

Microstructural and Protein Subunit Analysis: The Effect of Protease from Chayote, Pineapple Peel and Biduri Leaves as Natural Tenderizer on Mutton, Beef and Buff

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Abstract

Mutton, buffalo and beef meats have a tough texture and sometime is hard to eat. Naturally, enzymatic processing using plant-based compound, including, chayote (*Sechium edule*), pineapple (*Ananas comosus*) and biduri (*Calotropis gigantea*) contains abundance protease that potential to be developed. This research aims to compare the effect chayote, pineapple peel and biduri leaves to tenderize mutton, beef and buff meat at various doses. The sample meats, mutton, beef and buff were obtained from upper leg (round) part. The chayote, pineapple peel powder and biduri leaves was processed into powder and used to smear meats surface with the concentration of 5, 10 and 15 % of natural tenderizers powder from total meat wight (w/w), for 60 min. After treatment the Barford protein analysis using Bio-Rad protein assay was conducted to measure protein concentration, continued with SDS-PAGE analysis using Laemlli method. The structural observation was performed using Scanning Electron Microscope (SEM). The result performs hydrolysis process using pineapple and biduri leaves on mutton and beef produce light protein bands below 26, 34 and between 55 to 43 kDa. The application of natural tenderizer, including pineapple peel, biduri leaf and chayote peel is effectively tenderize the mutton, beef and buff meat. Furthermore, the pineapple peel and biduri leaves powder are the most potential tenderizer for mutton and beef. However, buff is most least tenderized meat among other and it may need an extra dosage or incubation period to get appropriate result.

Keywords: Enzymatic processes, Meat tenderness, Microstructural, Protease, Protein profiles

Introduction

Mutton, beef and buff are most common consumed meats in Indonesia. The meats have a dense collagen and myofibril structure that make it tough and hard to eat especially from active muscle [1,2]. Moreover, meats toughness is also influenced by the muscle type and origin [3], animal's age, water-holding capacity and sex [4]. In fact, the meats commonly cooked in high temperature for long duration that may destroy the nutrition, time-consuming and vanish the flavour [5]. Furthermore, those reason become main trigger that increases consumer demand for tender meat from calves. It will certainly threaten the availability of livestock for sustainable consumption, afterwards young animal's meat is more expensive [6-8]. To solve those problems, tenderizing method should be developed to discover an easy, economic and applicable technique.

The common techniques in culinary are by fermentation, sous-vibe cooking, freeze or dry-aging process in controlled temperature at least for 2 - 4 weeks may improve the meats' taste and nutrition [5,9]. However, those techniques are time consuming and may not applicable in high moisture and tropical condition, as like as in Indonesia. High humidity and warm condition may provide suitable environment for flesh-eating bacteria to growth faster, rotting the meat, change the flavour and make it inedible [10,11]. Another solution is by using natural active protease that can be collected from plant, including chayote (*Sechium edule*) [12], pineapple (*Ananas comosus*) [13] and biduri (*Calotropis gigantea*) [14]. The chayote's fruit is well known and widely used for various dishes, then pineapple is commonly consumed for dessert, however the fruit peel and sap are discarded. Then, the biduri plant is used for

decorative plant, while the flower is used for accessory, even though the leaf contains abundance protease.

Previous research reported that the protease in chayote fruit sap has a higher activity than other plant parts, relatively. Ratnayani *et al.* [15] stated that protease activity in chayote is more effective compared to papaya sap. On the other hand, protease also can be found from pineapple fruit and well known as bromelain [16]. However, the pineapple flesh fruit is main consumable part, but the peel is removed and rubbish. Even though, the pineapple peel contains abundance bromelain and potentially used as tenderizer after being processed [16,17]. An alternative abundance protease can be collected from decorative plant biduri (*Calotropis gigantea*), which has not been explored much. Furthermore, protease characteristic in biduri plant may indicated strong enzymatic activity due to exopeptidase [18,19]. This enzyme breaks down polypeptides at the amino or carboxyl terminal depends on whether the single or dipeptide of amino acid is released from the amino or the carboxy terminal, resulting in long-chain peptides and amino acids [20,21]. However, the effective dosage and application of those plants as tenderizer are still needed to be explored to maintain the quality, taste and texture of the meats.

A proper understanding about tenderizer effect and process can be well understanding scientifically using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and SEM methods [22]. The result from SDS-PAGE and SEM analyzes provide information on protein bands formed after treatments regarding of the hydrolyzed protein weight. In further, SEM analysis provides a microstructural image of muscle fibers and collagen tissue, which are responsible for meat thickness, that describe enzyme effect on protein breakdown. The microstructure and protein weight comparison study of those 3 plant-based proteases effect on mutton, beef and buff needs to be investigated to get proper tenderizing technique in economical and easy materials in Indonesia. This research aims to compare the effect of giving proteases from chayote, pineapple peel and biduri leaves to tenderize mutton, beef and buff meat at various doses.

Materials and methods

Preparation of natural tenderizers powder

The chayote and pineapple were obtained from local market, then the biduri leaves was collected from university's garden. Fresh-mature chayote and pineapple were washed and dried, then peeled lengthwise shapes. The biduri leaves were taken from the leaves in 4th position from the shoot. Chayote peel, pineapple peel and biduri leaves were cut into small pieces and dried using vacuum drying tube at a temperature of 50 °C for 12 h following procedures [23]. After dried the materials were grinded and sieved using a 100-mesh sieves, then used as natural tenderizer powders.

Tenderizer treatment on meat with chayote (*Sechium edule*) peel powder, pineapple peel powder and biduri leaf powder

The meats were obtained from the upper thigh (round) because it is assumed that the muscle part is actively used and has a hard texture. The meats were cut across the direction of the meat fibre into 3×3×1 cm³ square shape then weighed to determine the natural tenderizer powder weigh that should be used compare to the meat weight. The treatment was conducted by covering cube-shaped meats using natural tenderizer powder with a concentration 5, 10 and 15 % from total meats weight (w/w), respectively. Then, all tenderizers-covered meats was incubated at room temperature (25 - 28 °C) for 60 min [23].

After natural tenderizer application, the meat was brushed until clean and rinsed slowly by soaked in the 1 L of aquadest for 3 time. The cleaned meat was then dried and cut into thin slices by removing approximately 1 mm of the surface layer that contacts with the natural tenderizer powder during tenderizing process. Cleaned-meats were taken as much as 1×1×1 cm³ square shape and prepared for SEM analysis. The remaining meats were then cut into small pieces and weighed as much as 3 g and mashed using a mortar, separately. Each ground meats were put in 9 conical tubes, then added 8 mL of phosphate buffered saline (PBS) per each tubes and centrifuged at 6,000 rpm for 20 min. The supernatant from each sample was taken and used for protein concentration measurement and SDS PAGE analysis.

Scanning Electron Microscope (SEM)

Prepared meat samples (mutton, beef and buff) were dried using oven at temperatures of 50 °C up to 55 °C for 72 h). Each sample meats were then cut 1 mm of thickness and glued on sample holder covered using carbon tip, then inserting into an auto-fine coating tool for sample surface using gold colloidal coating. Meat-surface microstructure condition was captured using SEM JEOL JSM-6510LA (JEOL Ltd: Tokyo, Japan) under manufacture procedures.

Separating meat protein using SDS-PAGE

Protein concentration was calculated using the Bradford method following the research procedure of Kartika *et al.* [23]. The supernatant of each meat from previous procedure was taken as much as 2 μL and then added with 798 μL of distilled water, and 200 μL of Bio-Rad Protein Assay containing Coomassie brilliant blue R250 (CBB), cat. number 5000002 (Bio-Rad: California, USA), respectively. The total protein measurement was conducted based on the Bradford dye-binding method for protein assay by following standard procedure [24,25]. The assay is used with samples having protein concentrations between 200 and 1,400 $\mu\text{g}/\text{mL}$ (20 - 140 μg total). The sample and reagent were then vortexed to homogenize and incubated at room temperature for 10 min. Protein concentration was measured using a spectrophotometer with a wavelength of 595 nm. Protein concentration standard curve was obtained from protein standards using graded concentration from 1 to 10 $\text{ng}/\mu\text{L}$ of bovine serum albumin (BSA).

Protein separation using SDS-PAGE was conducted by following Laemmli [26] procedure. Acrylamide gel was prepared by diluted 29 g acrylamide and 1 g N,N'-methylenebisacrylamide in 100. mL of H_2O then filtered and placed in duran flask. The SDS-PAGE was prepared sceptically and following standard procedure. The prepared samples were mixed with buffer 5 \times , then heated for 2 min in boiling water and cooled in ice-water. Sample running was conducted using electric current with a voltage of 100 mA and 50 V. The gel was taken and stained with 0.25 % CBB for 30 - 60 min, then destaining for 4 times until the colour fades, and replaced with a 10 % glacial acetic acid solution. Then pressed using plastic and dried for 48 h in a dark room. To determine the molecular weight of a protein by calculating the Rf which was then plotted on the standard curve of protein molecular weight, the value of Rf as the X axis and the logarithmic value of the molecular weight as the Y axis.

Data analysis

The protein concentration was then analysed statistically using 2-way ANOVA test and continued with Tukey test. The ANOVA analysis was determined by the research design, the independent variables were consisted of natural tenderizers as 1st factorial and it different concentration for 2nd factorial. All of the statistical analysis was conducted using SPSS-21v with significant value ≤ 0.050 at confident interval (CI) = 95 % and significance level (α) ≤ 0.050 . Meats protein structure was observed using SEM and analysed qualitatively by describing the observed condition.

Results and discussion

Based on the analysis, 15 % of chayote peel powder is the most effective natural tenderizer for tendering the mutton and beef. While, 10 % of chayote peel powder is the significantly effective in tendering the buff. The natural tenderizers application on mutton, beef and buff significantly affect the protein concentration of the meats ($p \leq 0.001$). Total protein concentration has increased alongside with the increase of natural tenderizer concentration. However, the protein concentration in the mutton was not different significantly after treated with 10 and 15 % of all natural tenderizer (**Table 1**). It is indicating that high concentration of chayote, pineapple and biduri leaves powder has a same effect on mutton protein.

Table 1 Natural tenderizer powders effect on detected protein concentration of mutton.

Treatment	Protein concentration ($\mu\text{g}/\mu\text{L}$)			<i>p</i> -value ⁱ
	CP	PP	BL	
Control	16.49 \pm 0.31 ¹	16.49 \pm 0.30 ¹	16.49 \pm 0.30 ¹	0.001
5 %	15.22 \pm 0.35 ^{a2}	16.16 \pm 0.51 ^{b2}	20.04 \pm 0.58 ^{c2}	
10 %	12.60 \pm 0.19 ^{a3}	15.99 \pm 0.50 ^{b3}	17.31 \pm 0.57 ^{c3}	
15 %	12.61 \pm 0.13 ^{a3}	15.97 \pm 0.51 ^{b3}	17.91 \pm 0.56 ^{c3}	
<i>p</i> -value ⁱⁱ		0.001		

Note: CP = chayote peel powder, PP = pineapple peel powder, BL = biduri leaves powder; Alphabet codes: ⁱ) *p*-value for mean similarity of protein concentration based on the natural tenderizer dosages; ⁱⁱ) *p*-value for mean similarity of protein concentration based on the natural tenderizer material; ^{a-c}) indicates significantly different among tenderizer materials; number sign (¹⁻³) the different number indicates significantly different among concentration treatment, and not significant for same number. Statistically, *p*-value for variable interaction between concentration and tenderizer type is 0.001 at CI = 95 % and $\alpha = 0.050$.

The protein concentration decreased in the 10 and 15 % of chayote and pineapple peel powder compare to control mutton. However, it experienced the highest increase in application of biduri leaves treatment in all concentration, especially in 5 % of biduri leaves treatment. The increase in protein concentration indicates number of protein that separated from main polypeptide bond in the meat protein which was detected by Bio-Rad protein analysis. This shows that the high concentration of protein indicates the increasing amount of protein released from muscle fibres. Based on the analysis, the application of biduri leaf powder had better ability to break polypeptides and significantly different from that of pineapple peel powder and biduri leaf (F-score = 40.162; p -value = 0.000). However, the application of high-concentration natural tenderizer may damage primary structure of protein to release peptide bonds in the N amide or C carboxyl groups and release amino acids [20]. It is may the reason, why the protein was detected in small amounts.

In contrast to mutton meat, the application of natural tenderizers powder to beef did not show a significant difference in the variation in concentration of the natural tenderizers powder (F-score = 1.560; p -value = 0.225) (Table 2). However, a significant difference was shown by the interaction effect between chayote peel powder in high concentration of 15 % (F-score = 4.090; p -value = 0.030). It is known that the treatment of pineapple peel powder and biduri leaves has the ability to break down polypeptides in beef better than chayote peel powder and related to the previous research from Fawzya and Chasanah [18].

Table 2 Total protein beef meat after coated with natural tenderizers.

Treatment	Protein concentration ($\mu\text{g}/\mu\text{L}$)			p -value ⁱ
	CP	PP	BL	
Control	18.20 \pm 0.45 ¹	18.20 \pm 0.45	18.20 \pm 0.45	0.225
5 %	15.14 \pm 1.70 ^{a2}	17.49 \pm 0.49 ^b	17.84 \pm 0.57 ^b	
10 %	18.68 \pm 0.83 ¹	17.13 \pm 0.53	18.51 \pm 0.56	
15 %	10.19 \pm 0.81 ^{a3}	18.20 \pm 0.44 ^b	18.43 \pm 0.77 ^b	
p -value ⁱⁱ		0.030		

Note: CP = chayote peel powder, PP = pineapple peel powder, BL = biduri leaves powder; Alphabet codes: ⁱ) p -value for mean similarity of protein concentration based on the natural tenderizer dosages; ⁱⁱ) p -value for mean similarity of protein concentration based on the natural tenderizer material; ^{a-c}) indicates significantly different among tenderizer materials; number sign (¹⁻³) the different number indicates significantly different among concentration treatment, and not significant for same number. Statistically, p -value for variable interaction between concentration and tenderizer type is 0.075 at CI = 95 % and α = 0.050.

The difference result in protein concentration indicates that natural tenderizer application should be adjusted according to meat types. The statistical results showed that the natural tenderizer, chayote peel, pineapple peel and biduri leaves did not differ significantly when applied to buff meat (F-score = 0.569; p -value = 0.574). In addition, the higher the concentration of natural tenderizers also did not have an impact on increasing the protein concentration (F-score = 9.091; p -value = 0.000) as presented in Table 3.

Table 3 Total protein buffalo meat after coated natural tenderizer.

Treatment	Protein concentration ($\mu\text{g}/\mu\text{L}$)			p -value ⁱ
	CP	PP	BL	
Control	18.78 \pm 0.00 ¹	18.78 \pm 0.00 ¹	18.78 \pm 0.00 ¹	0.001
5 %	16.99 \pm 0.85 ¹	18.61 \pm 0.51 ¹	15.80 \pm 0.86 ²	
10 %	17.86 \pm 0.88 ¹	18.47 \pm 0.51 ¹	18.62 \pm 0.59 ²	
15 %	14.63 \pm 1.05 ²	15.33 \pm 0.41 ²	17.27 \pm 0.77 ¹	
p -value ⁱⁱ		0.574		

Note: CP = chayote peel powder, PP = pineapple peel powder, BL = biduri leaves powder; Alphabet codes: ⁱ) p -value for mean similarity of protein concentration based on the natural tenderizer dosages; ⁱⁱ) p -value for mean similarity of protein concentration based on the natural tenderizer material; ^{a-c}) indicates significantly different among tenderizer materials; number sign (¹⁻³) the different number indicates significantly different among concentration treatment, and not significant for same number. Statistically, p -value for variable interaction between concentration and tenderizer type is 0.038 at CI = 95 % and α = 0.050.

The decrease in protein concentration after 10 and 15 % of natural tenderizer doses treatments may have a short polypeptide or even disjointed into amino acids. This is also seen in the results of SDS-PAGE visualization which shows changes in the cut of mutton, beef and buff protein bands after treatment. Based on the visualization results, the meat samples treated with natural tenderizer powder had various protein band sizes, especially in the pineapple peel and biduri leaves powder treatments (**Figure 1**). Therefore, further proteomic analysis is needed to detect the protein type that produced from the hydrolysis process by the proteases in chayote, pineapple peel and biduri leaves.

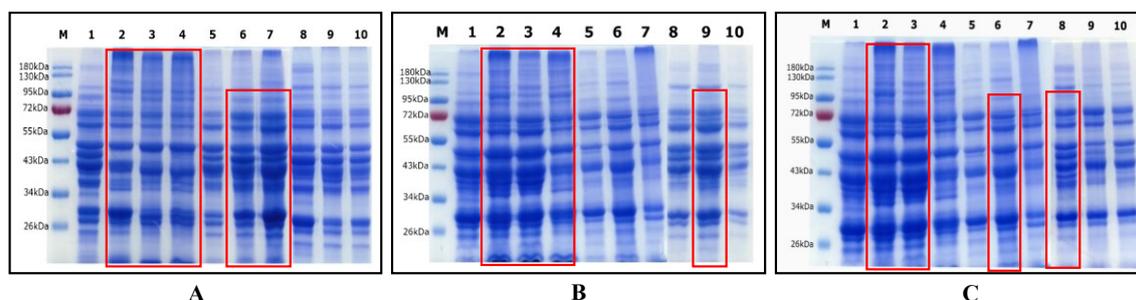


Figure 1 SDS-PAGE visualization of mutton (A), beef (B) and buff (C) protein band after natural tenderized treatment. M = protein marker; 1 = protein control; 2 - 4 = pineapple peel powder with concentration 5, 10 and 15 %, respectively; 5 - 7 = biduri leaves powder with concentration 5, 10 and 15 %; 8 - 10 = chayote peel powder with concentration 5, 10 and 15 %. The red boxes show the thick protein band indicating high concentration of enzymatic-digested protein.

The protein fragments produced from the pineapple peel powder treatment with a concentration of 10 - 15 % had the thickest bands compared to other treatments (**Figure 1**). Hydrolysis process using pineapple and biduri leaves on mutton and beef produce light protein bands below 26, 34 and between 55 to 43 kDa (**Figures 1(A)** and **1(B)**, code 2 - 7). The addition of pineapple and biduri leaves is the most effective tenderizers for mutton and beef in hydrolysing protein because they have higher band depletion along with the addition of w/w variation, than chayote. Then, low enzymatic activity performed of all tenderizers on buff, however, pineapple was better to digest buffalo meat that produces protein bands measuring 43 - 34 to 130 - 95 kDa, followed by biduri leaves powder that produce protein bands 130 - 95 to 43 - 34 kDa, and 1 protein band that weighs between 34 - 26 kDa.

Hydrolysis of peptide bonds results in a decrease in molecular weight and an increase in protein solubility because the structure changes to become smaller. This indicates a high concentration of protein that was successfully cleaved by bromelain [27]. Bromelain is one of the endopeptidase class enzyme that mostly abundance found in Bromeliaceae plant family [28]. The content of the enzyme bromelain in pineapple peel can denature the collagen protein in the meat and change the arrangement of the fibres into shorter fragments, so that the muscles are easier to separate and the meat becomes tender [29,30].

The enzymatic activity of biduri leaf was also observed high, especially at concentrations of 5 and 10 % biduri leaves powder of total meat weight. This is probably due to the high content of exopeptidases which hydrolyze terminal peptide bonds and release single amino acids or dipeptides from the peptide chain [31]. Another research results showed that the use of biduri leaves was able to hydrolyze carp protein up to 23.09 kDa and release 15 types of amino acids such as aspartic acid which reached 8.36 % and lysine 8.20 % [32]. The hydrolysis process by a natural tenderizer can increase the nutritional value of meat because it cuts polypeptides into amino acids so that they are easily absorbed by the body [33,34].

The high amount of isolated and detectable protein was indicated by meat spoilage. The results of observations using SEM showed enzymatic cutting activity which was confirmed by damage to muscle fiber and collagen tissue in mutton, beef and buff. The results showed that the greatest damage was caused by the activity of pineapple peel powder and biduri leaves powder at various concentrations (**Figures 2-4**).

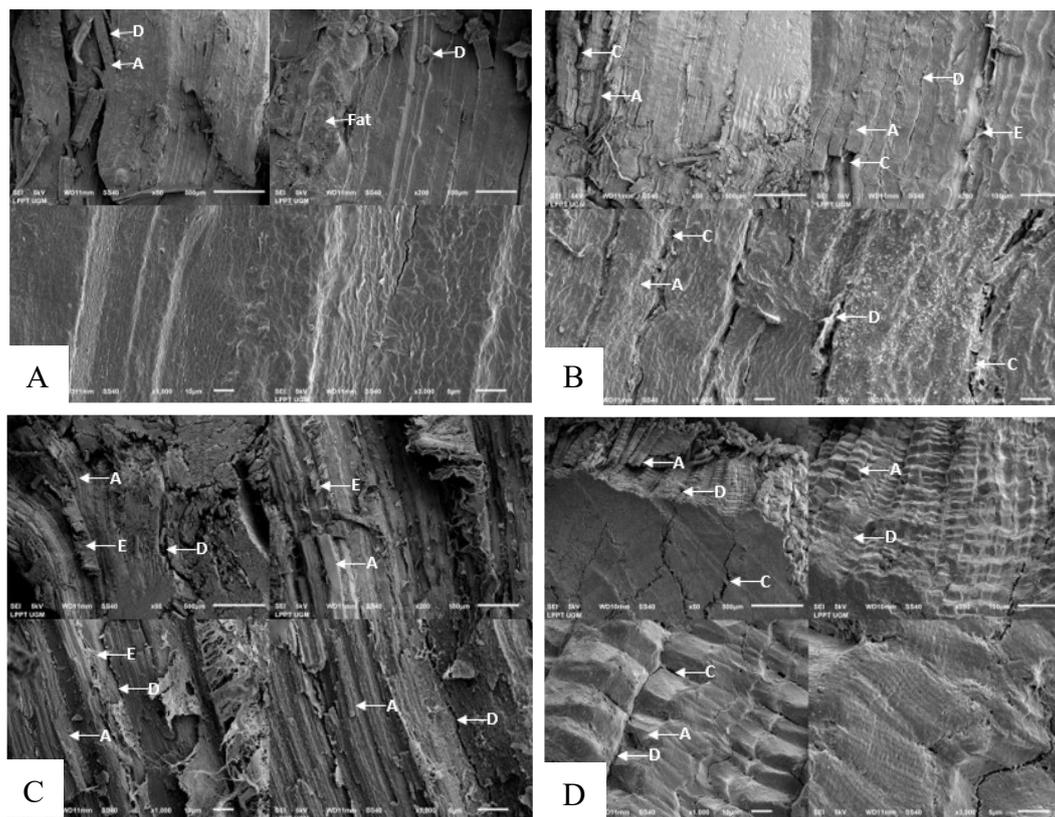


Figure 2 Microstructural condition of mutton after treatment using chayote peel powder (A), pineapple peel powder (B) and biduri leaves powder (C). The alphabets and arrows in with colour indicate: A = muscle fibre; B = Z-disk; C = connective tissue: Endomysium; D = connective tissue: Perimysium; E = collagen.

The softening process in mutton using pineapple peel powder results in greater collagen damage than the effects caused by biduri leave or chayote peel powder. The muscle fibre structure of mutton was damaged and the distance between the muscles was wider than the control meat. In control mutton that, which was not got treatment, has tighter and denser muscle fibres and the connective tissue is still intact. In line with those result, natural treatment in beef performs destructed muscle fibres and collagen, especially using pineapple peel extract [35] and biduri leaves powder treatment [36]. Meat protein structural conformation and physical changes were observed by the clafed and cracked surface of the meat (**Figure 3**).

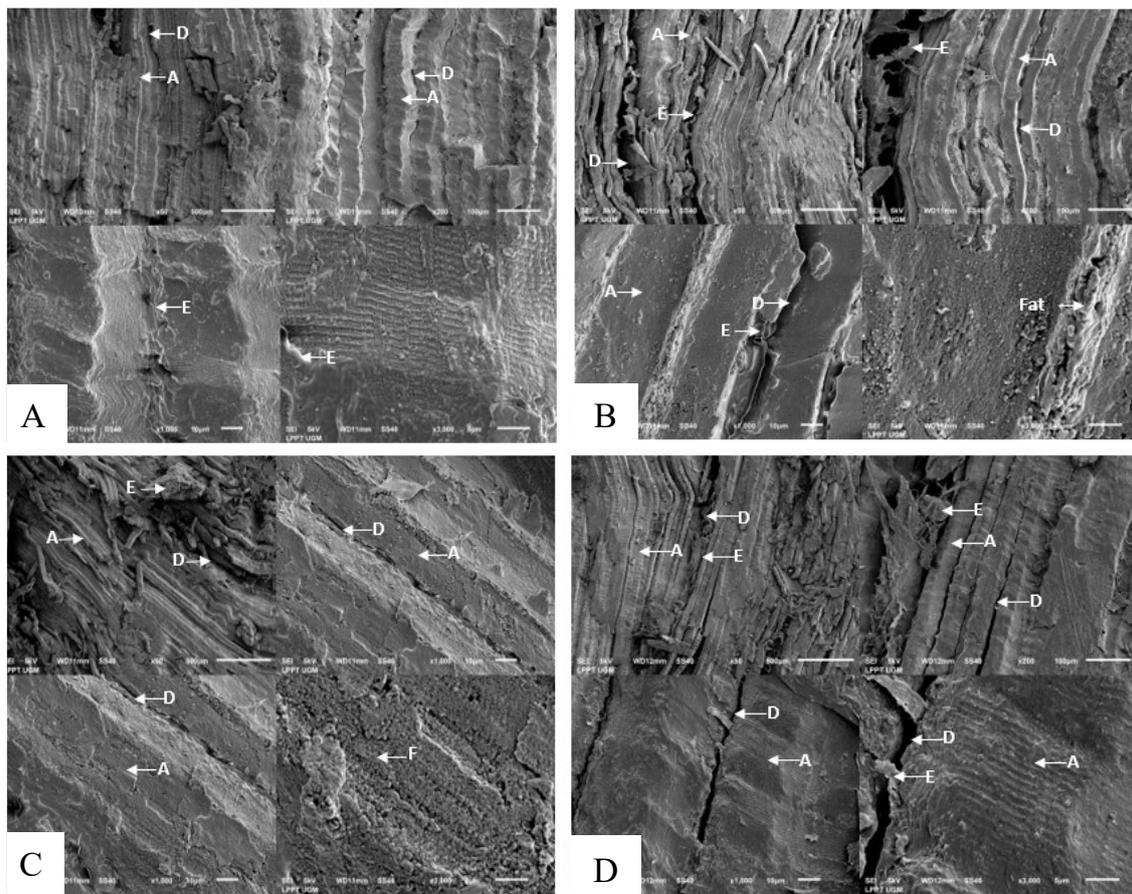


Figure 3 Microstructural condition of beef after chayote treatment (A), pineapple peel (B) and biduri leaves (C). The alphabet in with colour indicates: A = muscle fiber; B = Z-disk; C = connective tissue: Endomysium; D = connective tissue: Perimysium; E = collagen.

Based on the SEM results, it can be seen that beef with pineapple, biduri leaves and chayote peel pineapple undergoes collagen hydrolysis, respectively. Compared to the treatment groups, beef control showed that the muscles were bound together by connective tissue (collagen). Then, the hydrolysis process may breaks perimysium, interstitium layer and stretch the muscles fascicles further apart. The treatment of beef with chayote also experienced hydrolysis of connective tissue (collagen), but the damage to the connective tissue was lighter when compared to the pineapple peel and biduri leaves treatment. The arrangement of muscle fibers is tighter and the perimysium is very narrow. Treatments using chayote, pineapple peel and biduri leaves showed hydrolysis of connective tissue and protein making up meat compared to control beef. In contrast, high performances of natural tenderizer hydrolysis activity in buff was only showed in pineapple peel and biduri leaves powder (**Figure 4**).

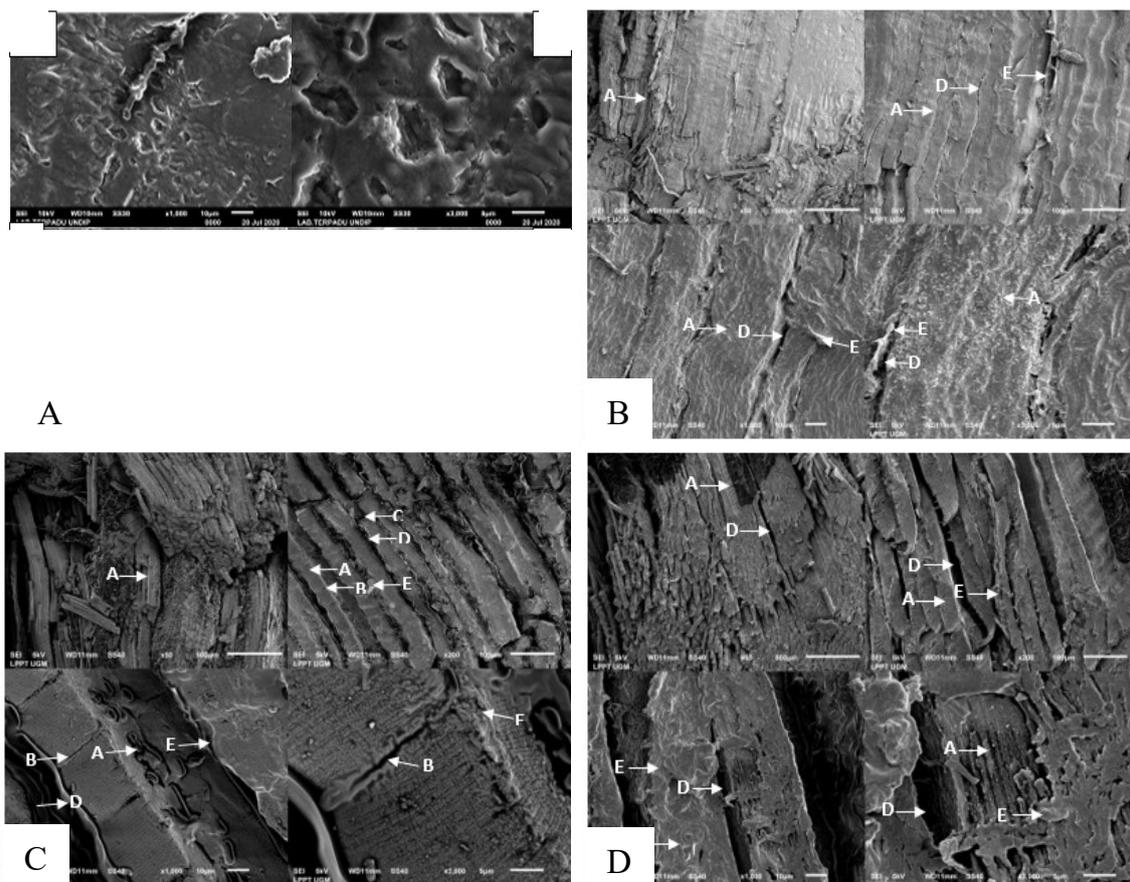


Figure 4 Microstructural condition of buff after chayote treatment (A), pineapple peel (B) and biduri leaves (C). The alphabet in with colour indicates: A = muscle fiber; B = Z-disk; C = connective tissue: Endomysium; D = connective tissue: Perimysium; E = collagen.

The buff condition after given with pineapple and biduri leaves shows damaged connective tissue that makes muscle fibres wider stretched, compared to chayote peel powder treatment. In fact, chayote peel powder treatment on buff shows narrower crack. It also on line with the low measured protein concentration and SDS-PAGE result that indicate low enzymatic activity of chayote peel powder. This result reveals that the chayote peel powder may has a lower protease concentration than that of the pineapple peel and biduri leaves.

The protease content in pineapple peel powder is dominated by bromelain which has a resistance of up to 83 % of active enzymes at 50 and 100 °C at 40 °C for 1 h. In addition, the proteolytic activity of bromelain is relatively stable at room temperature [35]. This is the reason why pineapple peel powder has better protein digestion results than biduri and chayote in all meats. Several studies have also shown that bromelain requires a free sulfhydryl group from the side chain of the amino acid cysteine to carry out hydrolysing activity [37]. Bromelain hydrolyzes meat proteins by breaking the peptide bonds through deprotonation of thiols at the active site of the enzyme by amino acids adjacent to basic side chains, such as histidine residues. Through this activity the sulfur anionic cysteine is deprotonated and reacts with the carbonyl carbon of the substrate. release the amine terminal [38]. Therefore, pineapple proteases have strong activity so that they can damage muscle protein in mutton, beef and buff.

Meanwhile, proteases in biduri are exoproteases that work effectively at a temperature of 55 °C with a pH of 7 [18]. Biduri leaves powder contains L-cysteine protease which has the same activity as bromelain, however, requires a higher temperature to conduct catalytic process than bromelain [39]. More over the temperature range of proteolytic activation of bromelain is wider than that protease in *Calotropis* [39]. It explains that the activity of pineapple peel powder is better than biduri leaves powder in hydrolyzing meat protein.

Furthermore, in contrast to pineapple peel and biduri leaves powder, the proteolytic enzymes in chayote may be dominated by the activity of hydrophilic proteases, including serine-protease [40,41]. The activity of this enzyme is catalysed by the presence of serine, histidine and aspartate which play different roles in protein hydrolysis. Serine has an -OH group which is able to act as a nucleophile, and break the carbonyl carbon group from the peptide bond. While the -OH group of serine is accepted by nitrogen histidine. The presence of -OH on the histidine nitrogen then attracts the carboxyl group of the aspartic acid and increases electronegativity in severing the N-terminal end of the C-carbonyl [42]. Based on this explanation, the proteases contained in the natural tenderizer are exopeptidase groups that directly damage peptide bonds. However, the difference in proteolytic results was probably caused by enzyme concentration, powder dose used, temperature and incubation time. Therefore, further analysis is needed to determine the type and concentration of proteases contained in each natural tenderiser, so that the enzymatic mechanism that occurs can be clearly identified.

The ability of proteases to cut peptide bonds in the primary chain causes the production of small protein bands as observed in the SDS-PAGE results. On the other side, the enzymatic activity may breaks the specific protein from the main muscle fasciculus. It shows that isolated protein after treatment seems to have the same weight with muscle protein, including actin (43 kDa), tropomyosin (36 kDa), troponin T (32 kDa), troponin I (21 kDa), myosin light chain (20 - 16 kDa), troponin C (18 kDa) and parvalbumin (12 kDa). The actin and myosin are the most abundant proteins found in muscle that together with tropomyosin and actomyosin complex provide strength for muscles to contract and responsible for meat tenderness [43-45].

In addition to the type of enzyme, the concentration or dose used for tenderizing meat affects the levels of enzymes used to hydrolyze meat. In other words, the more doses of natural tenderizer used, the higher the enzyme levels and the more tender the meat will be. In addition, several factors contribute to meat tenderness such as intramuscular fat content, muscle contraction activity, connective tissue, animal age, muscle parts and postmortem proteolysis processes. Furthermore, the use of a natural tenderizer also needs to consider the type of meat, the results of this study indicate that pineapple, biduri leaves and chayote are more effective in softening mutton and beef than buff. This is because mutton has finer muscle fibers, while beef has coarser fibers due to larger and denser fascicles. Meanwhile, buffalo meat has a denser muscle fiber structure and less fat than beef [46].

Conclusions

The application of natural tenderizer, including pineapple peel, biduri leaf and chayote peel is effectively tenderize the mutton, beef and buff meat. However, giving 10 - 15 % of chayote peel extract is significantly most effective compare to other natural tenderizers. Contained enzyme in natural tenderizer mostly dominated by exopeptidase, such as cysteine-protease and serine-protease that have the ability to hydrolyse polypeptide bond in primary structure of protein. The pineapple peel and biduri leaves powder is the most potential tenderizer for mutton and beef. However, buff is most least tenderized meat among other and it may need an extra dosage or incubation period to get appropriate result. Further research about costumers respond should be conducted to evaluate the tenderizers effect on favorable meats' flavour, taste and texture. In this research, measuring proteases concentration in the natural tenderizer and elimination of protein-destroying substances step should be added for further research. It is very important to ensure that the results of the study are pure effects of protease activity.

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