cooking

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ABSTRACT

This study examines the effects of sous-vide cooking with varying concentrations of sodium tripolyphosphate (STPP) on the physicochemical, microstructural, and sensory characteristics of frozen buffalo loins. Samples were prepared with 0.15% and 0.30% STPP, alongside a control group without STPP, and cooked using a two-stage sous-vide process at 45°C for 3 hr, followed by 60°C for an additional 3 h. The results demonstrated that STPP significantly influenced pH, cooking loss, water-holding capacity (WHC), colour properties, Warner-Bratzler shear force (WBSF), microstructure, and sensory characteristics. Raising STPP concentration improved WHC and lowered cooking loss. STPP generated a pH rise, which delayed the denaturation of myoglobin and resulted in the sous-vide-cooked buffalo loins having a redder appearance. Scanning electron micrograph (SEM) supported the notable increase in tenderness with STPP addition, by showing structural changes in the muscle fibres; 0.3% of STPP samples displayed noticeably looser fibres and a thinner perimysium. The 0.3% STPP samples had a higher overall acceptability, texture, juiciness, and appearance score than the other samples, as indicated by the sensory evaluation using generic descriptive analysis (GDA) and principal component analysis (PCA). Therefore, it is recommended to incorporate 0.3% STPP into the sous-vide cooking process to optimize the eating quality of the frozen buffalo loins.

Key words: Buffalo, PCA, SEM, sensory evaluation, sodium tripolyphosphate, sous-vide

INTRODUCTION

Sous-vide is a cooking method that involves vacuum-sealing and cooking meat at low temperatures for a long period. This method is popular because it preserves meat's nutritional and sensory qualities. Typically, sous-vide temperatures range from 40°C to 90°C, with cooking times varying from a few hour to several days, depending on the type of meat and the desired outcome (Ismail *et al.*, 2019). There are two main types of sous-vide cooking: single-stage and two-stage. Single-stage cooking maintains a constant temperature and time throughout the process. In contrast, two-stage cooking involves sequential heating at two different temperatures, where the first stage at a lower temperature maintains tenderness, and the second stage at a higher temperature enhances flavour and ensures food safety (Trbovich *et al.*, 2017).

Malaysians often consume frozen buffalo meat. Buffalo meat imported from India is cheaper compared to local beef (Sharma *et al.*, 2023). The taste of buffalo meat and beef may sometimes be indistinguishable (Naveena & Kiran, 2014). For sous-vide cooking, frozen cuts pose distinct difficulties. The formation of ice crystals, cold shortening, and thaw rigour in frozen meat can damage muscle fibres, affecting texture and water retention (Dang *et al.*, 2021). Post-cooking, frozen buffalo meat tends to toughen more than beef (Neath *et al.*, 2007). The processing of buffalo meat affects sensory properties such as tenderness, juiciness, and acceptability, which are crucial for customer satisfaction (Dang *et al.*, 2021). However, there is still a lack of scientific articles on buffalo meat quality, especially in connection to sous-vide cooking.

Phosphates are essential in the preparation of meat and meat products because they have a significant impact on texture and water retention by moving proteins from their isoelectric point. Phosphates are essential in the preparation of meat and meat products because they have a significant impact on texture and water retention by moving proteins from their isoelectric point. During rigour development, lactic acid accumulation lowers the pH (Zhang *et al.*, 2018), while cooking slightly increases it (Roldán *et al.*, 2014). To mitigate pH fluctuations, sodium tripolyphosphate (STPP) is employed during sous-vide cooking. This is essential for the prevention of protein denaturation and the avoidance of meat toughness (Ruslan *et al.*, 2023). Phosphates can also increase the ionic strength of meat, widen the gaps between muscle fibres, and decrease cooking losses. Phosphates can decrease lipid oxidation and prolong the shelf-life of meat by forming bonds with metal ions (Glorieux *et al.*, 2017). A previous study by Roldán *et al.* (2014) used phosphate concentrations of 0.2% and 0.4% in sous-vide lamb loins, while Nik Zainal Abidin *et al.* (2024) and Ruslan *et al.* (2023) incorporated phosphate at 0.15% and 0.3% in sous-vide beef. However, according to Malaysian Food Regulations 1985, the maximum permissible amount of phosphate in meat products must not exceed 0.3% (Food-Act-281, 2023). Hence, the objective of this study was to investigate the physicochemical, microstructural, and sensory

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properties of frozen buffalo loins during two-stage sous-vide cooking and STPP at various concentrations (0%, 0.15% & 0.3%).

MATERIALS AND METHODS

Sampling and cooking procedure

The frozen buffalo loins (Black Gold brand, India) stored in a freezer at -18°C were collected from a local market in Kampung Raja, Terengganu. The temperature of the loins was checked with an infrared thermometer (62 Max Mini Infrared Thermometer, Fluke, Eindhoven, The Netherlands) before they were randomly selected ($n=15$). Then, the loins were placed in an ice box under aseptic conditions and transported to the laboratory. Following overnight thawing in a refrigerator at 4°C , the loins were trimmed of subcutaneous fat and visible connective tissue, and sliced into steaks of 2 cm thickness with an average weight of $100\text{ g} \pm 5\text{ g}$. Each steak was injected and targeted at five different points with three concentrations of STPP (0%, 0.15% & 0.30%) using a meat injector, with an injection depth of 1 cm. After injection, the steaks were individually vacuum-sealed in oxygen-impermeable nylon food-grade bags and subjected to a tumbling process at 4°C for 1 hr and speed set at 8 rpm to ensure even distribution of the phosphate. Subsequently, the sous-vide cooking process was conducted using the sous-vide precision cooker (ANOVA, San Francisco, CA, USA) and consisted of an initial stage at 45°C for 3 hr, followed by a secondary stage at 60°C for an additional 3 hr (Table 1). After cooking, the steaks were rapidly cooled by immersion in ice water for 1 hr before analysis.

Table 1. Temperature, time, and STPP concentration for two-stage cooking

Temp. 1	Time 1	Temp. 2	Time 2	STPP concentration (%)
45°C	3 h	60°C	3 h	0.00
				0.15
				0.30

pH, cooking loss, and water-holding capacity

The pH of the meat was measured using a benchtop pH meter (AE150; Fisher Scientific, Waltham, MA, USA). The meter was calibrated with standard buffer solutions of pH 4 and 7. To determine the pH, 2 g of cooked steak was chopped and homogenized in 10 mL of distilled water. Cooking loss was calculated by comparing the steak's weight before (W_b) and after cooking (W_{sv}). Water holding capacity (WHC) was analysed based on the method described by Joo (2018). For this, 3 g of cooked steak was weighed and placed between two pieces of Whatman no. 1 filter paper, covered with aluminium foil, and pressed with a 2 kg load. The pressed sample was then weighed, and the WHC was recorded as the percentage of the water loss.

$$\text{Cooking loss (\%)} = (W_b - W_{sv}) / W_b \times 100\%$$

Colour properties

The colour of the meat was assessed using a Konica Minolta Colorimeter (Chroma Meter, CR-300, Japan) by measuring five different random spots on the surface of the cooked steak. The instrument was calibrated with a white ceramic plate ($Y = 93.5$, $X = 0.3132$, $y = 0.3198$). The values for L^* (lightness), a^* (redness), and b^* (yellowness) were evaluated.

Warner-Bratzler shear force (WBSF)

WBSF was determined according to the American Meat Science Association guidelines (AMSA, 1995) using a double-arm texture analyser (Stable Micro System, London, UK). Cooked steak samples (1 cm width \times 1 cm thickness \times 3 cm length) were cut parallel to the myofibers, with only muscles free of visible fat used for analysis. A cutter blade with a 73°V cut (HDP/BSW) was used, fitting through the platform. The meat was positioned on the platform and sliced parallel to the muscle fibres using settings: pre-test speed: 5.00 mm/sec; test speed: 2.00 mm/sec; post-test speed: 10.00 mm/sec; travel distance: 50 mm. The maximum peak force recorded during the test was used to determine the shear force values. The WBSF value was reported in kg.

Scanning electron microscopy (SEM)

The steak sample underwent a 48 hr freeze-drying process and was subsequently cut into small cubes with a diameter of 0.5 cm. The dried samples were gold-coated using a sputter coater (Magnetron MA0600, Tucson, AZ). The surfaces were examined perpendicular to the fibres using a JSM-IT500THR scanning electron microscope (Jeol Ltd., Tokyo) at magnifications ranging from 30 to $200\times$.

Sensory evaluation

The UniSZA Human Research Ethics Committee (UHREC) approved the study protocol (UniSZA/UHREC/2024/640) for the sensory evaluation of sous-vide buffalo meat. The evaluation session was conducted with 30 semi-trained panellists at the sensory laboratory, School of Food Industry, Universiti Sultan Zainal Abidin (UniSZA). The General Descriptive Analysis (GDA) method was used to evaluate the samples. Panellists were served three sous-vide buffalo meat samples, each with a different phosphate concentration. The samples were diagonally cut into 1 cm thick slices, with one slice served to each panellist. Each panellist performed the evaluation individually in isolated booths, with the samples presented in random order and identified by three-digit random codes. Plain water was provided for palate cleansing between samples and before the evaluation. Panellists identified the sensory attributes and responded to the questionnaire, rating each sample on a 12-cm GDA scale for appearance, colour, texture, juiciness, and overall acceptability attributes. Each attribute was marked on the scale with a vertical line to indicate the perceived intensity.

Statistical analysis

Statistical analysis was conducted with three replications for each analysis. Analysis of variance was performed on buffalo sous-vide treated with STPP concentrations (0%, 0.15% & 0.3%) to assess significant differences in pH, cooking loss, WHC, colour properties, and WBSF using one-way analysis of variance in SPSS version 29 (IBM Corp., Armonk, NY, USA). The collected data were presented as mean \pm standard deviation. An independent sample of Duncan's test with a significance level of $p < 0.05$ was used to compare mean scores between treatments. Principal component analysis (PCA) was conducted to analyse the sensory data, identifying the characteristics that explained most of the variation between sensory attributes (appearance, colour, texture, juiciness & overall acceptability) and treatments (STPP concentrations). PCA was performed using XLSTAT software (Addinsoft, 2022, New York, NY, USA).

RESULTS AND DISCUSSION

pH analysis, cooking loss and water-holding capacity

Phosphate is commonly added to meat to buffer pH changes during processing. In this study, the initial pH of raw buffalo meat averaged 5.96. Fresh-cut meat typically has a near-neutral pH (approximately 6.7 for buffalo & 6.4 for beef) (Neath *et al.*, 2007); however, postmortem metabolism in muscle tissue leads to lactic acid accumulation, resulting in a decrease in pH. The incorporation of phosphate, which carries a negative charge, led to a slight increase in pH, shifting it away from the isoelectric point. After cooking with different concentrations of sodium tripolyphosphate (STPP), the pH further increased, as shown in Table 2. This observation aligns with previous research, which indicates that cooking generally raises the pH of meat (Roldán *et al.*, 2014), which aligns with the findings presented in Table 2. Additionally, Neath *et al.* (2007) reported that the postmortem pH of buffalo meat (6.7) at 40 min postmortem was significantly higher than that of beef (6.4). While the ultimate pH of beef reaches 5.4 after 24 hr, buffalo meat requires 48 hr to reach the same pH level. According to Valin *et al.* (1984), the slower postmortem pH decline in buffalo meat compared to beef is likely due to buffalo being less susceptible to stress than cattle. However, the statistical analysis conducted in this study revealed that the inclusion of STPP (phosphate concentrations) had a significant impact on the pH values of cooked buffalo meat ($p < 0.001$). This finding is consistent with the results of Roldán *et al.* (2014) in lamb loin sous-vide, where the pH was also significantly influenced by the addition of phosphate ($p = 0.018$).

Table 2. pH, cooking loss, and water holding capacity of sous-vide cooked buffalo at different STPP concentrations

STPP concentration (%)	pH	Cooking loss (%)	WHC (%)
0	6.08 \pm 0.08 ^c	21.61 \pm 0.46 ^a	80.64 \pm 0.73 ^c
0.15	6.30 \pm 0.09 ^b	19.26 \pm 0.26 ^b	83.06 \pm 0.68 ^b
0.3	6.74 \pm 0.05 ^a	17.74 \pm 0.51 ^c	86.30 \pm 0.89 ^a
P_{STPP}	$p < 0.001$	$p < 0.001$	$p < 0.001$

Mean \pm SD.

^{a-c} Means value within different letters in the same column referring to significant differences ($p < 0.05$).

P_{STPP} means the significant effect of STPP concentrations on pH, cooking loss, and WHC.

Results for cooking loss and WHC of sous-vide buffalo loins treated with different concentrations of STPP are shown in Table 2. Both cooking loss and WHC were significantly affected by the STPP ($p < 0.001$). With the higher concentration of STPP (0.3%), there was a corresponding and significant decrease in cooking loss. Specifically, at 0% STPP, the cooking loss was 21.61%, while at 0.3% STPP, it dropped to 17.74%. Similarly, the pattern seen in the WHC was consistent with the trend in the cooking loss of buffalo loins, which was significantly enhanced by the inclusion of STPP. The WHC was first measured at 80.64% with 0% STPP, and this value rose significantly to 86.30% at 0.3% STPP. The decrease in cooking loss seen when using higher concentrations of STPP can be related to the phosphate's capacity to enhance the WHC of meat proteins (Ruslan *et al.*, 2023). The addition of alkaline STPP to the meat results in an increase in pH, which in turn causes the pH to mitigate further away from the isoelectric point of the meat proteins. Consequently, the WHC is improved. The decreased protein-protein interactions that result from the increased electrostatic repulsion among meat proteins at higher pH levels create additional space for water retention (Glorieux *et al.*, 2017). Also, STPP has a role in controlling muscular contraction and protein cross-linking by forming complexes with metal ions in the muscle tissue. This mechanism enhances water retention inside muscle fibres (Morris *et al.*, 2019). The synergy between STPP concentration, pH adjustment, and protein interaction underscores the crucial function of phosphates in augmenting the quality of sous-vide buffalo loins by promoting their moisture retention and reducing cooking losses.

Colour properties

The colour properties of buffalo loins cooked using the sous-vide method are presented in Table 3. In the present study, the concentrations of STPP showed no significant effects on the L^* values ($p = 0.249$), indicating that STPP concentrations did not influence the overall lightness of the sous-vide buffalo loins. This differs from findings in other studies, where phosphate-induced pH increases had a more significant impact on meat lightness. For instance, Ayub and Ahmad (2019) and Roldán *et al.* (2014) observed that the addition of phosphates resulted in a lower L^* values, causing the meat to appear darker. In those cases, the pH changes were substantial enough to affect the protein structure in the meat, allowing deeper light penetration and a noticeable reduction in L^* values. Contrarily, the present study did not observe such a pronounced effect of STPP concentrations, indicating a more stable lightness across treatments.

The colour variations in the sous-vide buffalo loins were primarily driven by changes in the redness (a^*) values, which significantly increased with higher STPP concentrations ($p < 0.001$). The a^* value rose from 11.24 (0% STPP) to 12.71 (0.15% STPP) and continued to increase to 15.39 (0.3% STPP). Heme iron, which is bound within the myoglobin molecule, serves as the key determinant of the red colour in fresh meat. Factors that impact heme iron content, such as processing conditions and phosphate addition, clearly influence the redness values. According to Lawrie (2006), the myoglobin denaturation and

the endpoint cooking temperature critically influence the a^* values of cooked meat. The myoglobin denaturation begins at 55°C and continues until 80°C Hunt *et al.* (1999), with the present study cooking temperature (60°C) falling within this range of myoglobin denaturation. Additionally, Cheng and Ockerman (2003) observed that roast beef treated with 0.5% phosphate had significantly higher heme iron content compared to samples without phosphate, indicating that phosphate can slow down myoglobin denaturation. This finding is in tandem with the current study, where 0.3% STPP resulted in a redder appearance. Also, this observation is further supported by Ahn and Maurer (1989), who found that phosphate addition increases a^* values while reducing the oxidation potential in meat.

The b^* values showed a significant decrease with increasing STPP concentrations ($p=0.008$). The direct impact of phosphate on b^* values has been considered negligible in several studies (Önenç & Kaya, 2004; Roldán *et al.*, 2014), but the influence is more closely linked to heat-induced protein denaturation at higher temperatures. Although the role of phosphate in modifying the meat's redox state is limited, the decreases in b^* values may align with STPP's ability to stabilize myoglobin, thereby reducing the conditions that promote metmyoglobin formation.

Table 3. Colour properties (L^* , a^* , b^*), and Warner-Bratzler shear force of sous-vide cooked buffalo at different STPP concentrations

STPP concentration (%)	Colour properties			WBSF (kg)
	L^*	a^*	b^*	
0	53.01±0.17 ^a	11.24±0.16 ^c	10.29±0.12 ^a	11.56±0.06 ^a
0.15	53.36±0.90 ^a	12.71±0.49 ^b	9.69±0.22 ^a	9.57±0.53 ^b
0.3	52.30±0.82 ^a	15.39±0.18 ^a	9.06±0.47 ^b	8.05±0.42 ^c
P_{STPP}	$p = 0.249$	$p < 0.001$	$p = 0.008$	$p < 0.001$

Mean±SD.

^{a-c} Means value within different letters in the same column referring to significant differences ($p<0.05$).

P_{STPP} means a significant effect of STPP concentrations on colour properties and WBSF.

Warner-Bratzler shear force

The WBSF values show a significant decrease with increasing concentrations of STPP (Table 3). The lower WBSF values indicate a marked improvement in meat tenderness. The significant reduction in WBSF values with higher STPP concentrations ($p<0.001$) underscores the effectiveness of STPP in improving meat texture. Our result aligns with the findings by Shi *et al.* (2021), who found that phosphate improves tenderness by dissociating actin-myosin complexes and breaking down connective tissues. Furthermore, Ismail *et al.* (2019) linked water loss after cooking to the toughness of sous-vide beef, which leads to the shrinkage of muscle fibres. The addition of STPP seems to have counteracted these effects by increasing water retention. This is further corroborated by Li *et al.* (2013), who observed a strong correlation between lower shear force values and higher water retention, reinforcing the improved tenderness observed in the current study.

In this study, the initial lower cooking temperature of 45°C was specifically selected to create optimal conditions for proteolytic enzymes to degrade muscle proteins before the meat was exposed to a higher temperature of 60°C in the second stage of cooking. This approach is in line with the research of Lawrie (2006) and Myhrvold *et al.* (2011), who indicated that cooking meat at 45°C to 49°C for 4 hr can significantly improve tenderness. Within this range of temperature, proteolytic enzymes are highly active, efficiently breaking down myofibrillar and connective tissues in the muscle tissue. Furthermore, Neath *et al.* (2007) found that the slower pH decline observed in buffalo meat than beef might contribute to enhanced tenderness, as it provides a more conducive environment for protease activity. The application of a lower initial cooking temperature in this study likely promoted proteolytic activity, which combined with the use of STPP, contributed to significant improvements in buffalo tenderness. This improvement is evidenced by the lower WBSF values observed at higher STPP incorporation (0.3%) compared to those without STPP ($p<0.05$).

Scanning electron microscopy (SEM)

The microstructural changes in buffalo sous-vide treated with different concentrations of STPP are shown in Figure 1. The images, captured at 50× magnification, reveal detailed structural characteristics of the perimysium and fibrils after transverse sectioning of the sous-vide buffalo meat. In Figure 1a, representing the sample without STPP, the muscle fibres are tightly packed, and the fibrils appear densely arranged, with minimal disruption to the connective tissue structure. The arrow in Figure 1a highlights the largely intact connective tissue, which likely accounts for the greater force required to cut through the 0% STPP sample, as indicated by higher WBSF values, reflecting a tougher texture. Similarly, Chian *et al.* (2021) reported a reduction in the diameter of cooked beef muscle fibres and a noticeable increase in extracellular spaces after sous-vide cooking at 60°C. This observation aligns with previous findings that cooking induces transverse shrinkage of muscle fibres between 40°C and 60°C (Boland *et al.*, 2019), which corresponds to the cooking temperatures used in this study (45°C & 60°C).

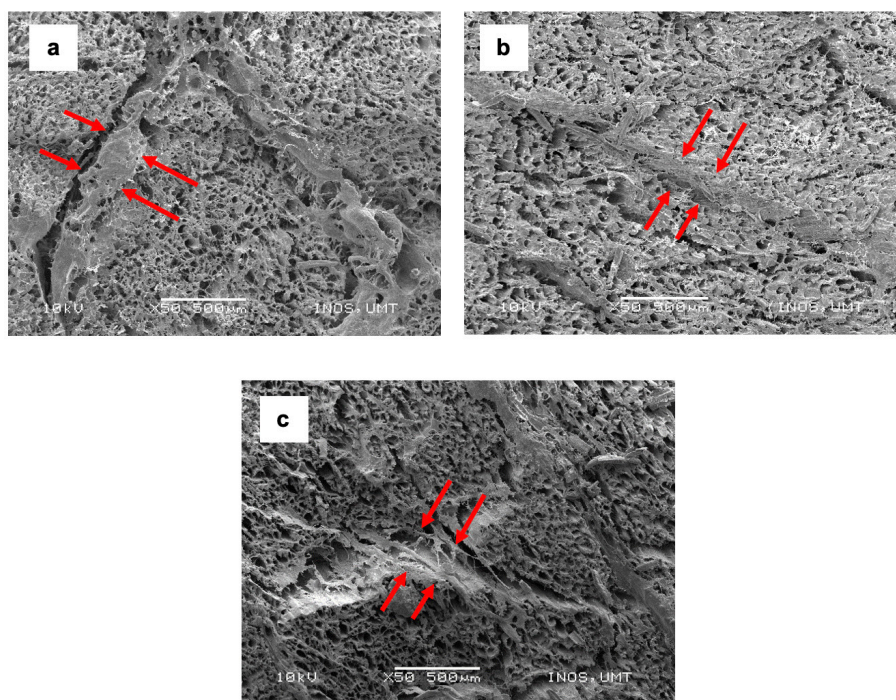


Fig. 1. Scanning electron micrographs of sous-vide buffalo: a) 0% STPP, b) 0.15% STPP, c) 0.3% STPP. Magnification: 50 \times .

Figures 1b and 1c illustrate the effects of STPP on the buffalo meat structure. The sample treated with 0.15% STPP exhibits a muscle fibre arrangement that remains similar to the untreated sample, with a dense appearance. However, the 0.15% STPP treatment induces noticeable alterations in the perimysium, characterized by partial degradation of the intramuscular connective tissue when compared to the 0% STPP sample. In contrast, the sample treated with 0.3% STPP (indicated by the arrow in Figure 1c) displays significant thinning and dissociation of the perimysium. The application of 0.3% STPP results in pronounced fibril shrinkage and increased porosity within the meat structure, particularly evident in the freeze-dried samples. The porous structure observed in Figure 1c suggests that STPP at this concentration effectively retains moisture within the muscle fibres, a process that becomes apparent following water removal through sublimation (Nowak & Jakubczyk, 2020). The increased porosity and marked thinning of the perimysium in Figure 1c indicate substantial degradation of the intramuscular connective tissue, likely contributing to the lower WBSF values observed in Table 3. The alteration in microstructure suggests that STPP induces protein degradation upon heating (Vaudagna *et al.*, 2008). This loosening of muscle fibres in the 0.3% STPP-treated samples suggests a corresponding enhancement in tenderness.

Sensory evaluation

The principal components analysis (PCA) illustrated in Figure 2 reveals that the first principal component (F1) accounts for 55.47% of the variance, while the second principal component (F2) explains 26.41% together capturing 81.88% of the total variance in the sensory evaluation data. This indicates that these two components effectively represent the majority of the variation observed. The red vectors represent the sensory attributes evaluated: colour, appearance, juiciness, texture, and overall acceptability. The direction and length of these vectors reflect the strength of the association between each attribute and the principal components, as well as their influence on the sensory evaluation outcomes. F1 is primarily associated with appearance and colour, indicating these attributes are the most influential in this component. Conversely, F2, though less prominent, still contributes to the overall variation, with a slight positive association with overall acceptability, texture, and juiciness.

The green samples (0% STPP) are more scattered across the plot, highlighting variability in sensory evaluations when no STPP treatment is applied. These samples do not exhibit a strong association with any specific sensory attributes. The red samples (0.15% STPP) cluster around the origin and in the lower right quadrant, suggesting a less pronounced influence on colour and appearance, as indicated by their proximity to the centre of the plot. These samples also show weaker associations with texture, juiciness, and overall acceptability. In contrast, the blue samples (0.3%) are more closely associated with overall acceptability, texture, juiciness, and appearance, with mean values of 8.79, 8.17, 7.94, and 8.79, respectively. These results indicate that a higher concentration of STPP positively impacts these attributes. The PCA plot suggests that samples treated with 0.3% STPP exhibit higher sensory quality, while the 0% and 0.15% STPP samples show greater variability in their sensory attributes and less pronounced effects on the sensory qualities of sous-vide buffalo meat. As discussed above, STPP is employed to enhance moisture retention, tenderness, and colour properties in cooked buffalo meat. Insufficient STPP (as in the 0% or 0.15% treatments) may result in meat that lacks structural and moisture-retaining benefits, leading to lower associations with desirable sensory attributes.

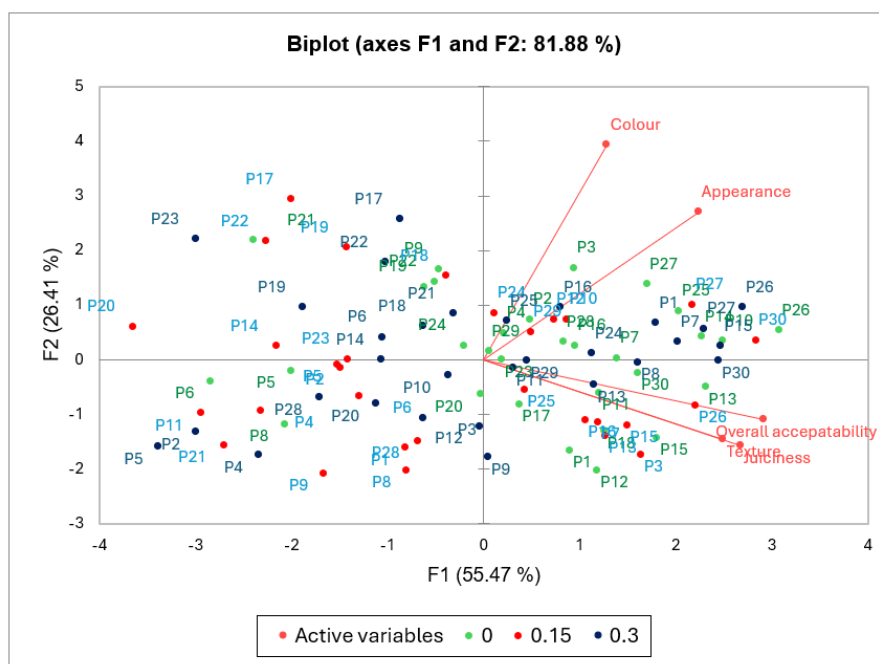


Fig. 2. Principal component analysis (PCA) of the sensory evaluation of sous-vide buffalo loins treated with 0%, 0.15%, and 0.3% STPP.

CONCLUSION

The combination of sous-vide cooking and STPP addition significantly improves the quality of frozen buffalo loins by enhancing water-holding capacity, reducing cooking loss, preserving colour properties, and increasing tenderness. The microstructural analysis confirmed that STPP led to a looser fibre structure and thinning of the connective tissue, resulting in better texture. The sensory evaluation further supported these results, showing higher scores in overall acceptability, texture, juiciness, and appearance for the 0.3% STPP-treated samples. Despite buffalo meat's naturally tougher texture, these findings suggest that STPP can effectively enhance its eating quality.

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ETHICAL STATEMENT

The study protocol for the sensory evaluation was approved by the UniSA Human Research Ethics Committee (UHREC) under reference number UniSA/UHREC/2024/640.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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