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Effect of High-Pressure Processing on the Quality of Beef and Buffalo Meat

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ABSTRACT

The present study evaluated the physicochemical and textural attributes of fresh beef and buffalo meats with applied high-pressure processing. Both types of meat were pressurised at 300 and 600 MPa at 20 for 2 min and compared to the untreated samples as the control. The pH values, water holding capacity (WHC), moisture content, cooking loss, yield, colour, Warner-Bratzler shear force (WBSF), and texture profile analysis were examined. The increase in pressure showed a significant ($p < 0.05$) increase in pH and moisture content for both types of meat. The lightness (L^*) values of beef and buffalo were increased with an increase in pressure applied. Meanwhile, the redness (a^*) and yellowness (b^*) values of both types of meat decreased when higher pressure was applied. The hardness and chewiness of treated beef samples increased compared to the control, while no significant differences were observed for buffalo. WBSF of cooked meat were reduced with an increase in the pressure applied for both meat types. In conclusion, the meat applied with 300 MPa pressure showed more effects on tenderisation with minimal changes in the physical appearance.

Keywords: High-pressure processing, meat processing, physical appearance, tenderisation, water holding capacity

INTRODUCTION

High-pressure processing (HPP) is one of the novel, alternative non-thermal processing methods that expanding with great success (Bolumar et al. 2021). In the past decades, the application of HPP to the food system has become widely known in the meat industry (Bajovic et al. 2012). Approximately 25-30% of the total HPP units installed in the food industry are represented by the meat industry (Tonello 2010). One of the main reasons for this phenomenal impact is because of the high commercial value of meat and meat products causing the investment in HPP units to be relevant and considerable. Based on several studies, pressurisation treatment takes place between 100 to 1000 MPa at

temperatures between 0 to 120°C (Hayashi 2002; Tonello 2018). As for meat products, they are commonly processed between 400 to 600 MPa with a short processing time of 3 min at chilled temperature to ensure vegetative pathogenic and spoilage microorganisms are decreased by more than 4 log reductions (Bolumar et al. 2021).

Despite the initial objective to reduce the microbial load in meat, HPP also affects the meat texture, which could reduce the meat's toughness (Mohd Azmi et al. 2023). Bolumar et al. (2021) noted that the most effective HPP conditions for pre-rigour muscle were around 200 MPa at 30 to 35°C for about 4 min. However, the conditions depend on the species and animal types. For example, it was reported that beef and lamb were effectively treated below 200 MPa and above 200 MPa for pork. The optimisation of pressure for tenderisation could

be related to the impacts on colour changes, pressure above 200 MPa caused myoglobin denaturation (Ma and Ledward 2004; Bak et al. 2017). Morton et al. (2017) reported that beef steaks treated at 175 MPa had a lower shear force value by 60%, thus showing an improvement in meat tenderness. Meanwhile, Souza et al. (2011) reported a decrease in shear force value for pork chops by 30% after being treated with pressure 225 MPa at 10 to 35°C for 180 s.

In contrast, some researchers stated the opposite trend of increasing shear force value with increasing pressurisation (McArdle et al. 2011; Sikes and Warner 2016). These authors found that a combination of high pressure and high temperature (>25°C) is required to ensure post-rigour meat tenderisation. In addition, some reports showed an increase in meat toughness after high pressure was applied at lower temperatures for several meat species (Duranton et al. 2012; Grossi et al. 2014). Studies reported that the optimum conditions to apply to the post-rigour muscle were at 100 to 200 MPa with a temperature of 60 to 80°C (Ma and Ledward 2004; McArdle et al. 2013). Additionally, Sikes and Tume (2010) reported that if higher pressure (>200 MPa) at 60°C was applied to post-rigour beef muscle, it toughened the meat. Moreover, since the colour of fresh meats is dependent on the structural proteins including myosin and water-soluble proteins which are high-pressure sensitive, hence the meat treated with high-pressure appeared to look cooked in the eyes of consumers.

Several conditions need to be considered during the HPP treatment, such as temperature, pressure level, and processing time, because these factors can affect the labile protein nature, especially, in raw meats (Bolumar et al. 2021). Depending on the settings, HPP will have various impacts on meat appearance and other quality attributes, which has so far limited a much larger deployment of HPP technology in the meat industry. In addition to that, different types of meat may have different effects when treated with HPP. Therefore, the present study aims to evaluate the effect of different levels of pressure during high-pressure processing on the physicochemical and textural properties of different types of fresh red meats.

MATERIALS AND METHOD

Materials and sample preparation

Topside meat cuts of beef and carabeef (buffalo meat) of identical age groups of 7-8 years were obtained from a local supplier, Wira Food Trading Sdn, Bhd, Selangor. The meat was cut into 2.54 cm x 2.54 cm x 1.5 cm cubes, and the weight, pH value, and colour were measured and recorded. All samples were vacuum-sealed in polyethylene bags and stored at -18°C. The samples were thawed at 4°C for the treatments. Samples were divided into a control group (no

pressure treatment) and a pressure treatment group (300 and 600 MPa) with triplicates in each treatment.

Pressure treatment

The meat pieces were subjected to two different pressure levels, 300 and 600 MPa, for 2 min (Sun et al. 2019). The pressurisation in both conditions was carried out in high-pressure processing equipment (Hiperbaric 55, Hiperbaric USA, Miami, FL; 55 L vessel; 200 mm diameter inside the vessel; throughput of 270 kg/h) with chilled water (12-16°C) as the pressurising medium. The HPP unit is located at the Food 8, Faculty of Food Science and Technology, UPM, Malaysia. The samples were kept within a cylindrical basket before being subjected to a high level of isostatic pressure transmitted by water. The sample holder was then inserted inside the stainless-steel pressure vessel which was hermetically closed. The control sample (unpressurised) was chilled at 4°C for 24 h before being kept at -18°C together with the pressurised samples. All samples (control and pressurised) were thawed at 4°C for 24 h the day before the analyses.

Product yield and moisture content

The product yield was calculated by noting weight differences before (W_1) and after (W_2) high-pressure treatment.

$$\text{Product yield (\%)} = W_2/W_1 \times 100$$

Moisture content was measured in triplicate using a moisture analyser (Model MX-50, A&D Company Ltd., Tokyo, Japan).

pH measurement

The pH values of meat samples were measured by homogenizing approx. 1 g of meat samples with 10 mL of distilled water using a homogeniser (Model T 18 D S2, IKA Inc., China). The pH measurement was carried out with a pH meter (Model Jenway 3505, Bibby Scientific Ltd., UK) (Asyul-Izhar et al. 2023).

Colour measurement

Colour measurements were taken with a Chroma meter (Model CR-410, Konica Minolta, Inc., Japan) with a standard white plate ($X=97.83$, $Y=81.58$, $Z=91.51$). The L^* , a^* and b^* -values were determined as indicators of lightness, redness and yellowness, respectively (Ming-Min and Ismail-Fitry). The colour measurements were taken from each surface of the samples in triplicate. Measurement of colour was taken for samples before and after treatment.

Water holding capacity (WHC)

A 1.5 g meat sample was placed in a centrifuge tube along

with filter paper (Whatman No 1) as absorbents. All the samples were centrifuged for 15 min and 4,000 g in a micro refrigerated centrifuge (Model 3740, Kubota Corporation., Tokyo, Japan) at 20°C and 4,000 g. The WHC was calculated as the amount of water retained in the sample per 100 g of water before centrifuging in triplicate (Ismail et al. 2022).

Cooking loss

Cooking loss was measured by assessing the value of transudation after pressurisation. Three treated meat samples from each treatment were weighed before and after cooking (Ismail-Fitry et al. 2008) at 180°C for 20 min in an electrical oven (Model BJY-E13KW-2BD, Berjaya Steel Product Sdn. Bhd., Malaysia) until the internal temperature of the meat reached 75 °C.

Texture profile analysis (TPA)

All meat samples were cut into small cubes with the measurement 2 cm x 2 cm x 1 cm and analysed using a texture analyser (Model TA-XT2i, Stable Micro Systems., UK). The probe used was a 50 mm diameter cylinder aluminium probe with a pre-test speed of 1.00 mm/sec, test speed of 5.00 mm/sec and post-test speed of 5.00 mm/sec (Ismail et al. 2021). Triplicate reading was taken for the data of hardness of force required to compress samples.

Warner-Bratzler shear force

Three 1.5-cm-diameter cylinders were cut off of the cooked meat in parallel to the longitudinal direction of the muscle fibres. The shear force was determined using a TA-XT2i texture analyser (Stable Micro Systems., UK) equipped with 1-mm-thick Warner-Bratzler blades. The speed of the blade ascent and descent was fixed at 2.00 mm/sec and its distance to the platform at 40 mm.

Statistical analysis

Minitab V21.4 software (Minitab Inc., State College, PA, USA) was used to analyse the collected data, and each analysis was implemented in triplicate. A 2-way ANOVA was performed on all variables measured using the general linear model (GLM) procedure to observe the interactions between factors (types of meat and pressure). One-way ANOVA was conducted for an individual factor if no interaction was observed after the GLM. Means were compared by Turkey's method ($P < 0.05$).

RESULTS AND DISCUSSION

pH values and water holding capacity (WHC)

Table 1 shows the pH values and WHC of the raw beef and buffalo meat incorporated with two different pressures; 300 and 600 MPa as compared to the control. Although no interaction ($P > 0.05$) between types of meat and pressure was observed for the pH value of treated and untreated meat, there were differences ($P < 0.05$) for the different types of meat used and the pressure levels individually. This is shown in the pH value result whereby the buffalo meat had a higher value (5.6 – 5.8) than beef (5.4 – 5.7). Interestingly, pressurising meat at 600 MPa resulted in higher pH values for both beef and buffalo meat than pressurising at 300 MPa and control.

The WHC showed an interaction ($P < 0.05$) between types of raw meat used for pressurising and varying pressure during HPP treatment. The WHC for beef and buffalo are significantly different ($P < 0.05$) within the same type of meat for each treatment. The meat treated with 300 MPa had higher WHC as compared to 600 MPa and control for both beef and buffalo.

According to a study conducted by Mc Ardle et al. (2010), the pH values of beef did not change at 200 MPa regardless of the temperature of the pressurisation. Contrarily, at higher pressure levels (300 and 400 MPa), increases in pH values were observed. This finding is in line with the current study where the pH value increased as the pressure applied to raw meat increased. The augmentation of pH values with the increased level of pressure during HPP is associated with protein denaturation as well as the reduction of acidic groups present in meat causing conformational changes (Sert and Coşkun 2022).

The functional properties of myofibrillar proteins are majorly affected by pressure. Chapleau et al. (2003) described that properties such as water binding, gelling ability and solubility are related to the ability of meat to hold water. The results in this study are similar to Ros-Polski et al. (2015) in chicken meat, Sazonova et al. (2019) in pork and Xue et al. (2017) in rabbit meat, who reported significant differences ($P < 0.05$) between control meat and pressurised meat in WHC due to more compact matrix of meat after pressurisation.

Similar findings were found in a study by Hong et al. (2006) who concluded that the improvement in WHC was correlated to the decrease in cooking loss. The water holding capacity may also be influenced by the pH value (Sazonova et al. 2017). The destabilisation of non-covalent bonds between proteins and overlapping could occur during HPP treatment. Moreover, Sun and Holley (2010) found that HPP caused the formation of hydrophobic and disulphide bonds after pressure was applied; thus, the tertiary structure of the protein was then replaced by protein-water interaction. In addition, collagen denaturation took place and was liberated into meat juice which could bind water (Sazonova et al. 2019).

Product yield, cooking loss and moisture content

There was an interaction ($P < 0.05$) for different types of meat and different applied pressures towards the percentage of yield for the beef and buffalo meat (Table 1). Both beef and buffalo samples treated with HPP showed lower ($P < 0.05$) yields compared to their respective control sample. However, the beef treated with 300 MPa pressure had a lower ($P < 0.05$) yield percentage compared to the 600 MPa treated sample. Buffalo meat, on the other hand, showed significant differences ($P < 0.05$) as the yield percentage decreased while the pressure level increased. The product yield was expressed as the percentage of weight difference before and after meat was subjected to different levels of pressure. In this study, high pressure caused a significant decrease in product yield, similar to a study by Hong et al. (2006), which showed a decrease in product yield percentage after HPP treatment.

Different types of meat and various pressure levels showed an interaction ($P < 0.05$) towards the moisture content of the cooked meats. As the pressure increased, the moisture content also increased for both cooked beef and buffalo meat. This was aligned with the cooking loss results. There was an interaction ($P < 0.05$) between the different meat types and pressure levels for cooking loss (Table 1). The HPP-treated samples had lower ($P < 0.05$) cooking loss than the control for both types of meat. However, the samples treated at 600 MPa had a higher percentage of cooking loss than 300 MPa. This finding is similar to several studies (McArdle et al. 2010; Kim et al. 2007), which reported an increase in cook loss after the level of pressure applied to beef muscle increased in the range of 200 to 400 MPa at 20 and 40°C. The cooking loss of meat was attributed to the decrease in the WHC. Jung et al. (2000) concluded that the higher the water losses, the more severe the shrinkage and changes in myofibrillar proteins occur at higher pressure levels. Hughes et al. (2014) also mentioned cooking causes an increase in the stiffness of the myofibrillar structure due to protein denaturation, which corresponds with greater water loss.

Colour analysis

The meat treated with different pressures showed an

interaction ($P < 0.05$) towards colour except for the b^* value (Table 2). The L^* values were significantly lighter for beef ($P < 0.05$) at 600 MPa (43.12) compared to 300 MPa (36.74) and control (23.94). A similar pattern was observed for buffalo meat where 600 MPa had significantly higher lightness ($P < 0.05$) compared to 300 MPa (36.42) and control (31.35). The a^* values for the untreated beef (9.28) were significantly higher ($P < 0.05$) compared to the treated samples, while no significant changes were observed for the buffalo samples. Both beef and buffalo showed higher b^* values compared to the treated samples at 300 and 600 MPa, respectively. Even though there was no interaction ($P > 0.05$) between types of meat and pressure towards the b^* values, there were interactions ($P < 0.05$) between types of meat and pressure individually.

Colour is greatly affecting the freshness and wholesomeness of raw meat which later incorporated with consumers' acceptance. Meat colour was affected by the types of meat, pressure levels, temperatures and myoglobin chemical state after the pressurisation process (Bolumar et al. 2021). Some studies showed a slight colour change at a pressure below 200 MPa. In contrast, meat appearance tends to be paler and lighter after applying pressure above 200 MPa due to protein denaturation and coagulation (Sert and Coşkun 2022). In this study, the HPP caused L^* values to increase similar to those reported by Jung et al. (2003) and Marcos et al. (2010). Light-scattering was increased due to the modification in the structure of meat, leading to changes in the proportions of light to absorb, refract and reflect; hence, the appearance of meat tends to be paler (Bolumar et al. 2021; Hughes et al. 2014).

The reduction in a^* values after HPP treatment was related to myoglobin content depletion and the formation of metmyoglobin (Sert and Coşkun 2022). Similarly, as a result of this study, there appears to be a reduction in redness (a^* values) and an increase in yellowness (b^* values) (Jung et al. 2003; Marcos et al. 2010). The findings were associated with the formation of ferric metmyoglobin and alterations of heme myoglobin (Carlez et al. 1995). Figure 1 shows the colour differences between raw and cooked beef and buffalo meat treated at different levels of pressure during high-pressure processing. Limited changes were observed in the colour of the meats after they had been cooked in an oven.

Table 1. pH values, water holding capacity (WHC), yield percentage, moisture content and cooking loss of different types of raw and treated meat with different pressures.

	2-way ANOVA			Beef			Buffalo		
	Type of meat*	Pressure**	Types of meat × pressure***	0 MPa	300 MPa	600 MPa	0 MPa	300 MPa	600 MPa
pH	<0.001	<0.001	ns	5.47 ± 0.03Cb	5.56 ± 0.03Bb	5.74 ± 0.03Ab	5.60 ± 0.07Ca	5.74 ± 0.03Ba	5.87 ± 0.01Aa
WHC (%)	<0.001	ns	<0.001	68.71 ± 0.31D	73.39 ± 0.39A	69.86 ± 0.23C	71.33 ± 0.51B	72.51 ± 0.26A	69.09 ± 0.13CD

Yield (%)	<0.001	<0.001	<0.001	97.19 ± 0.49A	85.28 ± 0.28D	88.86 ± 0.31B	97.62 ± 0.32A	87.04 ± 0.19C	84.39 ± 0.14D
Moisture content of cooked samples (%)	<0.001	<0.001	<0.001	71.49 ± 0.26C	72.16 ± 0.06BC	73.16 ± 0.38A	68.53 ± 0.61E	70.14 ± 0.25D	72.51 ± 0.16AB
Cooking loss (%)	<0.001	<0.001	<0.001	31.16 ± 0.40D	21.79 ± 0.18F	29.46 ± 0.50E	36.49 ± 0.07A	34.747 ± 0.07C	35.49 ± 0.06B

*If no interaction of factors is present, the posthoc analysis is for the type of meat factor ($p < 0.05$), where comparisons are made between beef and buffalo within the same pressure and indicated by lowercase letters ($p < 0.05$ for significant differences);

**If no interaction of factors is present, the posthoc analysis is for the pressure factor ($p < 0.05$), where comparisons are made between different pressures within the same meat type and indicated by uppercase letters ($p < 0.05$ for significant differences);

***If the interaction of factors is present ($p < 0.05$), the posthoc analysis is carried out for all the treatments and indicated by uppercase letters ($p < 0.05$ for significant differences);

ns = not significant. The values represent mean \pm SD; $n = 3$.

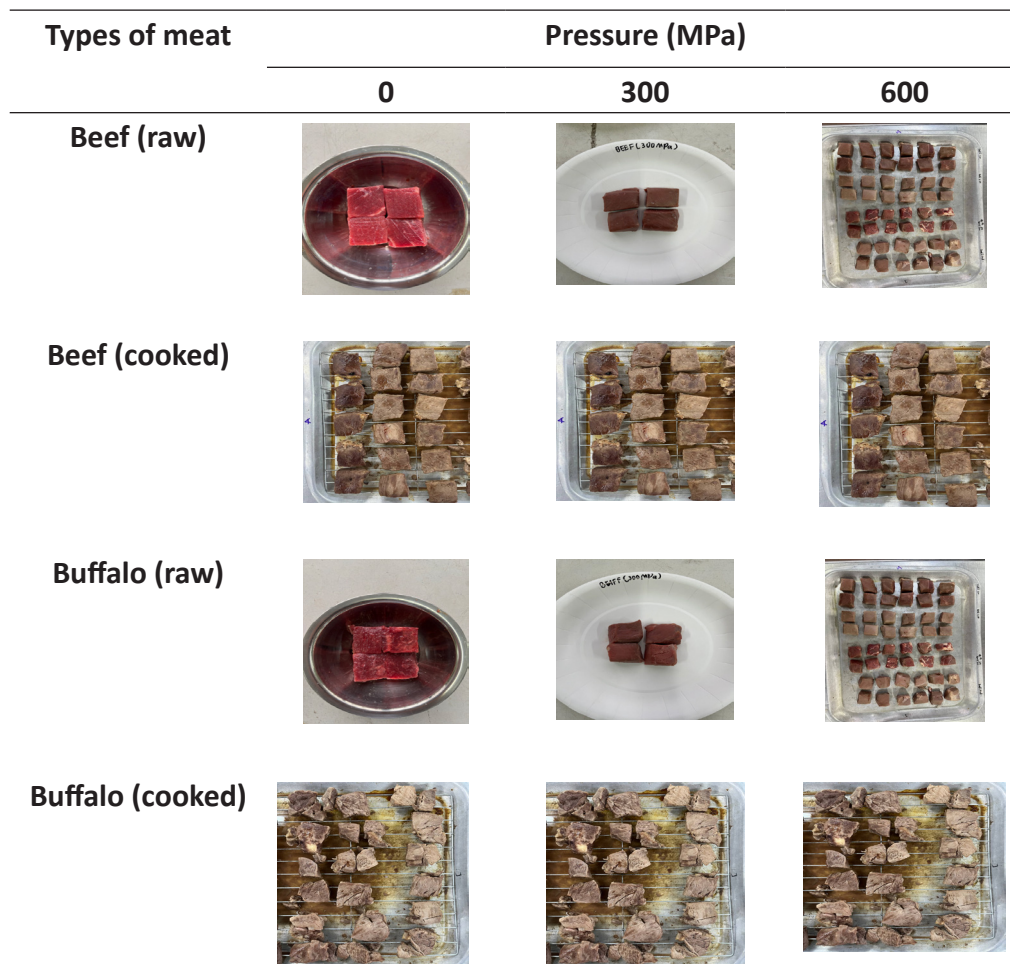


Figure 1. Colour changes of beef and buffalo pressurised with different levels of pressure for 2 min at 20.

Table 2. Colour (L^* , a^* and b^*) texture profile analysis (TPA) and Warner-Bratzler shear force (WBSF) of different types of cooked meat and different pressures.

	2-way ANOVA		Types of meat x pressure	Beef			Buffalo		
	Types of meat	Pressure		0 MPa	300 MPa	600 MPa	0 MPa	300 MPa	600 MPa
L^*	<0.001	0.002	<0.001	23.94 ± 1.66 ^D	36.74 ± 0.17 ^B	43.12 ± 2.02 ^A	31.35 ± 0.40 ^C	36.42 ± 0.30 ^B	40.50 ± 0.34 ^A
a^*	<0.001	0.001	0.050	9.28 ± 0.61 ^A	6.85 ± 0.74 ^C	7.40 ± 0.08 ^{BC}	9.51 ± 0.33 ^A	8.52 ± 0.51 ^{AB}	8.48 ± 0.23 ^{AB}
b^*	<0.001	0.002	ns	3.29 ± 0.14 ^{Bb}	3.93 ± 0.24 ^{Bb}	6.02 ± 0.68 ^{Aa}	4.80 ± 0.56 ^{Ba}	5.43 ± 0.37 ^{Ba}	6.52 ± 0.33 ^{Aa}
Hardness (g)	ns	ns	0.004	3765 ± 527 ^B	4952 ± 798 ^{AB}	5355 ± 414 ^A	5138 ± 504 ^{AB}	4676 ± 452 ^{AB}	3804 ± 233 ^B
Chewiness (g)	0.009	ns	0.017	8380 ± 870 ^B	16663 ± 3659 ^A	18938 ± 3286 ^A	19175 ± 2189 ^A	15646 ± 2878 ^A	17899 ± 551 ^A
Springiness	ns	0.024	ns	0.47 ± 0.02 ^{Ab}	0.53 ± 0.01 ^{Aa}	0.53 ± 0.06 ^{Aa}	0.61 ± 0.06 ^{Aa}	0.53 ± 0.04 ^{Aa}	0.62 ± 0.004 ^{Aa}
Cohesiveness	<0.001	0.003	0.004	0.47 ± 0.01 ^C	0.62 ± 0.02 ^B	0.65 ± 0.01 ^{AB}	0.61 ± 0.01 ^B	0.62 ± 0.02 ^B	0.68 ± 0.03 ^A
WBSF (N)	<0.001	<0.001	ns	102.40 ± 2.04 ^{Aa}	75.41 ± 8.57 ^{Ba}	82.70 ± 4.51 ^{Ba}	85.20 ± 2.12 ^{Ab}	66.31 ± 2.05 ^{Ca}	73.38 ± 1.74 ^{Bb}

*If no interaction of factors is present, the posthoc analysis is for the type of meat factor ($p < 0.05$), where comparisons are made between beef and buffalo within the same pressure and indicated by lowercase letters ($p < 0.05$ for significant differences);

** If no interaction of factors is present, the posthoc analysis is for the pressure factor ($p < 0.05$), where comparisons are made between different pressures within the same meat type and indicated by uppercase letters ($p < 0.05$ for significant differences);

***If the interaction of factors is present ($p < 0.05$), the posthoc analysis is carried out for all the treatments and indicated by uppercase letters ($p < 0.05$ for significant differences);

ns = not significant. The values represent mean ± SD; $n = 3$.

Texture profile analysis (TPA)

TPA is a common penetration test to determine the textural properties of muscle proteins. There were interactions observed ($P < 0.05$) between types of meat and different pressures for hardness, chewiness, and cohesiveness but no interaction ($P > 0.05$) for springiness (Table 2). At 600 MPa, beef had a higher ($P < 0.05$) hardness value compared to the control, while no changes were observed for the buffalo samples ($P > 0.05$) although the value showed a reducing trend as the pressure increased. The hardness of the meat showed a unique pattern for beef; the hardness increased with the increase of pressure; meanwhile, for buffalo, the hardness decreased with the increase of pressure. Meat hardness in this study showed an inclining trend for beef and declining for buffalo. These results were in line with a study by Ma and Ledward (2004) who found that meat hardness increased after high-pressure treatment at or above 200 MPa at 20°C

in beef muscle. However, Schenkova et al. (2007) reported a decrease in meat hardness after pressure is applied between 100 to 300 MPa at 10°C. The variation in results might be caused by different characteristics of the meat samples from different species.

The chewiness values for all the samples were higher ($P < 0.05$) compared to the untreated beef, similar to the cohesiveness results, where the untreated beef had the lowest ($P < 0.05$) value compared to others. The untreated beef also showed less springiness ($P < 0.05$) compared to the untreated buffalo meat but other treated samples showed no significant difference ($P > 0.05$). In the study, chewiness, springiness, and cohesiveness were recorded as comparable after HPP treatment. On the other hand, Fernandez et al. (1998) reported that springiness, chewiness and cohesiveness of treated meat at 400 MPa decreased, and the results were explained by the high-pressure protection of meat proteins from heat denaturation to some extent (Fernandez-Martin et al. 1997).

Warner-Bratzler shear force (WBSF)

No statistical interaction ($P > 0.05$) in affecting the WBSF was observed between beef and buffalo meat with different pressure levels (Table 2). Nevertheless, significant differences ($P < 0.05$) were observed towards the meats' shear force. The shear force of both meat is higher at 600 MPa (82.70 and 73.38) compared to 300 MPa (75.41 and 66.31). Consumers contend that tenderness is the most significant aspect among all the characteristics that define meat eating quality (Jung et al. 2000; Denoyellea and Lebihan 2003). WBSF values observed from this study showed pressurisation at 600 MPa resulted in higher WBSF than raw meat treated at 300 MPa. This is in line with other studies (Ma and Ledward 2004; McArdle et al. 2011), which found the same trend for beef (*M. longissimus dorsi*) treated at 400 and 600 MPa at 20 to 40°C. Jung et al. (2000) also reported an increase in meat toughness after being treated with HPP at 130 and 520 MPa at 10°C. At lower temperatures, the integrity of myofibrils is greater than the connective tissue which resulted in the reduction of WBSF and an increase in meat toughness. Dong Sun and Holley (2010) stated that the variation in muscle texture from high-pressure treatment was due to the rupture of hydrophobic and electrostatic interactions. Moreover, the stage of rigour, pressure levels, temperature and their combination are the factors that affect meat toughness and tenderness.

CONCLUSION

Based on this study, HPP effects can vary on different types of meat. The pH value and moisture content of both types of meat (beef and buffalo) increased with an increase in pressure levels. The yield percentage of beef treated at 600 MPa is higher than buffalo. In contrast, the buffalo meat treated at 300 MPa has a higher yield percentage than the beef. Moreover, both beef and buffalo meat treated at 300 MPa has higher water holding capacity than control and 600 MPa treated meats. Cooking loss for both types of meat decreased with an increase in pressure, especially at 300 MPa. In addition, both types of meat had significant increases in L^* values, decreases in a^* values and increases in b^* values. Warner-Bratzler shear force of the 300 MPa treated meat after cooking is lower than 600 MPa and control. The beef showed an increase in hardness with an increase in pressure, while buffalo meat showed a decrease in hardness with an increase in pressure. The results indicate that tenderisation of meat can be achieved when a lower pressure (300 MPa) is applied to the sample. However, pressurisation might influence other meat attributes such as binding properties, pH values and colour.

COMPETING INTEREST

The authors do not have any competing interests among themselves or others related to this research work.

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ETHICAL STATEMENT

Not applicable

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