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Research Article

Effect of High-Pressure Processing and Bromelain Application on The Quality Characteristics of Buffalo Meat

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ABSTRACT

The present study evaluated the effect of bromelain and high-pressure processing (HPP) on the physicochemical, tenderization, and microstructure of buffalo meat. The buffalo meat samples were marinated with 5% bromelain enzyme for 24 hr at 4°C followed by HPP treatment at 0 MPa, 100 MPa, and 300 MPa for 5 min at 20 ± 2°C, leading to six samples viz Control (without bromelain and with bromelain, no high-pressure treatment), 100 MPa (with & without bromelain) and 300 MPa (with & without bromelain). The pH and moisture content of buffalo meat improved when subjected to pressure and bromelain enzyme. The cooking yield was reduced for enzyme-treated meat, while there was no significant (p>0.05) difference for HPP-treated meat. Hardness and shear force reduced at 100 and 300 MPa compared to the control; however, they were not statistically significant (p>0.05). In addition, buffalo meat that was treated with 300 MPa had a more compact but ruptured fibre structure than the control. The sensory panellists preferred non-bromelain-treated meat due to the unpleasant taste of enzyme-treated meat. In conclusion, bromelain and HPP could improve buffalo meat tenderness, but the time and pressure of HPP need to be increased to get optimal tenderness while replacing bromelain with other types of enzymes to cater for the taste of the meat sample.

Key words: Buffalo meat, bromelain, high-pressure processing, meat structure, meat tenderization

INTRODUCTION

Asian buffalo (*Bubalus bubalis*) is a significant meat-producing animal in the tropical and subtropical regions of Southeast Asia. Buffalo meat, also known as carabeef, is rapidly becoming an important source of red meat and gaining popularity among consumers due to its lean characteristics, high protein content, low fat and cholesterol levels, health benefits, superior processing qualities, and price competitiveness (Di Stasio & Brugiapaglia, 2021). However, buffalo meat is known to be tougher than other types of meat and primarily results from the production of meat from aged, spent, or unproductive animals, which often leads to unsatisfactory quality. The toughness of the meat is determined by two factors: the connective tissue present in the meat and post-mortem processing conditions, which contribute to undesirable conditions such as meat shortening (Mohd Azmi *et al*., 2023).

The tenderness of the meat is a decisive factor profoundly affecting the eating quality, such as the palatability, juiciness, and chewability of meat and is considered the most important quality parameter of cooked meat (Mohd Azmi *et al*., 2023). For tough meat such as buffalo meat, post-mortem ageing is not sufficient to reduce the toughness to the desired level, and various exogenous proteases are used for tenderizing it (Ismail *et al*., 2018a). Among exogenous proteases, papain, bromelain, and ficin are commonly used to tenderize post-mortem meat by degrading both myofibrillar and connective tissue proteins (Maqsood *et al*., 2018). Bromelain is a combination of cysteine proteases isolated from the pineapple (*Ananas comosus*) plant's stem and fruit. It breaks down collagen more effectively than other vegetative enzymes (Mohd Azmi *et al*., 2023). However, the issue of excessive tenderization and lack of optimal concentration of these enzymes remains a major constraint in their application in meat. This has prompted the meat industry to explore other innovative technologies for meat tenderization.

Among the advanced technologies are high-pressure processing (HPP), pulsed electric field (PEF), and ultrasound treatment. HPP could reduce the toughness of meat by the pressure applied, denaturing the meat protein. However, higher pressure used

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could affect the physicochemical properties, such as changes in colour and sensory (Ma & Ledward, 2013). Similarly, PEF processing on beef resulted in improved tenderness but increased fat oxidation and saturated fatty acids (Kantono *et al*., 2019). Ultrasound increased the tenderness and fragmentation of meat but had a paler greyish-brown colour compared to the untreated samples (Peña-Gonzalez *et al*., 2019). These show that although advanced technologies can tenderize the meat, they still have limitations towards other meat quality parameters.

Regarding the HPP treatment, a combination of HPP with enzymes could be an alternative to enhance meat tenderization without jeopardizing the meat quality (Madhusankha & Thilakarathna, 2021). Furthermore, HPP could be used to reduce the processing time, space, and labour associated with traditional ageing without compromising the quality of the product (Morton *et al*., 2017). For example, an increased tenderness was observed in yak meat by applying HPP 50 MPa/15 min with papain (80U/mL) (Ma *et al*., 2019) and HPP with ficin in post-rigor tan mutton (Li *et al*., 2021). The optimal value of HPP application and protease enzymes varies with the type of meat, breed, age, and other pre- and post-slaughter processing.

Till now, limited studies have undertaken the effect of bromelain and HPP in tenderisation and other quality parameters in buffalo meat. The most related is a work by Santos *et al*. (2020), where they reduced the meat hardness using pre-treated pineapple core with HPP to promote the bromelain activity before marinating the beef. The range of HPP pressure proposed for tenderizing the meat is 100-400 MPa (Ma & Ledward, 2013). Meanwhile, the suitable concentration of bromelain is proposed to be between 3-5% (Orynbekov *et al*., 2024). However, a combination of both HPP and bromelain may optimize the tenderness while retaining other qualities of the meat, which so far has not been well established. There is a need to have insights into the various changes in the application of pressure and bromelain for getting the desired level of textural and microstructural in buffalo meat. Thus, the present study was undertaken to evaluate the effect of HPP (0, 100 & 300 MPa) and bromelain (0 & 5%) combined application on the various physiochemical parameters, sensory attributes, and microstructure of buffalo meat.

MATERIALS AND METHODS

Sample preparation

Frozen buffalo meat was purchased from Pak Cik Butcher in Bandar Baru Bangi, Selangor, and stored in the freezer (-18°C). Before the start of the experiment, frozen meat was thawed at 4°C and cut into a uniform cube size (3 × 3 × 3 cm), where all the samples had undergone the same controlled procedure of freezing and thawing to eliminate any confounding factors such as muscle damage due to ice crystal formation and drip loss. Based on preliminary experiments and available literature, two levels of bromelain (0% & 5%) (Orynbekov *et al*., 2024) and three levels of HPP application (0 MPa, 100 MPa & 300 MPa) (Ma and Ledward, 2013) were used in the present study. These levels of pressures of HPP and concentrations of bromelain were selected to optimize the effect towards meat tenderization without affecting the meat quality. At the same time, a lower pressure of HPP was tested to determine whether the bromelain can enhance the meat tenderness effect at a minimum pressure applied. A total of six samples, viz Control (without bromelain and with bromelain), 100 MPa (with & without bromelain) and 300 MPa (with & without bromelain), were prepared. The commercial bromelain (Take It Global, Sungai Jawi, Pulau Pinang) (CAS No: 9001-00-7; 100,000 U/g enzyme activity) was dissolved in distilled water to prepare a 5% concentration of bromelain solution. The meat samples were marinated with a bromelain solution at a ratio of 1:2 w/v. The meat chunks were placed in polypropylene bags after being thoroughly mixed manually and kept at 4 ± 1°C for 24 hr. The meat pieces were drained and vacuum-packed in polyethylene bags.

HPP was performed immediately after the packaging at 0 MPa, 100 MPa, and 300 MPa for 5 min at room temperature (20 ± 2°C) by using a pilot-industrial scale high-pressure equipment (Model 55, Hyperbaric, Burgos, Spain, pressure vessel- 200 mm inner diameter × 2000 mm length, 55 L capacity, maximum operating pressure - 600 MPa). Potable water served as the pressure transfer fluid, and the pressure release period was just a few sec after the pressure came up. The samples were cooked in an oven for 20 min at 150 ± 1°C, followed by tempering at room temperature and stored at 4°C for further evaluation of quality parameters. A flowchart of the experimental design of the study can be referred to in Figure 1.

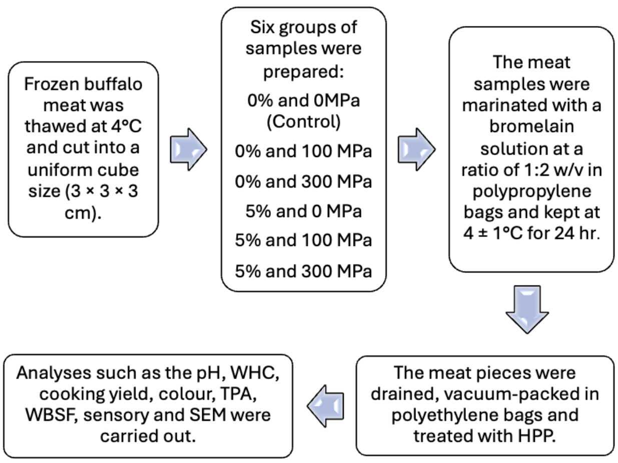

Fig. 1. The flowchart of the experimental design of the study.

Physicochemical attributes

The pH of the meat samples was determined by using a pH meter (PB-10, Sartorius, Germany) after homogenizing the buffalo meat sample (10 g) in the double distilled water (50 mL). The moisture content of meat samples was measured by using a moisture analyser at 105°C (AND MX-50, Tokyo, Japan). The cooking yield of meat samples was measured by measuring the weight of raw samples and weight after cooking samples in an oven for 20 min at 150 ± 1°C (Ismail-Fitry *et al*., 2008; Ismail *et al*., 2021).

The WHC of meat samples was determined by taking 1.5 g of sample in a 50 mL centrifuge tube followed by centrifugation for 15 min at 4000 × g centrifugation (Kubota 3740, Tokyo, Japan) at 20°C. The WHC was calculated by measuring the weight of the sample before and after centrifugation and expressed as the quantity of water retained per 100 g of water in the sample before centrifugation (Ramle *et al*., 2021).

The surface colour of the samples was measured by using a colourimeter (CR-410, Konica Minolta, Japan). The findings were represented as CIE (Commission Internationale de l'Eclairage) L*, a*, and b* parameters. The parameters used to assess colour are the L* value (lightness), which varies from 0 to 100 (from black to white); a* value (redness), which ranges from green to red (a* > 0); and b* value (yellowness), which ranges from blue to yellow (b* > 0).

Texture profile analysis (TPA) and Warner-Bratzler shear force (WBSF)

A texture profile analyser (TA.HD plus, Stable Micro Systems, United Kingdom) was used to assess the texture profile analysis (TPA) of cooked buffalo meat samples. Three samples from each treatment were cut into cubes (3 × 3 × 3 cm) and placed in the centre of the compression plate. Using a cylindrical probe, the test samples were compressed twice continuously to 50% of their original height (diameter 75 mm) and analysed for hardness (kg), springiness (mm), cohesiveness (ratio), gumminess (kg), and chewiness (kg. mm) (Ming-Min & Ismail-Fitry, 2023).

The shear force value of samples was determined by using a Warner-Bratzler shear blade linked to the Texture Analyzer (TA-XT2, Stable Microsystem System Ltd., UK). The samples were cut perpendicularly in the longitudinal direction of muscle fibres. The meat samples were put down on the table underneath the V-shaped blade and were sliced as the blade descends through the table's slit at a consistent pace. The WBSF values were reported as the highest peak of the shear test's force-distance curve or the maximum shear force.

Scanning electron microscopy

Double-sided tape was used to attach and secure the diced meat on an aluminium mounting stub. Using a scanning electron microscope (LEO 1455 VPSEM, Cambridge, UK) with a 250× magnification, thin layers of the meat were examined as per Asyrul-Izhar *et al*. (2021).

Sensory evaluation

A sensory evaluation of cooked beef pieces was performed by 50 untrained panellists using a hedonic scale with a rating of 1 for dislike extremely and 9 for like extremely. Before the sensory assessment, each meat sample was grilled on each side within 6 min and given in a random sequence. The panellists were asked to rate the food based on its colour and appearance, flavour, tenderness, juiciness, and overall acceptability.

Statistical analysis

Using Minitab version 19, two-way ANOVA was used to analyse all data (Minitab, Statistical Software, USA). By using two-way ANOVA, the interaction between the pressures and bromelain could be determined. The effect of one independent variable (e.g. HPP pressure) on the dependent variable (e.g. colour) differs depending on the level of the other independent variable (e.g. bromelain concentration). The significance of the differences between the means for the various treatments was determined using Tukey's test (n=3). For all comparisons, a significance threshold of (P=0.05) is considered acceptable.

RESULTS AND DISCUSSION

pH value

The present study noted an interaction effect between pressure and enzyme (P<0.05) (Table 1). The buffalo meat treated with 300 MPa without enzyme has the highest pH value, whereas untreated (control) meat exhibited the lowest value, 6.11 and 5.31, respectively. The pH values of 300 MPa + 0% enzyme, 0 MPa + 5% enzyme, 300 MPa + 5% enzyme, 100 MPa + 5% enzyme, and 100 MPa + 0% enzymes were not significantly different (P>0.05). In addition, the pH values of the buffalo meat treated with the enzyme without HPP were higher (p<0.05) than those of the control without the enzyme. Our findings agree with the higher pH reported on HPP application (Morton *et al*., 2017) and upon treatment with bromelain (Ismail *et al*., 2018a). Meat possesses a natural buffering capacity, meaning it can resist pH changes, and the addition of bromelain, an enzyme with acidic properties, initially lowers the pH. However, the meat's buffering capacity can then lead to pH stabilization or a slight increase as enzymatic reactions proceed (Mennah-Govela *et al*., 2020). The decrease in pH could lead to a decrease in protein reactive groups, which are important for water-retaining characteristics (Ismail *et al*., 2018b).

Moisture and WHC

There was no interaction (P>0.05) recorded between pressures and enzymes for the moisture and WHC values of buffalo meat. There were significant variations (P<0.05) in the moisture content for the three pressures utilized, 0 MPa, 100 MPa, and 300 MPa, respectively (Table 1). The bromelain treatment did not alter WHC significantly (P>0.05). Ismail *et al*. (2018b) reported that the WHC of enzyme-treated samples were not significantly different from control samples. According to Ketnawa and Rawdkuen (2011), the decreased WHC in bromelain enzyme-treated samples is most likely due to the denaturation of myofibrillar proteins, which play a role in water retention. Bromelain enzymes might hydrolyze these proteins into tiny peptides or amino acids, making them ineffective. The 300 MPa without enzyme meat samples exhibited the highest moisture content, followed by 100 MPa and 0 MPa without enzyme. Similarly, 300 MPa with the enzyme has the highest moisture content, followed by 100 MPa and 0 MPa with the enzyme. This could be due to the availability of more hydrophilic sites in the buffalo meat samples under the application of HPP. A similar increase in the moisture content and WHC of pork samples treated with 300 MPa and 400 MPa was also reported by Korzeniowski *et al*. (1999).

Table 1. pH, moisture content, cooking yield and water holding capacity of buffalo meat with different pressures and with and without bromelain enzyme (Mean±SD)*

Analysis	Pressure × Enzyme	Without enzyme			With enzyme		
		0 MPa	100 MPa	300 MPa	0 MPa	100 MPa	300 MPa
pH	0.024	5.31 ± 0.10^{b}	5.67 ± 0.09^{ab}	6.11 ± 0.02^{a}	6.06 ± 0.07^{a}	5.85 ± 0.56^{ab}	5.96 ± 0.07^{ab}
Moisture content (%)	ns	63.95 ± 1.50^{Ab}	66.64 ± 1.55^{Aab}	69.21 ± 1.92^{Aa}	61.60 ± 1.59^{Ab}	66.88 ± 1.00^{Aa}	67.90 ± 0.44^{Aa}
Cooking yield (%)	ns	84.78 ± 2.73^{Aa}	85.92 ± 1.43^{Aa}	80.84 ± 2.33^{Aa}	61.11 ± 3.61^{Ba}	66.70 ± 4.61^{Ba}	65.14 ± 10.84^{Aa}
Water holding capacity (%)	ns	81.78 ± 5.59^{Aa}	81.78 ± 5.43^{Aa}	83.33 ± 2.00^{Aa}	74.67 ± 2.31^{Aa}	75.78 ± 3.91^{Aa}	80.22 ± 3.42^{Aa}

*The interaction of factors was established ($P<0.05$), therefore, post hoc analysis was carried out for all the samples row-wise with different small letters(a-b) showing significant differences ($P<0.05$) ($n=3$). If no interaction was established ($P>0.05$), the post hoc analysis was carried out for the pressure and enzyme factor, separately; means with similar small letters(a) row-wise show no significant differences ($P>0.05$) between different pressures with the presence and absence of enzyme; means with different capital letters(A-B) row-wise show significant differences ($P<0.05$) between presence and absence of enzyme at the same pressure.

Cooking yield

The cooking yield values for buffalo meat recorded a significant ($p<0.05$) decrease upon enzymatic treatment (Table 1). This could be attributed to the breakdown of myofibrillar tissues due to enzymatic treatment. Similar findings were also reported in bromelain-marinated beef (Ketnawa & Rawdkuen, 2011). Interestingly, there was no significant variation ($P>0.05$) in the cooking yield of buffalo meat when various pressures were applied. Similar findings of non-significant change in the cooking yield of poultry meat at 300 MPa application were reported by Kruk *et al*. (2011). Further pressure application was observed to decrease cooking yield in post-rigor tan mutton up to 350 MPa (Li *et al*., 2021) and bovine semitendinosus muscle up to 300 MPa (Kim *et al*., 2007).

Colour analysis

There were no interactions ($P>0.05$) between the various pressures utilised and the presence or absence of enzymes for the L* and a* values of colour analysis of uncooked buffalo meat (Table 2). However, there was a significant increase ($P<0.05$) in L* and a* values with the increase in the pressures utilised, as well as the presence and absence of enzymes individually. When 300 MPa pressure was tested in the absence of the enzyme, the L* value was found to be the highest (lightest) compared to the control. There was no significant difference ($P>0.05$) observed between the L*, a*, and b* values of the HPP-treated buffalo meat sample at 100 MPa in the absence of enzyme and the control. Similar findings of increasing L* value with increasing pressure and no significant difference among samples at 100 MPa were also reported in bovine semitendinosus muscle by Kim *et al*. (2007). Further, the presence of bromelain caused a significant ($P<0.05$) increase in L* values. Similar increases in L* values were reported in bromelain-treated beef samples by Nadzirah *et al*. (2016). This could be due to the deformation and breakdown of muscle fibre and the surface becoming mucous, leading to colour fading (Doneva *et al*., 2015).

The a* value of samples increased with increasing pressure from 100 MPa to 300 MPa. This could be due to a reduction in the metmyoglobin formation on HPP application leading to an increase in redness (Jung *et al*., 2003). For b* value, an interaction ($P>0.05$) was detected between the various pressures that were applied as well as the presence or absence of enzymes. Samples treated with 300 MPa in the absence of enzyme had the highest b* value. Similar higher b* values in beef in beef treated upon applying pressure at 300 MPa were reported by Marcos *et al*. (2010). Further, the enzymatic treatment was observed to increase the b* values of buffalo meat samples, which could be due to the accumulation of marinade on the sample surface as observed by Ismail *et al*. (2018b).

After cooking the HPP-treated samples, L* and a* values showed a difference ($P<0.05$) for the presence and absence of enzymes individually. However, the b* value demonstrates a significant difference ($P<0.05$) for the various pressures utilised as well as with the presence and absence of enzymes in each sample following HPP treatment. In the present study, HPP-treated enzyme samples had lighter, redder, and yellower colours. This could be due to the formation of metmyoglobin from myoglobin due to oxidation upon heat treatment (Huang *et al*., 2017). However, in the present study, enzyme-treated samples that were exposed to HPP would not completely be oxidised during cooking, leading to a higher result for a* values.

Table 2. Colour of buffalo meat before HPP, after HPP and after cooked with different pressures and with and without bromelain enzyme (Mean±SD)*

Analysis	Pressure × Enzyme	Without enzyme			With enzyme		
		0 MPa	100 MPa	300 MPa	0 MPa	100 MPa	300 MPa
Colour (after HPP)							
*L**	ns	30.38 ± 2.59^{Ab}	33.28 ± 1.06^{Ab}	39.64 ± 1.12^{Aa}	36.49 ± 1.63^{Bb}	38.29 ± 0.82^{Bb}	45.28 ± 1.30^{Ba}
*a**	ns	8.03 ± 2.60^{Ab}	12.06 ± 1.29^{Aab}	16.01 ± 0.90^{Aa}	10.01 ± 0.34^{Ab}	13.86 ± 1.98^{Aa}	13.91 ± 0.55^{Ba}
*b**	0.008	2.99 ± 0.38^{d}	4.57 ± 0.59^{bcd}	8.07 ± 0.48^{a}	3.53 ± 0.98^{cd}	5.18 ± 1.27^{bc}	5.72 ± 0.45^{b}
Colour (after cooked)							
*L**	ns	38.10 ± 5.25^{Aa}	35.22 ± 2.04^{Ba}	38.74 ± 0.70^{Ba}	41.55 ± 2.94^{Aa}	40.59 ± 0.62^{Aa}	42.77 ± 1.73^{Aa}
*a**	ns	9.91 ± 2.50^{Aa}	9.18 ± 0.38^{Ba}	10.01 ± 0.97^{Ba}	12.45 ± 2.10^{Aa}	12.28 ± 1.73^{Aa}	13.42 ± 1.22^{Aa}
*b**	ns	5.65 ± 0.47^{Bab}	5.49 ± 0.82^{Bb}	7.17 ± 0.55^{Ba}	9.29 ± 1.39^{Aa}	8.69 ± 0.74^{Aa}	9.23 ± 0.81^{Aa}

*The interaction of factors was established (P<0.05), therefore, post hoc analysis was carried out for all the samples row-wise with different small letters(a-b) showing significant differences (P<0.05) (n=3). If no interaction was established (P>0.05), the post hoc analysis was carried out for the pressure and enzyme factor, separately; means with similar small letters(a) row-wise show no significant differences (P>0.05) between different pressures with the presence and absence of enzyme; means with different capital letters(A-B) row-wise show significant differences (P<0.05) between presence and absence of enzyme at the same pressure.

Texture profile and WBSF values

For the meat samples' hardness, cohesiveness, gumminess, and chewiness, no significant interactions (P>0.05) between the pressures employed and the presence or absence of enzymes were observed (Table 3). However, the springiness of the meat samples recorded a significant difference (P<0.05) for each enzyme's presence or absence, individually. The value of hardness and gumminess displayed a distinctive pattern (p>0.05), reducing at 100 MPa with the presence and absence of the enzyme in comparison to the control, then increasing at 300 MPa with the presence and absence of the enzyme. This could be due to severe denaturation of myofibrillar proteins at higher pressure, resulting in a lower capability to bind water. Similar findings of increased HPP increasing hardness at higher pressure application (above 400 MPa) were reported in bovine longissimus dorsi muscle (Marcos *et al*., 2010).

However, the overall impact of HPP on the hardness or softness of meat varies with the rigour stage, pressure, temperature, and the combination of these factors (Sun & Holley, 2010). The exogenous proteases improve the solubility of collagen and facilitate structural changes by acting on collagen cross-links. Most of the published studies reported the tenderising effect of pre-rigour meat treatment with low pressure (<200 MPa) application, whereas, for tenderization, post-rigour meat, HHP application at higher temperatures (40°C to 80°C) was reported effective (Giménez *et al*., 2015).

Meat tenderness and shear force are closely correlated, with an increase in shear force indicating a reduction in tenderness. There was no interaction between the various pressures utilised, and the presence or absence of enzymes for the shear force of the buffalo meat (P>0.05) was recorded (Table 3). However, at 0 MPa, there was a significant difference in the maximum shear force between the presence and absence of enzyme-treated samples (P<0.05). The shear force for enzyme-treated samples at 0 MPa was lower than for controls. Similar findings were reported by Ma *et al*. (2019) in papain-treated yak samples as compared to the control untreated samples.

Table 3. Texture profile analysis and Warner Bratzler shear force of buffalo meat with different pressures and with and without bromelain enzyme (Mean±SD)*

Analysis	Pressure × Enzyme	Without enzyme			With enzyme		
		0 MPa	100 MPa	300 MPa	0 MPa	100 MPa	300 MPa
Texture							
Hardness (kg)	ns	15.96 ± 5.73^{Aa}	11.38 ± 9.53^{Aa}	12.51 ± 9.04^{Aa}	17.03 ± 9.68^{Aa}	14.50 ± 3.30^{Aa}	14.76 ± 7.34^{Aa}
Springiness (mm)	ns	0.61 ± 0.04^{Aa}	0.78 ± 0.19^{Aa}	0.51 ± 0.13^{Ba}	0.74 ± 0.15^{Aa}	0.83 ± 0.08^{Aa}	0.90 ± 0.05^{Aa}
Cohesiveness (ratio)	ns	0.61 ± 0.01^{Aa}	0.62 ± 0.04^{Aa}	0.57 ± 0.10^{Aa}	0.52 ± 0.05^{Aa}	0.57 ± 0.01^{Aa}	0.57 ± 0.03^{Aa}
Gumminess (kg)	ns	9.85 ± 3.76^{Aa}	6.95 ± 5.47^{Aa}	7.66 ± 6.28^{Aa}	9.05 ± 5.41^{Aa}	8.23 ± 1.80^{Aa}	8.51 ± 4.48^{Aa}
Chewiness (kg.mm)	ns	6.13 ± 2.72^{Aa}	5.01 ± 3.24^{Aa}	4.46 ± 4.36^{Aa}	7.25 ± 4.90^{Aa}	6.81 ± 1.19^{Aa}	7.81 ± 4.34^{Aa}
Shear force							
Maximum shear force (kg)	ns	9.67 ± 1.54^{Aa}	7.58 ± 1.87^{Aa}	8.24 ± 2.75^{Aa}	5.34 ± 1.27^{Ba}	4.46 ± 1.23^{Aa}	5.82 ± 0.39^{Aa}

*The interaction of factors was established (P<0.05), therefore, post hoc analysis was carried out for all the samples row-wise with different small letters(a-b) showing significant differences (P<0.05) (n=3). If no interaction was established (P<0.05), the post hoc analysis was carried out for the pressure and enzyme factor, separately; means with similar small letters(a) row-wise show no significant differences (P<0.05) between different pressures with the presence and absence of enzyme; means with different capital letters(A-B) row-wise show significant differences (P<0.05) between presence and absence of enzyme at the same pressure.

Further, in the present study, the shear force value of HPP-treated meat decreased at 100 MPa and rose at 300 MPa in the absence of enzymes. This result is consistent with Ma *et al*. (2019), who reported that the maximal shear force initially reduced and subsequently rose with rising pressure levels. Therefore, high pressure might increase the sheer force of meat, leading to cell dehydration and cell ageing (Grossi *et al*., 2012). Additionally, meat that received HPP treatment in the presence of an enzyme displays a lower shear force value than meat that received HPP treatment in the absence of an enzyme, but the statistical analysis reveals no discernible difference. This result is consistent with Ma *et al*. (2019), who reported that papain-treated yak meat followed by HPP decreased the maximal shear force by 35.70%. The HPP disrupts muscle protein structure in meat, leading to denaturation, unfolding, and reconfiguration, making proteins more susceptible to enzymatic breakdown and resulting in a more tender texture (Bolumar *et al*., 2021). Protease enzymes, like bromelain, effectively break down muscle proteins and connective tissues in meat. When combined with HPP, the high-pressure environment enhances the release and activation of the enzyme, making proteins more accessible for breakdown. HPP also improves enzyme penetration into the meat matrix, creating micro-channels that allow for uniform tenderization. This synergy results in a more pronounced tenderization effect, as HPP increases the catalytic efficiency of proteases, leading to consistent and effective meat tenderization. This was reported by Schenková *et al*. (2007), where the partial muscle protein breakdown by enzymes, which improves HPP functioning, might be the reason for the synergistic association between HPP and enzymes.

Scanning electron microscopy analysis

Scanning electron microscopy (SEM) was used to detect alterations in the microstructure of treated samples (Figure 2, A-F). The control samples had well-organised bundles of myofibril and sarcomere tissues that were tightly connected. The structure of the treated sample with 300 MPa (E) was observed more compact but also showed ruptured fibre than the structure of the control sample (A). Similar findings of muscle fibres becoming finer and more compact under higher pressure when compared to control samples were also reported by Kim et al. (2007). This might be due to the aggregation or coagulation of specific sarcoplasmic proteins or the build-up of myofibrillar proteins after muscle fibre disintegration (Hatae et al., 1984). However, Souza et al. (2011) observed that HPP disrupted the tightly packed muscle cells, rupturing of I band and loss of the M line suggesting that HPP caused "space" in the myofibers which was an indication of structural damage and produced lower shear force value as a result of the bigger cell spacing of the muscle cells.

Additionally, in beef samples treated with bromelain, Ketnawa and Rawdkuen (2011) noted that there were damaged muscle fibres in various bundles, they were less connected, and there was a loss of muscle fibres contact. This demonstrates that another factor contributing to meat tenderization is the disturbance of the structure of the intramuscular connective tissue. The loss of muscle integrity and the degradation of meat proteins might be the cause of the reduction in shear force that was seen in beef that had been treated with papain at a concentration of 50 U/g for 60 min Ashie et al., 2002). Further, Ma et al. (2019) found that the cell structure of yak meat was more readily disrupted, the cell gap became more significant, and the cell size was changed to varied degrees when HPP was performed after papain treatment

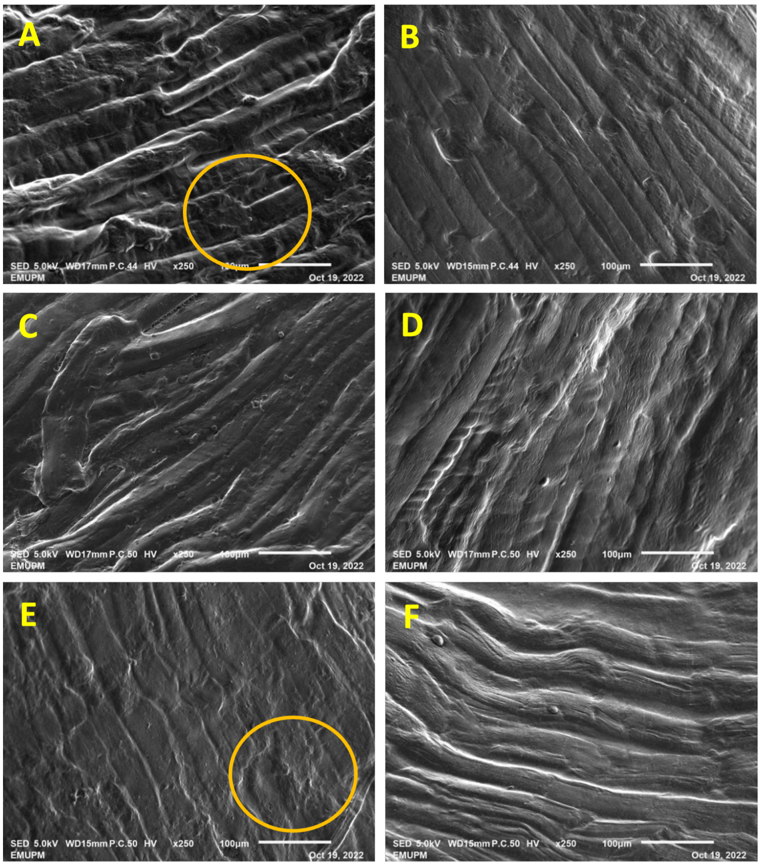

Fig. 2. Buffalo meat samples with the presence and absence of enzymes with different pressures used were observed at 250× magnification. Untreated buffalo meat (control) (A); buffalo meat with 0 MPa and presence of the enzyme (B); buffalo meat with 100 MPa and absence of enzyme (C); buffalo meat with 100 MPa and presence of the enzyme (D); buffalo meat with 300 MPa and absence of enzyme (E); buffalo meat with 300 MPa and presence of the enzyme (F).

Sensory analysis

There was no interaction ($P<0.05$) between the various pressures that were utilised and the presence or absence of enzymes in the process of influencing the sensory properties of buffalo meats (Table 4). HPP can preserve the sensory attributes of meat, including texture. Unlike traditional cooking methods that can lead to moisture loss and textural changes, HPP, when applied at a low pressure, helps retain moisture and maintain the original texture, making the meat more appealing to consumers. However, in terms of tenderness, juiciness, and overall acceptability of buffalo meats, significant differences were identified between the presence and absence of enzymes at the same pressure ($P<0.05$). Meanwhile, there were substantial changes ($P<0.05$) in colour and appearance, as well as flavour, depending on the various pressures that were utilised, as well as the presence or absence of enzymes separately. The panellists preferred ($P<0.05$) the buffalo meat that had not been treated with the enzyme in comparison to the meat that had been treated with the enzyme. This might be due to the unpleasant taste of the enzyme-treated meat that leads to low-scoring points. According to Zhao *et al*. (2020), excessive enzymatic treatment of beef with bromelain results in considerably raised amounts of ketones and odours, both of which contribute to a bitter taste owing to the generation of bitter peptides. Besides, statistical analysis did not observe noticeable differences between the untreated controls and the treated samples.

Table 4. Sensory characteristics of buffalo meat with different pressures and with and without bromelain enzyme (Mean±SD)*

Analysis	Pressure × Enzyme	Without enzyme			With enzyme		
		0 MPa	100 MPa	300 MPa	0 MPa	100 MPa	300 MPa
Colour & appearance	ns	7.30 ± 1.11^{Aa}	6.58 ± 1.58^{Ab}	6.94 ± 1.68^{Aab}	5.58 ± 1.69^{Ba}	4.90 ± 1.76^{Ba}	5.44 ± 1.49^{Ba}
Flavour	ns	6.96 ± 1.41^{Aa}	6.74 ± 1.44^{Aa}	6.40 ± 1.96^{Aa}	4.90 ± 1.97^{Ba}	3.90 ± 1.83^{Bb}	4.18 ± 2.00^{Bab}
Tenderness	ns	6.10 ± 1.80^{Aa}	6.08 ± 1.65^{Aa}	6.02 ± 1.92^{Aa}	5.22 ± 1.75^{Ba}	4.98 ± 1.94^{Ba}	5.34 ± 1.84^{Aa}
Juiciness	ns	6.04 ± 1.69^{Aa}	5.76 ± 1.76^{Aa}	5.88 ± 2.09^{Aa}	4.90 ± 1.87^{Ba}	4.20 ± 1.77^{Ba}	4.68 ± 1.78^{Ba}
Overall acceptability	ns	6.94 ± 1.54^{Aa}	6.56 ± 1.59^{Aa}	6.50 ± 1.78^{Aa}	5.06 ± 1.97^{Ba}	4.28 ± 1.81^{Ba}	4.64 ± 1.93^{Ba}

*The interaction of factors was established ($P<0.05$), therefore, post hoc analysis was carried out for all the samples row-wise with different small letters(a-b) showing significant differences ($P<0.05$) ($n=3$). If no interaction was established ($P<0.05$), the post hoc analysis was carried out for the pressure and enzyme factor, separately; means with similar small letters(a) row-wise show no significant differences ($P<0.05$) between different pressures with the presence and absence of enzyme; means with different capital letters(A-B) row-wise show significant differences ($P<0.05$) between presence and absence of enzyme at the same pressure.

In addition, the scores of all the sensory qualities of buffalo meats that were treated with the enzyme were considerably ($P<0.05$) lower than the scores of buffalo meats that were not treated with the enzyme. The present study agrees with Doneva *et al*. (2015), who observed that marination caused the surface of meat samples to become mucous. This observation might lead to a change in the sensory qualities, which would then result in lower ratings on the sensory assessment and, ultimately, be the least favoured. To overcome this, several steps could be taken to improve the sensory properties of the treated samples in the future. The concentration of the bromelain could be adjusted to prevent the unpleasant taste, provided the amount used is sufficient to tenderize the meat. The marination could also be enhanced by adding salt, sugar and other spices on top of the bromelain alone, which could improve the sensory experience.

CONCLUSION

The results demonstrate that buffalo meat treated with 300 MPa without enzyme has the highest pH, moisture content, and lightness, followed by 100 MPa and 0 MPa without enzyme. Bromelain treatment decreased the cooking yield of buffalo meat samples. The bromelain concentration which is currently being utilised was insufficient to statistically reduce the meat's hardness value. There were no interactions ($P<0.05$) between the pressures used and the presence or absence of enzymes for the meat samples' hardness, cohesiveness, gumminess, or chewiness. The shear force without HPP was lower for enzyme-treated samples than for controls. The microstructure results show that the treated sample's structure at 300 MPa was more compact than the control sample's structure. The sensory attributes of buffalo meat treated with HPP had significantly higher values than those of bromelain-treated samples. Since buffalo meat is tougher than some other types of meat, it is suggested to increase the pressure use up to 400 MPa or more. The bromelain concentrations also could be increased to more than 5%. By doing this, the desirable tenderness of buffalo meat could be achieved. Currently, to our knowledge, most food industries offer meat tenderization using enzymes (e.g. Enzyme Development Corporation - EDC and Ajinomoto Foods Europe) or HPP (e.g. Avure Technologies and Hiperbaric), separately. This study could offer a new alternative procedure for meat tenderization by combining both enzymes and HPP while maintaining the other meat quality parameters. However, more study needs to be carried out to optimize the enzymes' concentrations and types and HPP's pressures, durations, and temperatures in the future.

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ETHICAL STATEMENT

The privacy and rights of the panellists during the sensory evaluation session were protected. All the panellists were willing to participate voluntarily. They gave consent to join the study after the requirements and risks had been explained. They were also able to withdraw from the sensory session at any time. All the data will not be released without their knowledge.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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