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The combined effect of ultrasound treatment and papain on the quality properties of beef

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Abstract

Individual ultrasound treatment or adding exogenous enzyme treatment such as papain has been successfully proven to not only marinate but tenderise the meat. Incorporating both treatments at the same time could further tenderise the meat but the quality of the meat might be compromised. Hence, this study aimed to investigate the effect of ultrasound treatment coupled with papain solution on the quality properties of meat. Four different treatments were carried out on the meat samples; control (C), papain treated (P), ultrasound treated (US), and a combination of papain and ultrasound treated (USP). Meat treated with USP did not affect the physicochemical properties of the meat as no significant difference was found in water holding capacity (WHC), cooking loss and pH. Moreover, USP treatment was found to have effects on textural properties of the meat samples as positive linear relationships between the hardness of the treated meat with work of shear were found via Pearson correlation analysis. This is in line with the findings on the microstructure of the treated meat samples where tenderised meat samples exhibited disrupted muscle fibre. Thus, incorporating papain and ultrasound together improved the quality texture of the meat.

1. Introduction

For numerous years, livestock species including but not limited to poultry, sheep, goat, cow, and buffalo have been selectively bred and raised to satisfy human demand for protein consumption. Meat, being a primary source of protein, is not only abundant in this nutrient but also contains important minerals such as iron, zinc, selenium, phosphorus, and vitamin B complex (Pereira and dos Reis Baltazar Vicente, 2013). Accordingly, the Food and Agriculture Organization (FAO) has estimated that meat consumption in 2030 will reach 45.3 kg per capita. Beef, being the third most widely consumed meat in the world after pork and poultry, is expected to be a significant contributor to this increase (Ballin, 2010). Tenderness, along with flavour and juiciness, are examples of quality attributes that heavily influence consumer choices and overall meat palatability (O'Quinn, 2018). Additionally, tenderness has become a motivating factor for consumers to purchase meat, even at a higher price (Madhusankha and Thilakarathna, 2021). Meat tenderness is determined by several intrinsic factors, including connective tissue, sarcomere length, proteolytic potential, and the degradation of myofibrillar proteins. Beef is frequently considered tough, leading to a negative eating experience (Kandeepan *et al.*, 2009). Recently, there has been a growing interest in the use of tenderisers to improve the tenderness of tough meats, which has shown great potential and benefits in the meat industry.

The tenderisation of meat has been discovered through three distinct methods: mechanical, chemical, and enzymatic. In the meat industry, mechanical tenderisation is frequently employed through the utilisation of methods such as needle (blade) tenderisation, maceration, and tumbling processes, all of which involve mincing and crushing the muscle and conjunctive tissues (Davis *et al.*, 1975). Chemical tenderisation is accomplished through the injection of substances such as sodium chloride, sodium polyphosphate, and potassium lactate, all of which have been dissolved into water (Istrati, 2008). The enzymatic

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method of tenderisation utilises proteolytic enzymes such as papain, bromelain, or ficin. One of the most commonly employed post-mortem techniques for tenderizing meat is marination, which involves immersing, infusing, injecting, or tumbling meat with marinades (Ismail *et al.*, 2018). Papain, a plant proteolytic enzyme commonly found in raw papaya fruit latex, is a potent meat tenderiser and stabilising agent (Arvanitoyannis and Varzakas, 2008; Mohd Azmi *et al.*, 2023). Papain helps enhance the tenderness of the meat by primarily affecting the mucoproteins and collagen of connective tissue through the conversion of collagen suspensions into dense gels (Ionescu *et al.*, 2020).

Recently, there has been a surge in the emergence of novel and sophisticated technologies. Among these, ultrasound has emerged as a promising green technology, offering an alternative to conventional food processing methods (Jadhav *et al.*, 2021). The principle of ultrasound involves the utilisation of sound waves, in which the frequency exceeds the range of human auditory perception (Lin *et al.*, 2021). The acoustic waves with a constant ultrasound frequency produce acoustic cavitation. Subsequently, mechanical energy is imparted to the medium, resulting in the loss of some energy as heat and the formation of cavitation. Despite being categorized as a non-thermal process, mechanical friction generates heat due to mechanical vibrations during propagation, which can cause a temperature increase ranging from 1 to 10°C (Zhang and Abatzoglou, 2020).

In order to enhance the quality attributes of meat, it is possible to perform the combined application of ultrasound and enzyme. Furthermore, numerous studies have reported the outcomes demonstrated by ultrasound treatments, as well as the combined treatments involving ultrasound and other methods. However, only a limited number of researchers have focused on investigating the combination therapies of exogenous enzymes and ultrasound (Barekat and Soltanizadeh, 2017). The objective of this investigation was to evaluate the impact of ultrasound treatment coupled with papain solution on the quality properties of beef.

2. Materials and methods

2.1 Meat preparation

Beef preparation was performed by obtaining approximately 3 kg of beef from the Pasar Borong Selangor, Malaysia and stored at -18°C until sample preparation. The ultimate pH of the beef should be within the range of 5.5–5.8 before being selected for further treatment. The noticeable surface fat, silver skin and exterior connective tissues were trimmed off, and samples were sliced into cuboids with the size of 2 × 2 × 1.5 cm and randomly assigned for different treatments according to the experimental design. The meat pieces were packed individually, labelled, and kept frozen at -18°C for further analysis. Samples were prepared in triplicates.

2.2 Papain solution preparation

A total of 1 g of papain powder was diluted in 100 mL of distilled water. This solution was used during the ultrasound radiation treatment. Papain solution (1 % w/v) was prepared and exposed to ultrasound radiation at 500 W for 1 min.

2.3 Meat treatments

The treatments involved immersing samples in different types of solution for 24 hrs at a chilled temperature (4°C). The experiment involved dividing beef samples into four groups: a control group (C) which was immersed in distilled water with no treatment, a group treated with 1% (w/v) papain solution (P), a group treated with ultrasound (US), and a group treated with a combination of ultrasound and papain (USP) (Figure 1). The ultrasound equipment used was QSonica (Newtown, USA) with a fixed frequency of 20 kHz and a power of 500 W. The samples were rotated manually to ensure an even distribution of ultrasound waves. USP samples were treated for 1 min (7 s pulse on and 2 s off) at 50% amplitude.

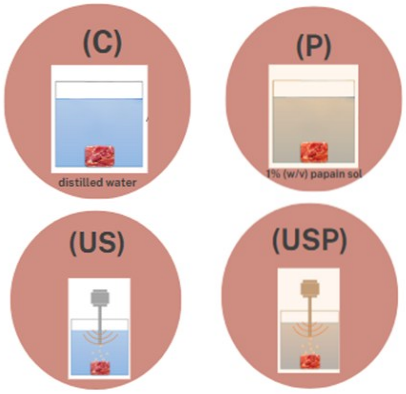

Figure 1. Illustrations of how meat samples were prepared according to the treatments. C: control, P: Papain-treated sample, US: ultrasound-treated sample, USP: Ultrasound and papain-treated sample.

2.4 Colour properties

The colour of the surface of the treated samples was measured using a chromameter (Konica Minolta CR-410, Japan) (Ismail *et al.*, 2022). The average score of triplicate experiments was recorded and expressed as CIE lightness (L*), redness (a*), and yellowness (b*).

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2.5 Warner Bratzler shear force analysis

The tenderness of the beef samples was measured using the Warner Bratzler shear force method, following the American Meat Science Association (AMSA) procedure. The samples were cooked in an airtight plastic bag, using a water bath (Memmert WNB14, Germany), at a temperature of 72°C for 20 mins. Following this, the samples were cooled for 10 mins at room temperature, and sliced into cubes 2.5 cm × 2.5 cm × 1.5 cm. The shear force was measured by shearing the cubes in a direction perpendicular to the muscle fibers, using a Warner–Bratzler shear force blade connected to a texture analyser (Stable Micro System TA-XT2, UK). The test compression load was set to 5 kg, and the crosshead speed was set to 100 mm/min. Shear force values were calculated using an average of triplicates for each sample.

2.6 Texture profile analysis

Texture Profile Analysis (TPA) was performed on the treated beef samples using a texture analyser (Stable Micro System TA-XT2i, UK) with modifications (Ramle *et al.*, 2021). The properties that were evaluated include the firmness, resilience, cohesiveness, gumminess, and chewiness of the samples. The P75 probe, with a plate diameter of 2.0 cm, was used. The samples, which were in the form of a cuboid (2 cm × 2 cm × 1.5 cm), were cut from the centre of each meat sample and placed in the middle of the compression plate. The equipment parameters were set as follows: measure force in compression, trigger type, auto— 20 g; pre-test speed, 2.0 mm/s; test speed, 8.0 mm/s; post-test speed, 8.0 mm/s; strain, 45%; the interval between two compressions, 2 s.

2.7 Water holding capacity

The determination of water holding capacity (WHC) in treated beef samples was conducted with a slight adjustment (Kahar *et al.*, 2021). The beef meat was precisely weighed at 1.5 g and subsequently placed in centrifuge tubes, which were then centrifuged at 4 000×*g* for 15 mins using a centrifuge (KUBOTA 3740, Japan). Post-centrifugation, the weight (g) of the sample was measured and compared to the weight (g) of the sample before centrifugation. The amount of released water was then divided by the original weight (g) of the sample before centrifugation to express WHC.

2.8 Cooking loss

The cooking loss of the treated beef samples was ascertained through the methods and equation in accordance (Jauhar *et al.*, 2020). The equation is as follows:

$$Cooking\ loss\ (\%) = \left(\frac{raw\ weight - cooked\ weight}{raw\ weight}\right) \times 100$$

2.9 pH

The treated beef samples were weighed 1 g and were subjected to 1:10 (w/v) homogenization with distilled water using a homogenizer (Heidolph, Germany). The pH values were then measured using a digital pH meter (Jenway 3505, United Kingdom).

2.10 Scanning electron microscopy

Cubes of the surface of the treated samples were obtained and then immersed in a 2.5% glutaraldehyde fixative solution in Sorensen's phosphate buffer, having a pH of 7.2, for 72 hrs at a temperature of 4°C. Following this, the samples were washed thrice with a sodium phosphate solution, with a pH of 7.2, for 10 mins at room temperature. The cubes were subsequently dehydrated through an ethanol series, which involved using ethanol with varying concentrations ranging from 30% to 100% twice, each for 20 mins. CO^2 was used as a transition fluid to dry the samples, which were then coated with gold-palladium via a fully automated sputter system (Joel Fine Coat Ion Sputter JFC-1100) to enhance conduction in the scanning electron microscope (JEOL JSM 6390 SEM). The characterisation of fibres and interfibrillar spaces was conducted through SEM imaging at 10 kV and 500× magnifications. Furthermore, the micrographs were analysed utilising ImageJ software.

2.11 Statistical analysis

The One-Way ANOVA method was employed to analyse all of the data using Minitab version 18 (Minitab, USA). The level of significance was set at $p < 0.05$ and Tukey's test was used to assess the significance level of the discrepancies between the mean values for different treatments. Correlation analysis by using Pearson's correlation matrix was also performed.

3. Results and discussion

3.1 Colour properties

Colour is important in determining the quality of the meat as it is a key factor in consumers' purchasing decisions and perception towards the freshness and quality of the meat (Jauhar *et al.*, 2020). Consumers commonly associate specific colours such as bright cherry red with the freshness of meat (Tomasevic *et al.*, 2021). Aside from red, the lightness of meat is also closely related to its freshness This study found raw beef samples exhibited significant increases in lightness (L*) value after being treated with papain and ultrasound

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individually or together (Table 1). This finding is aligned with a study that reported increasing in L* value has a negative impact on its quality, specifically, this increase exhibits an increase in free water that diffuses to the surface of the meat and results in an enhanced reflection of light (Suman *et al.*, 2013; Guo *et al.*, 2021). However, there were no significant differences in the a* and b* values of beef samples with different treatments compared to the control group ($p > 0.05$). The findings indicated that there was no visible impact on the chromaticity coordinates of the beef samples, especially redness and yellowness as a result of the varied treatments applied. More studies also reported the same findings (Dang *et al.*, 2022; Wu *et al.*, 2022). In addition, the combination of ultrasound and papain treatment in cooked beef samples led to a significant increase in their redness and yellowness ($p < 0.05$). This resulted in a similar finding in which utilising papain might be the factor of increased redness and yellowness of beef samples in this study (Pizarro-Oteíza *et al.*, 2020).

3.2 Warner-Bratzler shear force

The instrumental tenderness, which is also known as the Warner-Bratzler shear force (WBSF), refers to a test that aids in measuring the maximum force that replicates the purpose of a knife and the compression required to cut off or shear a meat sample (Novaković and Tomašević, 2017). The results in Table 2 indicated that there was no significant difference between the treatments. Nevertheless, the results highlighted a decreasing trend, indicating that the beef sample treated with ultrasound combined with papain solution had the lowest work of shear and max shear force, and demonstrated the potential effect of tenderisation. Ultrasound treatment can elevate the myofibril fragmentation index (MFI) which can effectively tenderise the beef (Kang *et al.*, 2017). Furthermore, the addition of papain enzyme in this treatment further enhances the tenderisation of beef due to its property to break down protein structure through protein hydrolysis (Barekat and Soltanizadeh, 2017).

3.3 Texture profile analysis

The assessment of organoleptic properties in food texture complexes involves the utilisation of texture profile analysis (Brandt *et al.*, 1963). Various parameters were measured to determine the textural profile of the beef samples, including hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience. Based on the results, the majority of the raw beef texture profiles did not exhibit significant differences, except for chewiness. The chewiness of raw beef samples treated with US and USP showed similarities but differed significantly from C and P. This is in line with a finding in which coupling both treatments significantly reduced chewiness, indicating a decrease in shear force (Changwei *et al.*, 2021). US and USP had significantly low chewiness due to the application of ultrasound which subsequently aids in protein denaturation, myofibril degradation and increased proteolysis (Xiong *et al.*, 2020). This enhanced the texture and tenderness of the meat (Stadnik *et al.*, 2008).

Based on Table 3, it was demonstrated that the cooked beef samples exhibited significant differences in hardness, springiness, cohesiveness, gumminess, chewiness, and resilience among the treatments. Notably, the USP treatment displayed the least hardness ($p<0.05$), whereas the controlled sample presented had the most hardness ($p>0.05$). Prior research has proven that ultrasound and papain enzymes provide a distinct advantage in enhancing meat tenderness without

Table 1. Colour properties of beef samples treated with ultrasound, papain or a combination of ultrasound and papain.

Treatment	Colour					
	Raw			Cooked		
	L*	a*	b*	L*	a*	b*
Control	31.90 ± 0.47^{B}	7.71 ± 1.10^{A}	0.53 ± 0.63^{A}	35.57 ± 1.15^{A}	8.69 ± 0.95^{B}	0.49 ± 0.62^{B}
Papain	34.30 ± 3.11^{AB}	7.72 ± 1.47^{A}	0.37 ± 0.76^{A}	34.98 ± 1.03^{A}	8.83 ± 0.36^{B}	0.91 ± 0.36^{B}
Ultrasound	36.93 ± 0.57^{A}	8.53 ± 0.97^{A}	0.33 ± 0.65^{A}	37.16 ± 0.35^{A}	10.62 ± 2.05^{AB}	1.54 ± 1.33^{AB}
Ultrasound + Papain	35.52 ± 0.25^{AB}	9.13 ± 2.65^{A}	0.80 ± 1.40^{A}	36.84 ± 1.72^{A}	13.34 ± 0.93^{A}	3.45 ± 0.44^{A}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

Table 2. Shear force analysis for beef samples treated with ultrasound, papain or a combination of ultrasound and papain.

Instrumental tenderness	Treatment			
	Control	Papain	Ultrasound	Ultrasound + Papain
Work of Shear (kg.s)	45.73 ± 2.31^{A}	38.19 ± 13.58^{A}	38.65 ± 14.68^{A}	37.04 ± 9.76^{A}
Max Shear Force (kg)	8.56 ± 0.12^{A}	6.69 ± 2.08^{A}	8.30 ± 2.59^{A}	6.43 ± 3.35^{A}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

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Table 3. Texture profile analysis on beef samples treated with ultrasound, papain or a combination of ultrasound and papain.

Texture profile	Raw samples							
	Treatments				Treatments			
	C	P	US	USP	C	P	US	USP
Hardness (g)	2670± 1021^{A}	1688± 358^{A}	1756± 959^{A}	1529.90±7 7.80^{A}	7167± 1811^{A}	6469± 1349^{A}	4028± 685^{AB}	3066± 641^{B}
Springiness	0.89± 0.14^{A}	0.98± 0.01^{A}	0.98± 0.01^{A}	0.74± 0.22^{A}	0.66± 0.02^{A}	0.60± 0.02^{B}	0.58± 0.02^{B}	0.55± 0.01^{B}
Cohesiveness	0.52± 0.04^{A}	0.53± 0.03^{A}	0.38± 0.09^{A}	0.39± 0.08^{A}	0.66± 0.55^{A}	0.55± 0.04^{B}	0.49± 0.52^{BC}	0.45± 0.01C
Gumminess	1369± 498^{A}	895± 240^{A}	604± 198^{A}	599± 155^{A}	4699± 988^{A}	3579± 997^{AB}	2009± 530^{BC}	1379± 291^{C}
Chewiness	1164± 205^{A}	871± 225^{AB}	590± 188^{B}	463± 252^{B}	3114± 627^{A}	2149± 540^{AB}	1170± 332^{BC}	756.30± 149.20^{C}
Resilience	0.34± 0.05^{A}	0.30± 0.06^{A}	0.24± 0.04^{A}	0.22± 0.05^{A}	0.34± 0.01^{A}	0.24± 0.05^{B}	0.21± 0.04^{B}	0.21± 0.02^{B}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$). C: control, P: Papain-treated sample, US: ultrasound-treated sample, USP: Ultrasound and papain-treated sample.

affecting other quality parameters (Stadnik *et al.*, 2008; Wang *et al.*, 2018; Peña-Gonzalez *et al.*, 2019). The mechanical work of ultrasound, which results in the rupturing of myofibrillar protein, disassociation of collagen macromolecules, and migration of proteins and other compounds, specifically contributed to the tenderisation of beef samples that were ultrasonicated (Stadnik *et al.*, 2008). The incorporation of papain solution into the ultrasound treatment further increased beef tenderisation. Papain's capacity to break down connective tissue and myofibril proteins resulted in an elevation of hydroxyproline and free amino acid content. Additionally, the USP treatment facilitated the breakdown of collagen and sarcolemma surrounding muscle fibres. It is important to note that meat tenderness is closely linked to collagen, as it exists in a substantial amount within the connective tissues (Ahmad *et al.*, 2020). This is further supported by the Pearson correlation analysis which had a significant positive linear relationship between the hardness of the beef samples and the work of shear of the beef samples ($p < 0.05$, $r = 1.000$) (Table 4).

3.4 Water holding capacity, cooking loss and pH

The WHC of food refers to its ability to retain water during processing, including the application of force, pressure, centrifugation, or heating. Table 5 indicates that no significant differences were observed among the treatments. However, there was an increase in water holding capacity on the beef samples treated with both ultrasound and papain enzyme. The same trend was exhibited in the cooking loss of the beef samples. The increase in cooking loss is due to the degradation of muscle fibre composed of protein. Papain enzyme denatures protein structures and fragments of myofibrils, leading to water reduction. Ultrasound treatment causes myofibril shrinkage and water movement, resulting in increased cooking loss (Botinestean *et al.*, 2018). USP treatment had the highest cooking loss percentage due to papain proteolysis and ultrasound treatment but is still acceptable as no statistical differences were observed. However, the pH of both raw and cooked beef samples significantly decreased ($p < 0.05$) after being treated with the combination of ultrasound and papain. This finding is in line with a study reported in which ultrasound-assisted

Table 4. Pearson correlation matrix between variables of treated beef samples.

Variable	Cooking loss	Water holding capacity	Work of shear	Shear force	Hardness	pH
Cooking loss	-					
Water holding capacity	0.557 (0.443)	-				
Work of shear	-0.874 (0.126)	-0.589 (0.411)	–			
Shear force	-0.555 (0.445)	-0.933 (0.067)	0.746 (0.254)	–		
Hardness	-0.875 (0.125)	-0.599 (0.401)	1.000 (0.000)	0.753 (0.247)	–	
pH	-0.142 (0.858)	-0.601 (0.399)	0.562 (0.438)	0.825 (0.175)	0.565 (0.435)	–

Pearson correlation coefficient, r, (p-value).

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Table 5. Water holding capacity, cooking loss and pH values of beef samples treated with ultrasound, papain or a combination of

Analysis	Treatment			
	Control	Papain	Ultrasound	Ultrasound + Papain
Water holding capacity (%)	64.05±3.04^{A}	65.35±0.91^{A}	63.74±1.71^{A}	66.67±3.28^{A}
Cooking loss (%)	21.97±5.66^{A}	26.17±7.72^{A}	29.78±1.85^{A}	32.44±2.59^{A}
pH of raw beef samples	6.76±0.06^{A}	6.16±0.07^{C}	6.73±0.14^{A}	6.49±0.05^{B}
pH of cooked beef samples	5.23±0.10^{A}	5.12±0.04^{AB}	5.03±0.07^{B}	5.08±0.05^{AB}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

enzyme treatment had a significant impact on the pH value of chicken breast meat (Cao *et al.*, 2021).

3.5 Scanning electron microscopy

The application of the scanning electron microscope (SEM) is prominent in examining solid object surfaces. Figure 2 highlights several differences in the structure of the treatments observed. The muscle fibre of the control sample demonstrated no discernible changes, while the other samples exhibited disruptions in fibre arrangement. The degradation of muscle fibre was particularly notable in the USP sample, where the cell membrane was ruptured. Additionally, meat tenderisation by USP resulted in the disruption of connective tissue structure in the beef sample (Ahmad *et al.*, 2020). Furthermore, ultrasound has the potential to affect the sarcomere structure, specifically around the I-band and the Z-line, due to the physical effect of the ultrasound treatment itself (Stadnik *et al*., 2008).

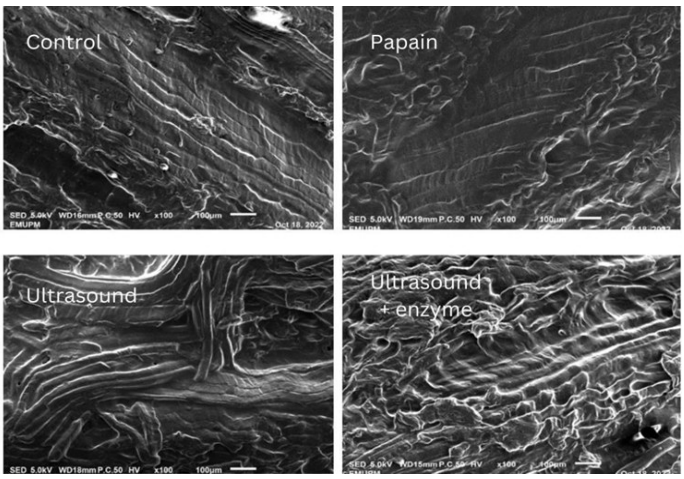

Figure 2. Scanning electron micrographs on treated beef samples.

4. Conclusion

In conclusion, the results of the study have demonstrated that the application of ultrasound in combination with papain enzyme treatment yields superior quality properties across almost all parameters, including colour properties, Warner-Bratzler shear force (WBSF), texture profile, water holding capacity (WHC), pH, and scanning electron microscopy (SEM). Therefore, it can be inferred that the use of ultrasound and papain enzyme treatment is a viable approach for enhancing the tenderness of beef without compromising other quality properties. Further research can enhance and extend the outcomes of this study. Alterations to the variables such as amplitude, holding time of ultrasound treatment, concentration of enzyme solution, diverse types of proteolytic enzymes, and size and type of meat sample can facilitate better results and validate this study. Moreover, sensory analysis can be carried out to determine the acceptability of treated samples by the consumers.

Conflict of interest

The authors declare no conflict of interest.

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