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Meat tenderisation effect of protease from mango peel crude extract

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Abstract

The objective of the present work was to investigate the effects of mango peel crude extract (MPCE) on meat tenderisation. MPCE possessed the proteolytic activity of 9,800 U/g. Beef top round (semimembranosus muscle) was incubated with MPCE (50 mL/100 g meat) for 1, 2, and 3 h at 25°C. Control (untreated) and control buffer (sample treated with phosphate buffer pH 7.5) were also prepared. The shear force value of the 2 h incubated beef samples significantly decreased 35% (from 94.4 to 61.2 N/cm^2), the total protein extractability significantly increased from 169.3 to 215.5 mg/g, the TCA-soluble peptides significantly increased from 85.9 to 119.3 µmole tyrosine/g, and the collagen solubility significantly increased from 8.7 to 15.7% as compared to the control samples. The 3 h incubated parameters were not significantly different from those of the 2 h. Scanning electron micrographs revealed that as the incubation period of MPCE increased, the integrity of muscle-fibre bundles decreased along with the gaps between these bundles. These results showed that MPCE was effective in tenderising otherwise tough meat, and can be used as an effective component for increasing the desirability of certain cuts.

Introduction

Meat tenderness has been identified as the primary determinant of eating-satisfaction among consumers. The meat industry is therefore likely to appreciate inexpensive and viable methods for tenderising (and thereby increasing the sales of) lower-value cuts (Miller *et al*., 2001). Many strategies have been employed to improve post-mortem tenderness and enzymatic hydrolysis; and the use of protease has become of the more practical methods. Currently, there is growing interest in discovering novel meat-tenderising protease from both plant and microbial origins.

Exogenous protease is one of the more effective methods for tenderising meat. Protease, derived from plants such as papain, bromelain and ficin, has long been used at household and industrial levels. It should be noted that, in either case, care should be exercised when using protease as its potency can result in over-tenderised and therefore less desirable meat. Protease could be extracted from plants and microorganisms. One example is the Kachri (*Cucumis trigonus* Roxburghi) fruit, traditionally used as a meat tenderiser in the Indian sub-continent, which contains a cucumicin-like (cucumicin EC 3.4.21.25) serine protease activity (Asif-Ullah *et al*., 2006). Other sources of protease, such as those from ginger rhizomes (*Zingiber officinale*) have also garnered substantial interest due to their collagenolytic activity. Choi and Laursen (2000) studied two distinct ginger proteases (GP-I and GP-II) revealing that both belong to the papain family of the cysteine protease.

Mango peel constitutes 15-20% of the entire fruit and becomes little more than waste whether consumed individually, sold retail, or processed by the food and beverage industries. Peel by-product becomes either animal feed or is merely discarded as solid waste into landfills. In an effort to maximise the potential of this by-product, Ajila *et al*. (2007) analysed the potential benefits of various compounds found in both raw and ripe mango peels. High levels of several beneficial compounds such as antioxidants, polyphenols, carotenoids, dietary fibre, pectin and vitamins were found therein, as well as various valuable enzymes, including serine protease. When dried, both raw and ripe mango peel displayed significant amounts of serine protease activity ranging from 4573 to 11173 U/g, with ripe peel having higher levels than raw (Ajila *et al*., 2007). Published research has focused primarily on either enzyme purification or the effects of drying methods in extracting protease from

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mango peel (Mehrnoush *et al*., 2011; Amid *et al*., 2012). However, these works did not explore other potential uses of the protease. Therefore, the present work explored the efficacy of protease derived from mango peel crude extract as a meat tenderiser. The microstructural changes of meat by using scanning electron microscopy (SEM) as well as protein extractability and collagen solubility were also examined to better explain the tenderisation effect of protease from mango peel.

Our preliminary study clearly showed that the maximum force required to cut through top round beef (semimembranosus muscle) (88.15 N) was approximately 50% higher than that of the premium cuts [tenderloin (psoas major muscle) (38.2 N), top sirloin (gluteus medius muscle) (36.0 N), and ribeye (longissimus thoracis muscle) (38.7 N)]. Therefore, the present work was focused on the tenderising effects of protease on top round beef. It should be noted that, although tenderness is desirable to consumers, it is assumed that tenderising more than 50% may result in mushy meat and an unpalatable experience for most consumers.

Materials and methods

Ripe *Nam Dokmai* mango (*Magnifera indica* L. var. *Nam Dokmai*) peel was donated by a Thai mango processing plant. The obtained mango peel was collected from the peel of mangoes harvested at commercial stage (100-105 days after flowering). Top round beef (semimembranosus muscles, Kampaeng Saen beef breed, approximately 8-10 months old) was also obtained from a local Bangkok slaughterhouse. The samples were rapidly transported to the laboratory while wrapped in polyethylene bags where they were stored at 4°C overnight before use. All chemicals and solvents were of analytical grade.

Preparation of mango peel crude extract

The crude enzyme extract of mango peel was prepared following the method optimised by Amid *et al*. (2012) with slight modifications. Briefly, mango peel was cut into small pieces (3 mm × 3 mm). Mango peel (10 g) was then blended with 100 mL 0.1 M sodium phosphate buffer (pH 7.5) at 4°C for 2 min. This homogenate was then filtered through cheesecloth and centrifuged at 15,000 g for 15 min at 4°C. Finally, the supernatant was recovered as MPCE and kept at -40°C until further use.

Determination of proteolytic activity

The proteolytic activity of MPCE was determined following the method described by Mehrnoush *et al*. (2011). The proteolytic reaction mixture contained 0.5 mL MPCE, 0.5 mL 50 mM Tris-HCl buffer (pH 8.0) and 0.5 mL 1.5% (w/v) azocasein. The enzyme reaction was stopped by adding 0.5 mL trichloroacetic acid (30%, w/w). The mixture was kept for 20 min at room temperature. The supernatant was obtained by centrifugation at 13,400 *g* for 10 min, and was then filtered through a 0.45 μm nylon membrane filter. The protease activity was measured with a spectrophotometer (335 nm). One unit protease activity was defined under aforementioned conditions as the activity resulting from 0.01 absorbance unit at 335 nm per hour and expressed as U/g dry mango peel.

Preparation of beef samples

Top round muscles were aged for 7 d at 4°C, trimmed of all visible fat and connective tissues, and then cut into uniform slices of 8 × 6 × 2 cm parallel to muscle fibres starting from the proximal side (sample weight: 100 g ± 5 g). Five treatments (control, control buffer, 1 h MPCE incubation, 2 h MPCE incubation and 3 h MPCE incubation) were assigned randomly to each top round slice (six slices for each treatment). Each slice was pierced using a kitchen fork to facilitate the absorption of enzyme crude extract into the meat. The beef was then incubated with 50 mL MPCE in a zip-lock plastic bag (8 cm × 12 cm) for 1, 2, and 3 h at 25°C. A control beef sample without any treatment was kept at 25°C for 30 min, while beef incubated for 3 h in a 0.1 M phosphate buffer (50 mL/100 g meat) of pH 7.5 became the control buffer.

After their respective incubation times, three slices from each treatment were removed from their separate bags, homogenised, and prepared to determine their pH, water-holding capacity, protein extractability, TCA soluble peptides and collagen solubility. The microstructure of the intact raw beef was determined via SEM. The remaining three slices for each treatment were sealed with polyethylene bags and individually cooked in a water bath at 97 ± 2°C until a core temperature of 75°C was reached. After cooking, the cooked meat was cooled at room temperature for 30 min and weighed. Finally, the textural properties and cooking loss of each sample were determined.

Determination of textural properties and cooking loss of cooked beef samples

Each beef slice was further divided into 4-6 strips (1 cm × 1 cm cross-section) and cut parallel to the fibre direction. After placing the meat strips individually on the texture analyser (Stable Micro System, TA.XT Plus, London, UK), they were sheared perpendicular

to the muscle-fibre orientation with a sharp cutting blade probe (HDP/BS) at a 2 mm/s cross head speed with a 50 kg load shell. The maximum force required to cut the meat samples was measured in newtons (N) (Honikel, 1998). The textural measurement was run eight times for each sample. The cooking loss of each sample was calculated as the weight differences before and after cooking and expressed as a percentage of the initial sample weight (Zhang *et al*., 2017) as shown in the following equation:

$$\text{Cooking loss (\%)} = \frac{(\text{weight before cooking} - \text{weight after cooking})}{\text{weight before cooking}} \times 100$$

Determination of pH and water-holding capacity

Each beef slice was ground in a Moulinex HV6 grinder (Groupe SEB, Ecully Cedex, France). Minced meat (10 g) was homogenised with 50 mL distilled water, and pH was measured by pH meter (Orion 2 star, Thermoscientific, USA). Minced meat (20 g) was placed in a centrifuge tube containing 30 mL 0.6 M sodium chloride, and stirred with glass rod for 1 min. The tube was kept at 4°C for 15 min. The tube was then stirred again and centrifuged at 3,000 g for 25 min. The volume of supernatant was measured and amount of water retained by samples was expressed in percentage as water holding capacity (Naveena *et al*., 2004), as shown in the following equation:

$$\text{Water holding capacity} = \frac{(\text{vol. of NaCl added} - \text{vol. of supernatant})}{\text{vol. of NaCl added}} \times 100$$

Determination of protein extractability

The protein extractability of each raw beef sample was determined following the method described by Joo *et al*. (1999). The sarcoplasmic protein was extracted by homogenising minced meat (2 g) with 20 mL ice-cold 0.025 M phosphate buffer (pH 7.2). The total soluble protein was extracted by homogenising minced meat (2 g) with 40 mL ice-cold 1.1 M potassium iodide solution in a 0.1 M phosphate buffer (pH 7.2). Homogenates were centrifuged at 1,500 *g* for 20 min, and the protein concentration in each supernatant was determined by the Biuret method. Myofibrillar protein concentrations were calculated as the difference between the total soluble and sarcoplasmic protein values as shown in the following equation:

$$\text{Myofibrillar protein} = \text{total soluble protein} - \text{sarcoplasmic protein}$$

Determination of TCA-soluble peptide content

The TCA-soluble peptide content of each raw sample was measured following the method described by Benjakul *et al*. (2002). Specifically, 2 g sample were homogenised with 18 mL 5% (w/v) TCA for 1 min, and kept at 4°C for 1 h. The homogenised mixture was centrifuged at 8,000 *g* for 5 min. The soluble peptides in the supernatant were measured using the Lowry method (Lowry *et al*., 1951). The TCA-soluble peptides content was expressed as µmol of tyrosine/g of sample.

Determination of collagen solubility

Five grams of raw beef sample were placed in a 250 mL beaker covered with a Petri dish, and immersed in a water bath at 100°C for 30 min. The cooked meat was then removed from the beaker, cut into small pieces and homogenised with 50 mL distilled water at 4°C in a blender for 2 min. The extract was then centrifuged at 1,500 *g* for 30 min (Naveena *et al*., 2004). The supernatant and solid residue was hydrolysed separately with 6 N hydrochloric acid for 24 h at 100°C in a water bath. Aliquots of both hydrolysates were used for hydroxyproline estimation. Hydroxyproline was determined by measuring absorbance at 540 nm using spectrophotometer (PG Instruments Limited, T60, Leicestershire, UK) and referring to a standard curve. The collagen content was calculated from the hydroxyproline content using a multiplication factor of 7.14. The collagen content of the supernatant was considered soluble while that of the solid residue was insoluble, as shown in the following equatios:

$$\text{Collagen content} = \text{hydroxyproline content} \times 7.14$$

$$\text{Total collagen} = \text{soluble collagen} + \text{insoluble collagen}$$

$$\text{\% Collagen solubility} = (\text{soluble collagen} / \text{total collagen})$$

Scanning electron microscopy

The microstructure of the raw meat samples was determined using a SEM. Muscle samples (2-3 mm in thickness) were fixed overnight in a 2.5% (v/v) glutaraldehyde solution in a 0.2 M phosphate buffer (pH 7.2) at 4°C. Each sample was then rinsed for 1 h with distilled water and then dehydrated in acetone with a serial concentration of 20-100% (v/v). Critical-point drying of the sample was done using CO_2 as a transition fluid. The dried samples were mounted on a bronze stub and sputter-coated with gold. The specimens were observed using SEM with

a magnification of 350× at an acceleration voltage of 10 kV.

Statistical analysis

Results were expressed as mean ± standard error. A randomised block design (RCBD) was used to assess the effectiveness of MPCE in improving meat tenderness. Top round beef served as a block. From each block, one of five experimental units was randomly assigned to the five different treatments (control, control buffer, 1 h MPCE incubation, 2 h MPCE incubation and 3 h MPCE incubation). The entire experiment was run on three separate occasions (n = 3). Each measurement was run at least in duplicate. Statistical analysis was performed with the analysis of variance (ANOVA) using SPSS version 12. Significant difference among mean values from ANOVA was determined by Duncan's multiple range test, and was considered significant at $p < 0.05$.

Results and discussion

Protease activity of MPCE

The protease activity of prepared MPCE was 9,800 U/g. Our preliminary study showed that proteolytic activity of raw and ripe mango peel of different cultivars namely Nam Dokmai, Raed, Kaew Savoew and Kaew Kamin showed that both raw and ripe peels possessed overall good proteolytic activity. It was shown that the proteolytic activities varied according to cultivars and maturity levels ranged from non-detectable level in ripe Kaew Kamin to 11,675.95 U/g of raw *Nam Dokmai* peel (Dhital and Vangnai, 2013).

Texture properties and cooking loss

The instrumental texture measurement is an indicator of meat tenderness in which a lower shear force value is associated with more tender meat. Table 1 shows that the shear forces of all enzyme-treated samples significantly ($p < 0.05$) decreased as compared to their control; measured shear forces were reduced by 26.8, 35.1, and 35.8% for incubation times of 1, 2 and 3 h, respectively. The shear force required to cut samples decreased significantly ($p < 0.05$) when incubation time was increased from 1 to 2 h. However, there was no significant difference when the incubation time was extended from 2 h to 3 h. Several studies have been conducted to evaluate the efficacy of various plant crude extracts for tenderising meat such as ginger extract, Kachri extract (Naveena *et al*., 2004), kiwi fruit extract (Han *et al*., 2009), and pineapple extract (Ketnawa and Rawdkuen, 2011). All reports found a decrease in the shear force of the meat after applying these plant extracts. The decrease in shear force of MPCE-treated samples could be due to the proteolytic weakening of muscle proteins, as well as solubilisation of collagen, which could be explained by the measurements of protein extractability and collagen solubility as results shown in the following sections.

Cooking loss is an important parameter; it impacts not only on the final yield of product but also affects the quality of the meat, i.e. tenderness, juiciness, etc., and beef with a lower cooking loss is commonly found to be juicier (Toscas *et al*., 1999). The cooking loss values of MPCE-treated samples and the control are shown in Table 1. The results show that the cooking loss of the control and the control buffer samples were not significantly ($p < 0.05$) different. Similarly, the cooking losses of these control samples were not significantly ($p < 0.05$) different from the 1-h MPCE-incubated sample. However, the cooking loss values were observed to be significantly ($p < 0.05$) higher in the 2 h and 3 h MPCE-incubated samples. This may have been due to myofibrillar protein and/or connective tissue degradation leading to a loosening of the muscle structure, an increase in water evaporation during cooking, and ultimately an increase in cooking loss (Honikel, 2009). An increase in cooking loss in protease-treated beef steak compared to an untreated sample was also observed in other studies. Pietrasik and Shand (2011) found that a higher cooking loss was observed in samples treated with microbial protease. Similarly, the cooking loss of buffalo meat treated with 2% Kachri extract also increased, while the changes in a sample treated with a 5% ginger solution and a 0.2% papain solution was not significant (Naveena *et al*., 2004).

Table 1. Shear force, reduction in shear force and cooking loss of incubated beef samples (semimembranosus muscle) with mango peel crude extract (MPCE).

Treatment	Shear force (N/cm^2)	Reduction in shear force* (%)	Cooking loss (%)
Control	94.4 ± 0.91^{a}	-	40.9 ± 0.12^{b}
Control buffer	90.7 ± 0.85^{a}	-	40.8 ± 0.28^{b}
1 h MPCE incubation	69.0 ± 0.96^{b}	26.8 ± 1.02^{b}	40.3 ± 0.13^{b}
2 h MPCE incubation	61.2 ± 0.61^{c}	35.1 ± 0.64^{a}	41.9 ± 0.34^{a}
3 h MPCE incubation	59.9 ± 0.74^{c}	35.8 ± 0.79^{a}	42.1 ± 0.48^{a}

Control = beef sample without any treatment (kept at 25°C, for 30 min). Control buffer = beef sample incubated for 3 h in 0.1 M phosphate buffer (50 mL/100 g meat) of pH 7.5. *Control buffer was used to calculate the reduction in shear force for 1 h, 2 h, 3 h MPCE incubation. Data are mean of triplicates (n = 3) ± standard error. Means with different superscripts in a column are significantly ($p < 0.05$) different.

Table 2. The pH, water-holding capacity and protein extractability of incubated beef samples (semimembranosus muscle) with mango peel crude extract (MPCE).

Treatment	pH	Water-holding capacity (%)	Myofibrillar protein (mg/g)	Sarcoplasmic protein (mg/g)	Total soluble protein (mg/g)
Control	5.7 ± 0.70^{c}	15.3 ± 0.70^{b}	114.0 ± 1.37^{c}	55.3 ± 0.60^{c}	169.3 ± 1.29^{d}
Control buffer	6.2 ± 0.01^{b}	37.9 ± 1.17^{a}	116.0 ± 1.17^{c}	58.9 ± 1.18^{b}	174.9 ± 2.31^{c}
1 h MPCE incubation	6.1 ± 0.04^{b}	39.7 ± 0.47^{a}	130.9 ± 2.37^{b}	71.7 ± 0.42^{a}	202.6 ± 2.08^{b}
2 h MPCE incubation	6.3 ± 0.00^{a}	42.6 ± 1.64^{a}	142.6 ± 0.97^{a}	72.9 ± 0.83^{a}	215.5 ± 1.58^{a}
3 h MPCE incubation	6.3 ± 0.07^{a}	42.4 ± 1.63^{a}	143.7 ± 0.65^{a}	73.3 ± 0.61^{a}	217.0 ± 0.70^{a}

Control = beef sample without any treatment (kept at 25°C, for 30 min). Control buffer = beef sample incubated for 3 h in 0.1 M phosphate buffer (50 mL/100 g meat) of pH 7.5. Data are mean of triplicates (n = 3) ± standard error. Means with different superscripts in a column are significantly ($p < 0.05$) different.

pH and water-holding capacity

The pH of samples incubated with MPCE increased with increasing incubation time (Table 2). The pH values of 2 h and 3 h incubated samples significantly yielded the higher pH ($p < 0.05$) than 1 h incubated and control buffer samples. The water-holding capacity in MPCE incubated samples were in the range of 15-43%, considerably similar to those reported by Ketnawa and Rawdkuen (2011), which were 16-40%. Result (Table 2) shows that the water-holding capacity of control buffer sample was significantly higher than that of the control sample ($p < 0.05$). This was due to the use of phosphate buffer (pH 7.5) which gave the excess negative charges at high pH (Ketnawa and Rawdkuen, 2011). The water-holding capacities of all MPCE incubated samples were not significantly different with each other and control buffer sample ($p > 0.05$). The increase in water-holding capacity in the control buffer sample and MPCE incubated samples was caused by the increase of pH resulting from the use of phosphate buffer (pH 7.5). Increased pH of meat increases the net charge of actomyosin which allowed more intercellular space to further expand via electrostatic repulsion for additional water to be immobilised leading to the higher water-holding capacity (Yada, 2004).

Protein extractability

Immediately after exsanguination, muscle is both soft and pliable. However, after the onset of rigor mortis, meat toughens permanently; this is due to the formation of actomyosin within the muscle. Weakening of actomyosin by proteolytic enzymes has been reported to improve meat tenderness (Takahashi, 1996). As the actomyosin becomes loose or loses integrity, it can be easily extracted with a salt solution. Simply put, protein extractability increases as tenderisation increases. Researchers report that changes in myofibrillar (salt-soluble) protein extractability have a pronounced effect on meat tenderness while sarcoplasmic (water-soluble) protein extractability is less significant. The determination of both myofibrillar and sarcoplasmic protein extractabilities would help indicate the overall proteolytic activity of enzymes. Table 2 shows the myofibrillar, sarcoplasmic, and total protein extractabilities of the meat samples subjected to different treatments. Results show that a phosphate buffer (pH 7.5) alone could slightly improve the total protein extractability from 169.3 mg/g (control sample) to 174.9 mg/g (control buffer sample). All meat samples incubated with MPCE had higher sarcoplasmic and myofibrillar protein extractabilities than those of the control and control buffer samples. The higher values of myofibrillar and sarcoplasmic protein extractabilities in all MPCE-incubated samples were probably due to the hydrolysis of both myofibrillar and sarcoplasmic proteins by the proteolytic enzyme presented in MPCE. This most likely led to a loosening of the protein structure, which was easily extracted by water and salt solutions. These results agree with Sullivan and Calkins (2010) who found that protein extractabilities in actinidin-treated beef samples were higher than the control. Naveena *et al*. (2004) also found a marginal increase in the myofibrillar and sarcoplasmic protein extractability in papain, ginger extract and Kachri extract-treated buffalo meat samples. Among MPCE-treated samples, incubation of 2 h and 3 h resulted in significantly higher myofibrillar extractability than that of samples incubated for only 1 h. Longer treatment times allowed for greater enzyme interaction upon meat proteins and increased myofibrillar protein extractability. However, in the case of sarcoplasmic protein extractability, the three MPCE-incubated samples of different incubation times were not significantly ($p > 0.05$) different, possibly due to sarcoplasmic protein being more resistant to enzyme degradation than myofibrillar protein (Kang and Rice, 1970).

TCA-soluble peptides

The determination of TCA-soluble peptides has been used by many researchers to study the degree of hydrolysis of muscle proteins (Ketnawa and Rawdkuen, 2011). During measurement, long chain peptides and proteins are precipitated. TCA-soluble fractions contain some small peptides (molecular weight <10,000 D), free amino acids and other non-protein nitrogenous substance-like free bases (pyrimidines and pyridines) and nucleic acid (Sathe, 2012). Therefore, high levels of TCA-soluble peptides indicate a higher degradation of muscle protein by proteolytic enzymes. Several researchers have pointed out the positive relationship between TCA-soluble peptides and meat tenderness, specifically, the higher the level of TCA-soluble peptides, the greater the degree of meat tenderness (Ketnawa and Rawdkuen, 2011). The levels of TCA-soluble peptides in samples for each treatment are shown in Table 3. The TCA-soluble peptides of all MPCE-treated samples were significantly ($p < 0.05$) higher than those of the control and control buffer samples. An approximate 28% increase in TCA-soluble peptide content was found in samples treated with MPCE for 2 h as compared to the control buffer. The TCA-soluble peptides increased significantly ($p < 0.05$) when incubation time was increased from 1 h to 2 h. However, there was no significant difference when extended to 3 h as compared to 2 h.

Table 3. TCA soluble peptides, total collagen and collagen solubility of incubated beef samples (semimembranosus muscle) with mango peel crude extract (MPCE).

Treatment	TCA soluble peptides (μmol tyrosine/g)	Collagen solubility (%)
Control	85.9 ± 0.95^{c}	8.7 ± 0.24^{c}
Control buffer	84.3 ± 2.41^{c}	8.2 ± 0.34^{c}
1-h MPCE incubation	98.0 ± 1.21^{b}	12.9 ± 0.57^{b}
2-h MPCE incubation	119.3 ± 0.91^{a}	15.7 ± 0.21^{a}
3-h MPCE incubation	118.7 ± 0.69^{a}	16.5 ± 0.26^{a}

Control = beef sample without any treatment (kept at 25°C, for 30 min). Control buffer = beef sample incubated for 3 h in 0.1 M phosphate buffer (50 mL/100 g meat) of pH 7.5. Total collagen of all samples ranged between 6.3-7.2 mg/g. Data are mean of triplicates ($n = 3$) ± standard error. Means with different superscripts in a column are significantly ($p < 0.05$) different.

Collagen solubility

The presence of connective tissue and the degree of cross-linking between collagen molecules within a meat sample also influence the tenderness or toughness of meat. The cross-linkage between collagen molecules within muscle increases with the age and degree of physical activity of the animal, resulting in resistance to enzyme hydrolysis and cross-linkage solubilisation. The determination of collagen content and collagen solubility are important in understanding the activity of proteolytic enzymes on meat. Table 3 shows that the percentages of collagen solubility differed significantly ($p < 0.05$) among the samples. Collagen solubility increased from 8.7% (control sample) to 12.9, 15.7 and 16.5% in meat incubated with MPCE for 1 h, 2 h, and 3 h, respectively. In accordance with the results regarding shear force, collagen solubility significantly ($p < 0.05$) increased when incubation time increased from 1 h to 2 h. However, there was no significant difference when the incubation time was extended to 3 h. These results are in agreement with various previous works where the total collagen of meat remained unchanged but the solubility of collagen increased as the meat tenderised. Naveena *et al*. (2004) found that the collagen solubility of meat after treatment with various proteolytic enzymes from plant extracts and commercial papain increased from 6.6 to 12.9%. Kim *et al*. (2013) found that the total collagen solubility of beef treated with various levels of soy sauce increased from 2.5 to 40%. Oreskovich *et al*. (1992), Burke and Monahan (2003) and Naveena *et al*. (2011) also found a strong relationship between collagen solubility and meat tenderness.

Scanning electron microscopy

Scanning electron microscopy (SEM) was used to visualise the meat microstructure, especially muscle fibre bundles, whose integrity and compactness determine meat tenderness. Several researchers (Ramezani *et al*., 2003; Naveena *et al*., 2011; Kim *et al*., 2013) also used SEM to visualise the microstructure of meat in their studies of tenderisation. The SEM results are shown in Figure 1. In the SEM micrograph of the control sample, the muscle fibres bundles were very tightly arranged and seemed to be regular; this indicated a lack of disruption, having remained intact. However, in other MPCE-treated samples, gaps within the muscle structure increased with increased incubation time from 1 h to 3 h and a looser arrangement of the muscle fibres was also observed due to disruption and fragmentation. The solubilisation of perimysium, which contributed 90% of the total intramuscular connective tissue of the meat, is believed to be the main contributor of the intermuscular gaps and variations in connective tissue.

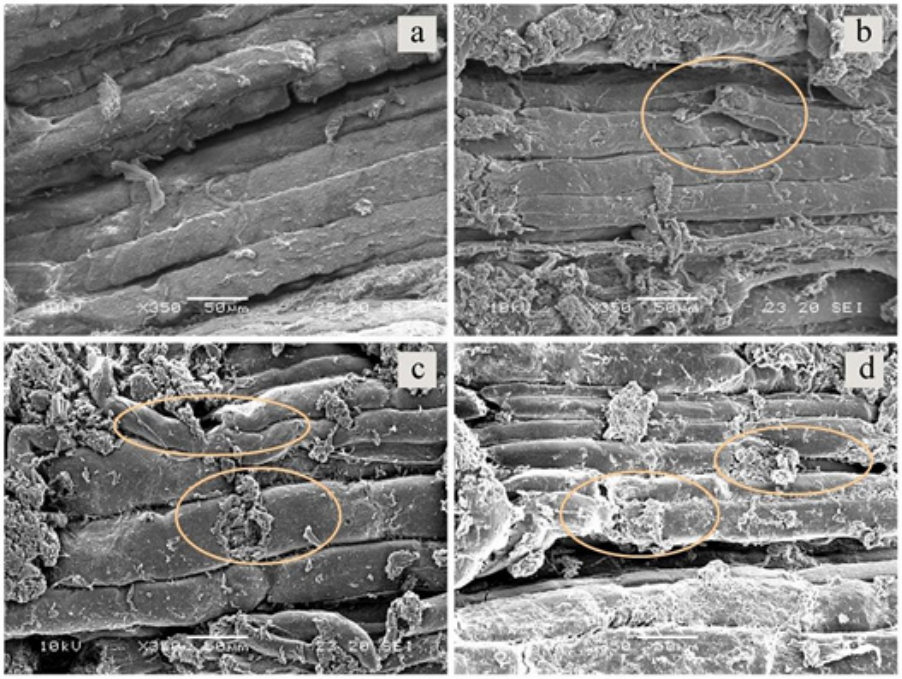

Figure 1. Scanning electron micrograph (SEM) of top round cut (bovine semimembranosus muscle) sample: control (a), and beef semimembranosus muscle samples incubated with mango peel crude extract (MPCE) for 1 h (b), 2 h (c) and 3 h (d), bar = 50 μm. Circles indicate some disruption and fragmentation of muscle fibres in MPCE-treated samples.

Conclusion

As indicated by the meats' shear force reduction, tenderness improved by 35% after 2 h incubation with mango peel crude extracts (MPCE). The improvement of tenderness could be understood from results of increased collagen solubility and myofibrillar fragmentation which were greatly explained by the increase of protein extractability and TCA soluble peptides. The degradation of myofibrillar protein and the disruption of connective tissues were clearly observed through scanning electron microscopy. Lastly, the tenderisation effect of MPCE could be attributed to various mechanisms especially increased proteolysis and collagen solubility as affected by incubation time. Therefore, mango peel has the potential to be utilised as an effective and inexpensive meat tenderiser at both industrial and retail levels.

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