



## QUALITY ATTRIBUTES OF BUFFALO MEAT USING PRECISION SOUS VIDE COOKING DEVICE

Chirasak Phoemchalard<sup>1</sup>, Tanom Tathong<sup>2</sup>, Pitukpol Pornanek<sup>3</sup>, Suthipong Uriyapongson<sup>4</sup> and Anusorn Cherdthong<sup>4</sup>

<sup>1</sup>Department of Agriculture, Mahidol University, Amnatcharoen Campus, Amnatcharoen, Thailand

<sup>2</sup>Department of Food Technology, Faculty of Agriculture and Technology, Nakhon Phanom University, Nakhon Phanom, Thailand

<sup>3</sup>Department of Animal Science, Faculty of Natural Resources, Rajamangala University of Technology Isan, Sakon Nakhon Campus, Sakon Nakhon, Thailand

<sup>4</sup>Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand

E-Mail: [chirasak.pho@mahidol.edu](mailto:chirasak.pho@mahidol.edu)

### ABSTRACT

This study compared the effects of single stage sous vide (55 or 59°C for 12 h), double stage sous vide (55 or 59°C for 6 h, then 70°C for 6 h), and conventional cooking (75°C for 30 min) on the physicochemical, textural properties, microbial, and odor of loin steak from swamp buffalo. Local markets provided a total of 25 buffalo beef samples. They were shipped, chilled, trimmed, cut, weighed, and pH and color checked. They were then vacuumed, cooked, chilled, and evaluated for quality, texture, safety, and odor. The findings showed that meat from TC75 looked lighter and yellower ( $P < 0.05$ ). Cooking with the SV55 treatment reduced cooking loss (CL), transversal shrinkage (TS), Warner-Bratzler shear force (WBSF), toughness, and almost all texture profiles ( $P < 0.05$ ). Multi-step sous vide treated samples had higher CL and longitudinal shrinkage (LS) ( $P < 0.05$ ), except for total color difference ( $\Delta E^*$ ). Meat from TC75 was tougher meat and stronger smell. All cooking procedures were justified as producing meat safe to eat. PCA may be used to determine the quality of beef prepared in various methods. We concluded that the buffalo meat in sous vide cooked at 55°C for 12 h had the potential desirable outcome of upholding moisture level and physical form, producing tender meat, lower carbon footprint, lesser odor of steaks than other treatments.

**Keywords:** buffalo, meat quality, sous vide, carbon footprint, electronic nose.

### INTRODUCTION

Market challenge for ready-to-eat food increases throughout the previous decades owing to changes in lifestyle habits, economic growth, and technologies [1]. Sous vide (SV), from the French, “under vacuum”, meaning cooking at low temperature for a long-time, is one alternative method for generating foods that are ready to eat to satisfy market demand. Traditional cooking is well known for using high-temperature cooking with an associated loss of nutrients, flavor, and color [2]. The key difference in the SV cooking technique is that it requires specific temperature regulation to produce reliable quality food results. Nowadays, SV has become more popular with home cooks due to a range of perceived attributes: simplicity, speed, safety, health and nutrient conservation, taste, waste reduction, flexibility, extended shelf life, and avoidance of over or undercooking [3]. Currently, SV is used throughout the industry; for meat, it typically uses a low temperature, between 50-80°C, for longer periods based on types of meat [4]. Potentially tough meat cuts become more tender with relatively long cooking times due to collagen melting into gelatin and lessening intercross connections between myofibers [5]. Cooking at low temperatures can also keep meat juicier and provide better flavor and tenderness [6]. The SV cooking conditions for meat mentioned by chefs are 58-63°C for 10-48 h, then cook at 75-80°C while serving meals [7, 8]. The maximum tenderness of tough beef cuts exceeded when cooked between 55-60°C for many hours or days [9]. However, differences in species, age, and connective tissue quantity and type require different cooking

treatments to be employed. It is generally agreed that the most common method of SV cooking meat is to use a single temperature and time combination. Yet, it is unlikely that more advanced SV cooking methods have been adapted for buffalo meat. A study conducted into the double cooking process found it beneficial for reducing the toughness of beef *supraspinatus* and *rectus femoris* [10] as well as beef and goat *semitendinosus* muscles [11]. Food odor is made up of a variety of chemical compounds that give food its distinct flavor and traits. In food production, precise estimation, optimal flavor classification, and accuracy of taste properties are essential. Electronic nose (e-nose) is now a fast and efficient system that that requires no specific sample planning and enables assessment and discrimination of a product's total volatile profile [10]. This system is made up of a variety of sensors that can simulate the scent. As a result, it is a favorable approach for food safety control and authentication. E-nose is a popular device for detecting and classifying odor and its strength by impersonating the human nose. It has already been stated that e-nose may be used to detect beef quality [11, 12], ensure authenticity [13, 14], meat freshness [15], or shelf-life [16-19].

Most papers investigating SV cooking have concentrated mainly on physicochemical, quality, and safety characteristics, but no carbon footprint and smell were reported. For this purpose, the study aimed to assess physicochemical, textural, microbial traits, carbon footprint, and smell of single- and double-step sous vide compared to the traditional cooking of buffalo beef.



## MATERIALS AND METHODS

### Meat Samples and Treatments

Five fresh loin cuts (*M. longissimus dorsi*) from culled female swamp buffalo cows (> 5-year-old) were obtained at 12 h post-mortem from a local butcher at Nakhon Phanom Province, Thailand. Meat samples were transported to Meat Laboratory and chilled. After 24 h post-mortem, the connective tissue and fat had been removed, and  $87 \pm 10$  g of 2.54 cm thick steak was cut and measured for pH (5.27-5.49) and color ( $L^*$ , 34.10-41.52;  $a^*$ , 16.79-22.47;  $b^*$ , 6.22-16.55;  $C^*$ , 17.89-24.80; and  $h^*$ , 20.26-25.15). The steaks were placed into  $15 \times 22$  cm food-grade pouches of 160  $\mu$ m of Linear Low-Density Polyethylene (LLDPE), and were packed under vacuum (DZ-500, Sammi Packing Machine Ltd., China). Twenty-five steaks were randomly assigned into one of five treatments consisting of 1) SV cooking with a single combination of temperature-time that was treated at 55 or 59°C for 12 h (SV55 and SV59), 2) SV cooking with a double combination of temperature-time that was first treated at 55 or 59°C for 6 h, then treated at 70°C for 6 h (MV55-70 and MV59-70), and 3) traditional cooking that was treated at 75°C for 30 min (TC75), respectively. All SV cooking was achieved in an 800 W circulating immersion cooker (SVJ-1000, Sous Vide Precision Cooker, China), holding the temperature and time as described above. The samples were kept at room temperature for a while and then immediately chilled for 24 h in the refrigerator at 4°C. Packs were disclosed, and the samples were removed, blotted, and evaluated for the changes of color and texture.

### pH and Instrumental Color Measurement

Meat pH value was measured using a pH meter supplied with an FC232D pH probe (HI99163, Hanna Instruments, USA). After cooking, meat samples were cooled in the refrigerator at 4 °C overnight, opened, surface-dried, and reweighed. Measurement of the surface color was completed in quintuplicates and expressed as average values using CR-400 Chroma Meter (Konica Minolta Sensing Inc., Japan) standardized with the specification white plate. The color values were measured in terms of lightness ( $L^*$ ), redness ( $a^*$ ), yellowness ( $b^*$ ), chroma ( $C^*$ ), and hue ( $h^*$ ); while, total color differences ( $\Delta E^*$ ) was calculated according to equation 1 [20].

$$\text{Total color differences } (\Delta E^*) = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

### Muscle Shrinkage and Cooking Loss

Longitudinal shrinkage (LS) of meat samples were determined from sample length before ( $L_1$ ) and after ( $L_2$ ) cooking with the relationship as equation 2. Transversal shrinkage (TS) of steak samples were determined from sample width before ( $C_1$ ) and after ( $C_2$ ) cooking with the relationship as equation 3 [21]. Based on variations in raw weight ( $W_1$ ) and cooked weight ( $W_2$ ), cooking loss (CL) was calculated using equation 4.

$$\text{Longitudinal shrinkage (LS)} = ((L_1 - L_2)/L_1) \times 100 \quad (1)$$

$$\text{Transversal shrinkage (TS)} = ((C_1 - C_2)/C_1) \times 100 \quad (2)$$

$$\text{Cooking loss (\%)} = ((W_1 - W_2)/W_1) \times 100 \quad (3)$$

### Warner-Bratzler Shear Force (WBSF)

The WBSF analysis was conducted following AMSA guidelines [22]. Six circular core parallel to the longitudinal muscle fiber was removed from each steak in diameter of 1.27 cm, and perpendicular sheared to longitudinal positioning of muscle fibers using a 'V' slot blade of TA.XT *plus* Texture Analyzer (Stable Micro System Ltd., Surrey, UK). Shear force (kg) and work of shear (kg.s) were recorded with a 240 mm/min test speed with a 50 kg load cell.

### Instrumental Texture Profile Analysis (TPA)

The Texture Analyzer was also used to conduct TPA experiments. Conditions for the testing were: P/50 cylindrical aluminum probe, test speed 1 mm/s, pre-test speed 1 mm/s, post-test speed 1 mm/s, compression (strain) 75%, time 5.0 s, trigger type auto, trigger force 5 g, and 50 kg load cell. Exponent version 6.1.16.0 was used to collect and calculate data.

### Microbiological Analysis

Total plate count (TPC) and *E. coli* and *coliform* (EC) bacteria were calculated by aseptically weighing 11 g pieces of meat, blending, and diluting in clean and sterile peptone according to Food Test Kit guidelines (B Smart Science Co. Ltd., Thailand). 1 mL of the solution was applied to the Compact Dry™ TC or EC's surface layer (Aerobic, Nissui Pharmaceutical Co. Ltd., Japan). A sterile bag was used to put the dishes, stayed for 24 h at 35°C. The results of the TPC and EC test are expressed in colony-forming units ( $\times 10^6$  CFU/g and CFU/g)

### Carbon Footprint Calculation

The carbon footprint of buffalo beef, plastic bags, and water was calculated. Before cooking, an AC Digital Wattmeter was connected to the SV machine to monitor electricity consumption to calculate the carbon footprint, as shown in equation 5.

$$\text{Carbon footprint (kg CO}_2\text{-e)} = [(\text{buffalo beef (kg)} \times 33.7 \text{ kg CO}_2\text{-e)} + (\text{electricity (kWh)} \times 0.5986 \text{ kg CO}_2\text{-e)} + (\text{plastic bag (kg)} \times 1.994 \text{ kg CO}_2\text{-e)} + (\text{tap water (L)} \times 0.2843 \text{ kg CO}_2\text{-e)} + (\text{transportation (km)} \times 0.3131 \text{ kg CO}_2\text{-e})] \quad (4)$$

where the emissions associated with buffalo meat was calculated using data obtained from Gerber *et al.* [23]; while, plastic bags, water, electricity and transportation were obtained from Thai National LCI Database, TIISMTEC-NSTDA (with TGO electricity 2016-2018) [24].



### Electronic Nose Analysis

Meat samples weighing around 15 g were prepared and measured for response sensing by the portable e-nose for food and beverage (Electronic Nose Co., Ltd., Thailand), where eight different gas sensors (Table-1) were incorporated into a chamber. The aromas analysis was performed in five cycles: reference time: 120 s, sample time: 30 s, and wash time: 60 s, using 1000 ml/min flow rate. The result of sensing response percentages is measured using CIMS NOSE 2.0 software.

**Table-1.** The lists of sensors used in the experimental study.

No.	Sensor models	Objective measurement
1	TGS 816	Butane, methane, propane
2	TGS 2600	Smoke, alcohol
3	TGS 823	Organic solvent vapors
4	TGS 2603	Methyl mercaptan, trimethylamine
5	TGS 826	Isobutane, ethanol, ammonia
6	TGS 2610	Propane, isobutane, methane
7	TGS 2620	Alcohol, organic solvent
8	TGS 2444	Ammonia

### Statistical Analysis

All statistical analyses were conducted using JASP v. 0.12.2 software (Jast Team, 2020). One-way ANOVA analyzed data obtained for pH, color, shrinkage, cooking loss, shear force, work of shear, texture profile

analysis, and odor. Significantly different results were subjected to post-hoc Tukey HSD tests for multiple mean comparisons. Means are considerably different if the  $P < 0.05$ . The result means and standard deviation are displayed. For carbon footprint, they were subjected to one sample t-test and setting the test values to 1.65. A total of 27 variables of meat quality were upload, processing, normalization, statistical analysis (correlation and PCA), and summary report using the web interface of MetaboAnalyst 5.0 (<https://www.metaboanalyst.ca>).

### RESULTS AND DISCUSSIONS

The pH, color, and differences are shown in Table-2. The samples in TC75 treatment provided lower pH than other SV treatments ( $P < 0.05$ ). Cooking treatments had affected almost all color values. Samples in TC75 generated higher  $L^*$ ,  $b^*$ , and  $C^*$  values, while  $\Delta E^*$  values were also higher and like SV treatment ( $P < 0.05$ ). An increase in the  $L^*$  value when increasing cooking temperatures had the same effect as shown by a previous study [26]. This result could be explained by myosin molecules denaturing induced noticeable changes in the opacity of the meat color [27].

In this study, cooking with SV decreased the  $a^*$  value by 41 % relative to fresh meat; however, there was similar among SV treatments ( $P > 0.05$ ). This effect was against the findings of other research [28, 29] which noted that higher temperature affected  $a^*$  value. Higher  $\Delta E^*$  means higher meat discoloration by oxidation of heme. Both double heat treatments showed significantly lower  $\Delta E^*$  than those of samples in single heat treatments and traditional cooking (9.68 vs 12.54 and 12.35).

**Table-2.** pH, color, and total color differences of sous vide cooked buffalo meat.

Items	Cooking treatments				
	SV55	SV59	MV55-70	MV59-70	TC75
pH	5.78±0.02 <sup>b</sup>	5.80±0.01 <sup>b</sup>	5.92±0.05 <sup>a</sup>	5.80±0.06 <sup>b</sup>	5.55±0.08 <sup>c</sup>
$L^*$	44.14±0.82 <sup>b</sup>	41.42±2.21 <sup>c</sup>	39.97±0.97 <sup>cd</sup>	38.09±0.61 <sup>d</sup>	46.89±0.71 <sup>a</sup>
$a^*$	11.21±1.35	11.02±0.71	12.19±0.05	12.32±0.10	12.14±0.31
$b^*$	15.14±0.55 <sup>a</sup>	14.87±0.42 <sup>a</sup>	13.80±0.24 <sup>b</sup>	13.40±0.28 <sup>b</sup>	15.32±0.84 <sup>a</sup>
$C^*$	18.89±0.45 <sup>ab</sup>	18.51±0.76 <sup>ab</sup>	18.42±0.21 <sup>b</sup>	18.20±0.27 <sup>b</sup>	19.55±0.84 <sup>a</sup>
$h^*$	53.55±4.16 <sup>a</sup>	53.50±0.98 <sup>a</sup>	48.54±0.38 <sup>bc</sup>	47.39±0.39 <sup>c</sup>	51.55±0.96 <sup>ab</sup>
$\Delta E^*$	13.11±1.26 <sup>a</sup>	12.21±0.29 <sup>a</sup>	9.91±0.65 <sup>b</sup>	9.42±0.29 <sup>b</sup>	12.43±0.65 <sup>a</sup>

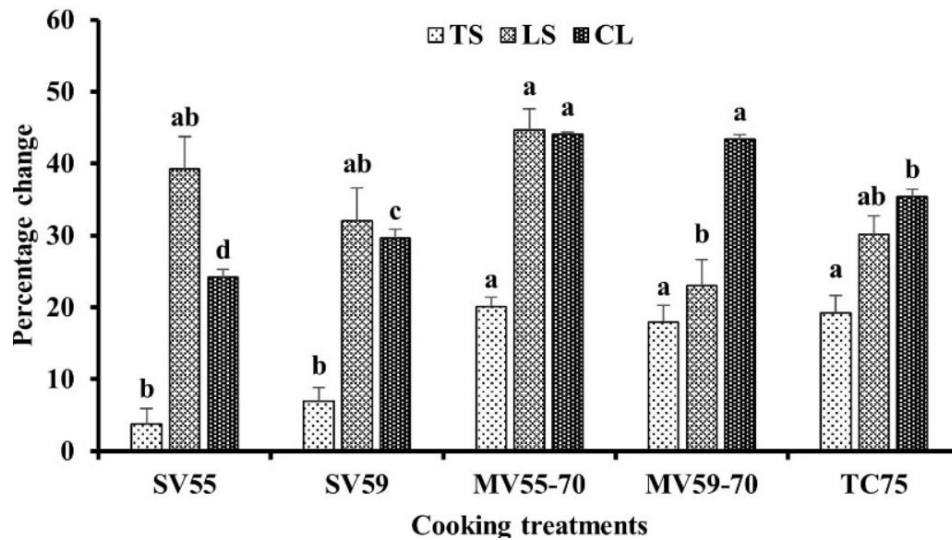
<sup>a-c</sup> Within a row, means with different superscript letters are significantly different ( $P < 0.05$ ).

The effects of cooking treatments on muscle shrinkage and cooking loss of buffalo meat are shown in Figure-1. The slightly higher TS ( $P < 0.05$ ) in samples cooked using double heats and TC75 were especially apparent, while only MV55-70-treated samples showed the highest LS than the other treatments ( $P < 0.05$ ). Also, CL produced higher values in the double heat treatments ( $P < 0.05$ ). The TS values of SV55 and SV59 were lower than the previous results [21]; however, the values in

MV55-70, MV59-70, TC75 samples, and the LS values of all treatments were greater than that report. This could be because the sample of steaks used in the current study varied from those whole muscles. Pearson's correlation coefficient (Figure-3) showed that CL was highly correlated with TS ( $r = 0.848$ ,  $P < 0.001$ ). This opposite to the findings of those who noted that the higher LS was associated with the highest amounts of CL [21]. Higher temperatures and longer cooking time increased CL in this



study in agreement with results from goat meat [28] and beef [7, 30].

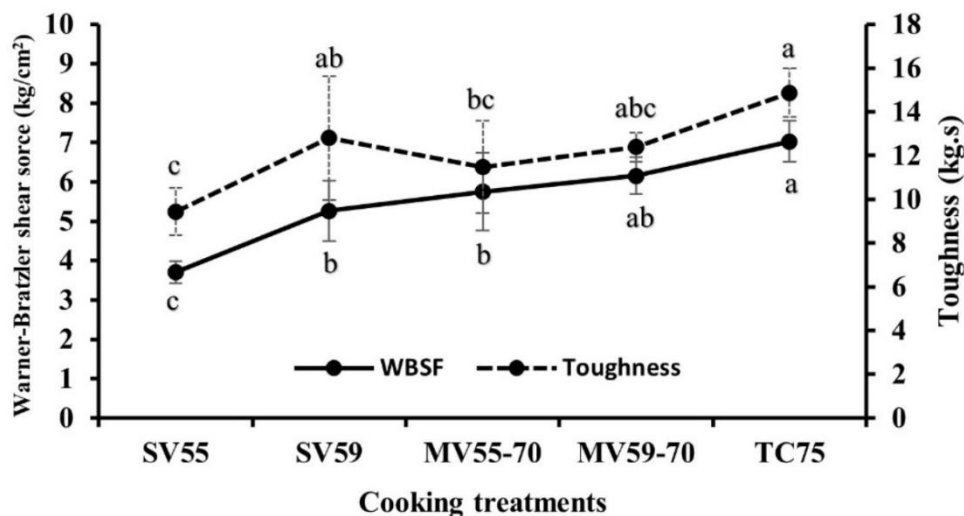


**Figure-1.** Means of percentage change of transversal shrinkage (TS), longitudinal shrinkage (LS), and cooking loss (CL) of sous vide cooked buffalo meat.

<sup>a-c</sup> Within the bar, different superscript letters are significantly different ( $P < 0.05$ ).

As shown in Figure-2, shear force values of sous vide cooked buffalo meat were lower than with TC75 ( $P < 0.05$ ). In addition to reducing the CL, SV55 also produced tender meat with significantly lower WBSF values ( $r = 0.60$ ,  $p < 0.01$ ). Relative to TC75, WBSF decreased by 47.53, 26.94, 16.93, and 14.25% when meat samples were treated with SV55, SV59, MV55-70, MV59-70, respectively. Similarly, cooking the beef at 55 or 60°C for 24 h considerably reduced SSF by 26-72% [31]. Also,

SV cooked beef at 55-70°C reduced WBSF by 6.24-26.54% and extended cooking treatment times decreased WBSF by 4.31-23.43% compared to traditional cooking [30]. According to Christensen *et al.* [26], the toughness of meat was found to reduce at temperatures between 50-60°C due to changes in the perimysium, while if the temperatures reached 60°C, the breaking force of single muscle fibers increased.



**Figure-2.** Shear force and toughness of sous vide cooked buffalo meat.

<sup>a-c</sup> Within a line, different superscript letters are significantly different ( $P < 0.05$ ).

Texture profile analyses of sous vide cooked buffalo meat are presented in Table-3. All variables were affected by different cooking treatments. Hardness, springiness, cohesiveness, gumminess, chewiness, and resilience of TC75 were significantly higher than with

other treatments ( $P < 0.05$ ). As illustrated in Figure-3, correlations were found between CL and hardness ( $r = 0.71$ ,  $P < 0.01$ ), gumminess ( $r = 0.66$ ,  $P < 0.01$ ), and between TS and both hardness ( $r = 0.80$ ,  $P < 0.001$ ) and gumminess ( $r = 0.76$ ,  $P < 0.001$ ). Changes in hardness may be influenced





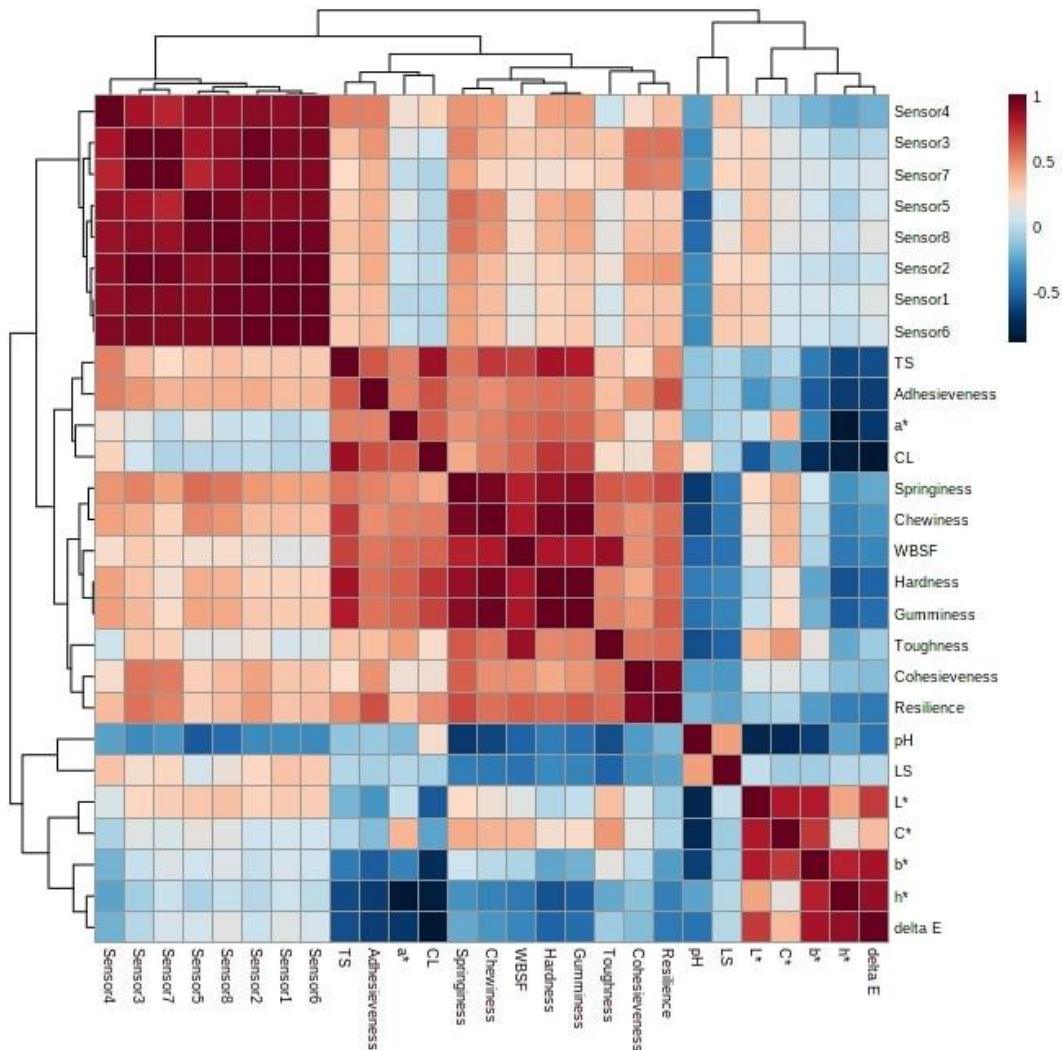
by the physical looseness of the perimysium in the endomysium-perimysium space [33]. The lower hardness

values associated with SV55 cooking may be explained by the higher degree of water holding capacity.

**Table-3.** Texture profile analysis (TPA) of sous vide cooked buffalo meat.

Items	Cooking treatments				
	SV55	SV59	MV55-70	MV55-70	TC75
Hardness (g)	142.52±48.78 <sup>c</sup>	308.05±63.02 <sup>c</sup>	720.72±18.89 <sup>b</sup>	1,007.17±163.78 <sup>a</sup>	1,106.34±183.94 <sup>a</sup>
Adhesiveness	-21.23±11.83 <sup>b</sup>	-9.25±7.68 <sup>ab</sup>	-5.75±0.43 <sup>a</sup>	-4.56±0.91 <sup>a</sup>	-5.69±0.86 <sup>a</sup>
Springiness	0.07±0.01 <sup>d</sup>	0.08±0.00 <sup>c</sup>	0.08±0.00 <sup>c</sup>	0.10±0.01 <sup>b</sup>	0.12±0.01 <sup>a</sup>
Cohesiveness	0.57±0.02 <sup>c</sup>	0.67±0.02 <sup>a</sup>	0.62±0.05 <sup>bc</sup>	0.63±0.01 <sup>ab</sup>	0.67±0.02 <sup>a</sup>
Gumminess	82.15±27.13 <sup>c</sup>	209.87±43.67 <sup>c</sup>	443.85±40.39 <sup>b</sup>	646.74±111.58 <sup>a</sup>	750.69±115.85 <sup>a</sup>
Chewiness	6.67±2.58 <sup>d</sup>	20.16±5.23 <sup>cd</sup>	38.43±4.78 <sup>c</sup>	69.18±14.57 <sup>b</sup>	96.19±20.85 <sup>a</sup>
Resilience	0.19±0.01 <sup>b</sup>	0.25±0.00 <sup>a</sup>	0.24±0.02 <sup>a</sup>	0.25±0.00 <sup>a</sup>	0.26±0.02 <sup>a</sup>

<sup>a-c</sup> Within a row, different superscript letters are significantly different (P<0.05).



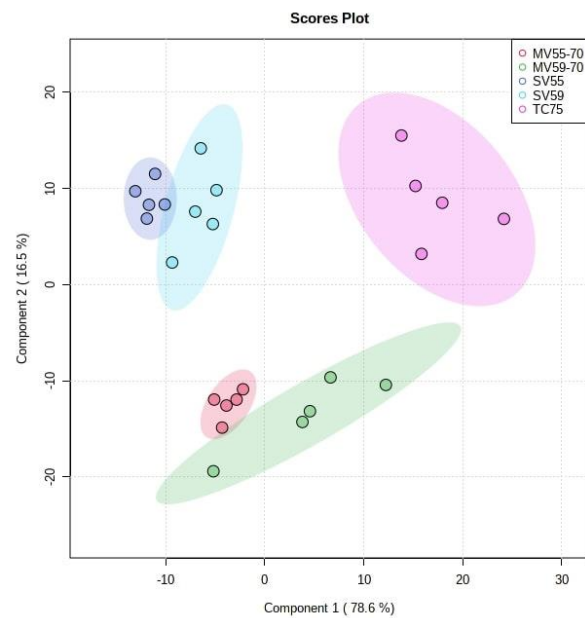
**Figure-3.** Correlation heatmaps of sous vide cooked buffalo meat.

The principal component analysis (PCA) is among the best efficient and straightforward feature extraction methods. It can use 2D or 3D maps to explain

the pattern of similarities between the observations and variables. In Figure-4, the 2D PCA analysis clearly showed that the first two PCs explain nearly 95.1% of the



total variation. The results of PCA show that classical cooking (upper right) is being considered separately from single step vacuum and multi-step vacuum cooking (lower). As a result, various classes of beef quality can be effectively categorized based on different cooking temperatures and times.



**Figure-4.** The explained variances are displayed in brackets with the scores plotted between the selected PCs.

Microbial analysis of sous vide cooked buffalo meat is shown in Table-4. Results revealed that total plate count of bacteria and *E. coli* as colony-forming units per gram in raw meat were positive. This means hygiene at local slaughterhouses needs to be improved and developed to meet the safety standards for meat production in accordance with the Department of Livestock Development policy. However, all the cooking methods adequately destroyed the high microbial load because the temperature and time used in this study were higher than 54.4°C and 2.5 h [3].

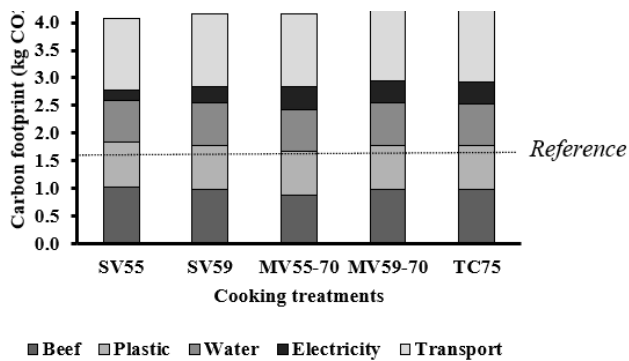
**Table-4.** Microbial analysis of sous vide cooked buffalo meat.

Items	Standard	Raw	Cooking treatments				
			SV55	SV59	MV55-70	MV59-70	TC75
Total plate count ( $\times 10^6$ CFU/g)	$\leq 9$	11.00 $\pm$ 5.72	ND	ND	ND	ND	ND
<i>Escherichia coli</i> (CFU/g)	$\leq 100$	195.67 $\pm$ 136.39	ND	ND	ND	ND	ND
<i>Coliform</i> (CFU/g)	$\leq 1000$	140.33 $\pm$ 121.35	ND	ND	ND	ND	ND

ND: not detected

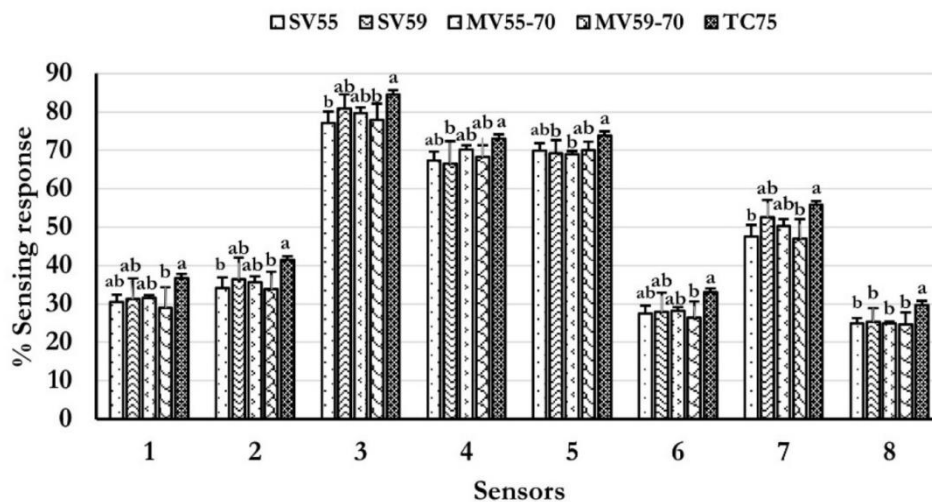
The carbon footprints of dairy, beef, lamb, and aquatic animal production vary considerably more than those of other foods due to the intensity and practices used in animal raising and fish farming worldwide varying greatly [34]. This means emissions output of buffalo meat production in East and Southeast Asia (70.2) is relatively higher than other regions of the World (21-53 kg CO<sub>2</sub>-eq/kg CW), due to lower productivity, lower reproductive efficiency, lower feed quality, lower dressing percentages (DP), longer raising time, etc. [23]. The DP of buffalo in Thailand is approximately 48% [35]. So, 1 kg of buffalo

meat produces 33.70 kg CO<sub>2</sub>-eq/kg meat. This footprint is greater than the footprint of finishing beef for 6, 6 - 12, or more than 12 months, and beef cut presenting 6.28, 7.16, 8.35, and 9.91 kg CO<sub>2</sub>-eq, respectively [24]. As shown in Figure-5, carbon footprints of the present study ranked 4.08-4.25 kg CO<sub>2</sub>-eq/kg; 31.24% of the values were generated from transport followed by meat (23.30%), bags (19.10%), water (18.16%), and cooking methods (8.20%), respectively. However, there were substantially higher in all treatments as compared to a study in beef (1.65 kg CO<sub>2</sub>-eq/kg) using the same procedure [30].



**Figure-5.** Carbon footprint of sous vide cooked buffalo meat.

The average values of the sensing response percentage from the eight sensors used in this test are



**Figure-6.** Sensing response of sous vide cooked buffalo meat.

<sup>a-b</sup>Within a bar, different superscript letters are significantly different ( $P < 0.05$ ).

## CONCLUSIONS

It is concluded that single-step sous vide cooking particularly SV55 could maintain pH and moisture content, reduce transversal shrinkage changes, lower shear force, hardness, odor, and carbon footprint of steaks better than those of double-step sous vide and traditional cooking. All cooking treatments had a lower risk of microbial contamination. Moreover, the quality of beef with different cooking methods can be classified by the PCA. Our results should be the basis for further confirmatory study to monitor carbon emissions and aroma changes throughout the cookery process, not just in terms of meat quality and safety.

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shown in Figure-6. Each sensor has a specific output response for cooked meat samples because different sensors react to various gases. For TC75 samples, all sensors showed a higher value ( $P < 0.05$ ). Sensing responses are stronger on TGS823 than on other sensors. TGS2603, TGS2620, TGS2600, TGS816, TGS2610, and TGS 72168 sensors are followed. Cooked beef can produce more volatile organic compounds (VOCs), such as organic solvent vapors, isobutane, ethanol, ammonia, methyl mercaptan, trimethylamine, alcohol, organic solvent due to the greater responsibility of the TGS823, TGS2603, TGS2600, and TGS2600 sensors. For fresh beef, sensors such as TGS2610, TGS2600, TGS2611, TGS2620, and TGS2602 are most suitable for monitoring their quality change during storage [15].

## CONFLICT OF INTEREST

We declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

C. Phoemchalard: conceptualization, data curation, formal analysis, investigation, methodology, writing - original draft, writing - review & editing. T. Tathong: recourses, supervision, validation. P. Pornanek: validation, writing - review & editing. S. Uriyapongson and A. Cherdthong: supervision, writing - review & editing.

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