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PROTEOLYTIC ACTIVITY OF *SECHIUM EDULE*, *COSMOS CAUDATUS*, *MEDICAGO SATIVA* IN MEAT TENDERIZATION

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Abstract
The number of plant proteases that still not applied is the subject of this research. This study was to test the effectiveness of plant protease in Sechium edule, Cosmos caudatus Kunth, and Medicago sativa L. in meat tenderization. The research included goat meat and beef that was sprinkled with extracts (15% w/w) of chayote fruit (Sechium edule), kenikir leaf (Cosmos caudatus Kunth), and Alfalfa sprout extract (Medicago sativa L.). The process was carried out at 50°C, for 30 and 60 min. SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) analysis was performed to see the degradation zone, while SEM (scanning electron methods) analysis was performed to inspect the condition of the meat connective tissue. The findings of the study showed that all three plant proteases were able to tenderize beef and goat meat. Cosmos caudatus Kunth extract showed the highest effectiveness in degrading beef proteins in the zone of 10–22 kDa (small peptide — troponin I) and α and β tropomyosin (33 kDa) with V_{Max} = 0.134 μg/μL/min and K_M = 17.05 μg/μL. In goat meat, the extract was only able to degrade the small peptide area and troponin C (10–17.5 kDa; V_{Max} = 0.087 μg/μL/min; K_M = 7.23 μg/μL). Conclusion: all three plant proteases proved to be effective in the process of beef and goat meat tenderization.

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Introduction

Meat industry players continue to strive for an environmentally friendly and natural way of tenderizing meat. The damage caused by the tenderization process can be minimized when compared to cooking or heating. Plant protease is the main choice related to this problem.

Proteases in plants are highly abundant as they are involved in the plant life cycle viz: physiology and development [1,2]. The widely used and researched plant proteases (Figure 1) are: cystein protease (CP), aspartic protease (AP), serine protease (SP), and metalloprotease (MP). Among these four groups, CPs prevail in usage and testing, namely papain and bromelain, although other types are insufficiently researched (capparin, asparagus protease, caricain, etc.).

Tantamacharik et al. [2] categorized the types of plant proteases that are often studied and those that studied a little. Plant proteases for the SP and AP groups are found in plant tissues such as tomato plant leaves [3], thistle flowers [4], cornpollen[5], potatoplantleavesandtubers[6], Arabidopsisthalianaseeds[7,8], cucumber [9], and flax seeds [10,11]. Different conditions in the SP (Asian pumpkin protease) and AP (Phytepsin) groups have not been widely researched.

In the MP group, only 2 types are known, namely metzincin and cotinifolin. It is possible that there are some plants that have not been identified. Among plant proteases, metalloproteases (MP) are the least characterized [12]. However, MP proteolytic activity was detected in several sources such as Arabidopsis thaliana [13], sorghum [14], soybean leaves [15], sugarcane [16], germinated corn [17], pea seeds [18], buckwheat seeds [19], and wheat [20]. This is inversely proportional to the genus Medicago. The lack of information about the genus is interesting for our research object.

On the other hand, research related to protease enzymes continues to grow. Figure 1 provides an overview of the lack of research on the latest protease sources. The search for protease sources is not something interesting. We collected 1000 journal articles from 2010 to 2023, then we mapped them using vosviewer. From the collected journals, we searched for the keyword "new plant protease", and found 120 out of 1000 papers (0.12%). This motivates us to use protease plants that have not been researched or were researched just a little.

In this study, plant protease (which has not been widely studied) was assigned to be used for tenderizing meat. We selected three groups that were slightly researched, namely SP (Asian pumpkin protease) in *Sechium edule*, aspartic protease (phytepsin) in *Cosmos caudatus* leaves and MP group in *Medicago sativa* L. The Siamese pumpkin *(Sechium edule)* was detected to contain asian pumpkin serine protease [21] but their use for tenderizing meat has not been studied until now. *Cosmos caudatus* is assumed to contain the enzyme phytepsin AP, while phytepsin is abundant in plants of the Asteraceae family [22]. *Medicago sativa* L. was proved to contain MP enzymes based on the metalloprotease

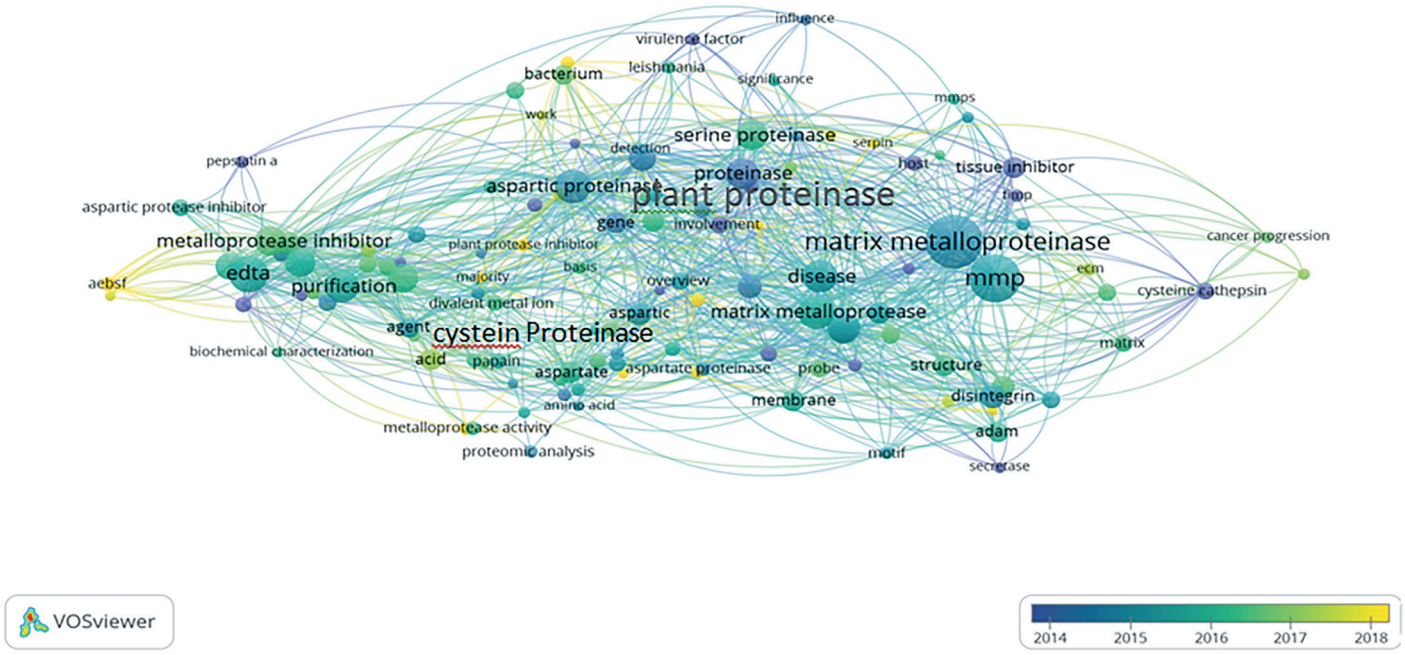

Figure 1. The development of research on plant proteases within 2010–2023

matrix genome sequence [12]. These three samples have the opportunity to be used in meat processing.

The aim of this study is to ascertain the efficacy of plant proteases in the three samples *(Sechium edule, Cosmos caudatus* Kunth*, and Medicago sativa* L.), which is supported by the preceding description.We tested its effectiveness based on: (i) enzyme kinetics through maximal speed (V_{max}) and Michaelis Menten constant (K_m) of protein degradation; (ii) the effects of plant proteases on to the areas of protein degradation in goat meat and beef; (iii) the impact of plant protease on collagen, perimysium, endomysium, and muscle connective tissue in beef and goat meat.

Objects and methods

Sample preparation

The materials used in this study included beef, goat meat, and extracts from chayote (Sechium edule), kenikir leaves (Cosmos caudatus Kunth), and alfalfa sprouts (Medicago sativa L.). The beef and goat meat were obtained fresh from a local market and stored at 4°C until use. Meanwhile, the plant materials were sourced from local farms, thoroughly washed with distilled water, and sun-dried for 48 hours. After drying, the plant materials were ground into a fine powder using a high-speed blender Cosmos CB-801 (Star Cosmos, Indonesia) before undergoing the extraction process. This procedure was conducted, referring to our previous research [23]. We extracted 150 g of dry powder with 100 mL of distilled water for 72 h to get a thick extract. After that, the final extract concentration used in the analysis was equal to 20% (20 mg/mL), which volume we achieved by filtering and evaporating it in an evaporator DLAB RE100-PRO (Wahana Hilab, Indonesia) for 1 h.

Thin slices of beef and goat meat (4 cm × 4 cm × 2 mm) were cut. 20% extract was applied to the beef, with the ratio of extract weight to meat weight. We allowed the meat to rest for 30 min before storing it for 60 min at 30–35 °C to avoid temperature fluctuations. Untreated beef (B) and untreated goat meat (GM) were used as control samples in this study. While the protein degradation kinetics analysis (SDS PAGE) was carried out at 30 and 60 min, the SEM test was undertaken at 60 min.

The sample codes are defined as follows:

B (Beef): Untreated beef (control).

B-C: Beef treated with *Cosmos caudatus Kunth* extract.

B-M: Beef treated with *Medicago sativa L.* extract.

B-S: Beef treated with *Sechium edule* extract.

GM (Goat Meat): Untreated goat meat (control).

GM–C: Goat meat treated with *Cosmos caudatus Kunth* extract.

GM–M: Goat meat treated with *Medicago sativa L.* extract.

GM-S: Goat meat treated with *Sechium edule* extract.

Protein level

Determination of protein content was counducted in accordance with Manzoor et al. [24]. Analysis was done using the biuret method with a UV–Vis spectrophotometer (Spectronic 200, Thermo Fisher Scientific, USA) at wavelength 595 nm. Biuret reagent used was 0.2 g $CuSO_4 \cdot 5H_2O$ and 0.6 g potassium tartrate mixed together in 50 mL of distilled water and 40 mL of 15% NaOH was added. The amount of protein content was determined-via the absorption of UV light.

Maximum speed (V_{max}) and Michaelis Menten constant (K_m)

The maximum speed (V_{max}) and substrate efficiency (KM) were calculated by the correlation between reaction rate (V) and substrate concentration (S). The Lineweaver-Burk equation was also used by [25].

$$\frac{1}{V} = \frac{K_m}{V_{max}} \cdot \frac{1}{S} + \frac{1}{V_{max}} \quad (1)$$

Given that $Y = bx + a$ and that the y- and x-axes on the graph are $1/V$ and $1/S$, respectively, $V_{max} = 1/a$ and $K_m = V_{max} \cdot b$

SDS-PAGE (sodium dodecyl sulfate — polyacrylamide gel electrophoresis) analysis

The procedure refers to the one of Association of Official Analytical Chemists [26]. Acrylamide gel electrophoresis was used in the analytical process. The concentration of the top (stacking gel) and bottom (separating gel) was 5% for the stacking gel and 12% for the separating gel. 200 V voltage, 15 mA/gel, and 60 min were used for the electrophoresis using a Mini-PROTEAN Tetra Cell-BIO-RAD.

After electrophoresis, 0.05% (w/v) coomassie blue R-250 was added to 15% (v/v) methanol and 5% (v/v) acetic acid to stain the gel. It was then microwave-heated for 30 seconds and allowed to incubate for 60 min. After the gel was submerged in a solution containing 30% methanol and 10% acetic acid, it was incubated for 2 h in a waterbath (Julabo TW12, Julabo GmbH, Germany).

SEM Analysis (Scanning Electron Methods)

This procedure is described by Koga et al. [27]. Meat structure was analyzed using SEM (ZEISS EVOMA10, Carl Zeiss Microscopy GmbH, Germany). The dried specimens were examined using a scanning electron microscope (S-4100; Hitachi, Japan) and placed on an aluminum platform that had been ion-sputter coated with platinum-palladium (E1030; Hitachi, Japan). The images were viewed with SE (Secondary Electron) detector, Working Distance 9.0 mm and EHT 16.00 kV at 500 x magnification (ZEISS EVOMA10, Carl Zeiss Microscopy GmbH, Germany).

Statistical test

IBM SPSS Statistics software of version 26 (SPSS Inc., Chicago, IL, USA) was used for the analysis. To see the variations in protein levels over time, we used an ANOVA test with Tukey HSD_ post hoc (x, y, and z). Changes in protein levels in each sample were observed using a, b, c, and d, respectively. The mean ± SD of the results was presented. $p < 0.05$ was used as the threshold for statistical significance.

Results and discussion

Enzyme kinetics

In Table 1, it can be seen that the change in protein content for between goat meat and beef did not show a significant difference ($p > 0.05$) at 0 min. Within 30 and 60 min, there was a significant effect ($p < 0.05$) on protein content and there was a significant difference in the three sample extracts. There was drastic degradation at 60 min, and mild degradation at 30 min. The protein content comparison scale to see the type of degradation (mild, moderate, and complete) can be seen in Figure 2.

Although there was a significant difference in the change of protein content at 30 and 60 min (Table 1), the researcher also tried to represent the difference in the form of color variations to easily classify the type of degradation. The representation of color variations in Figure 2a. was not clearly visible at 30 min. The significant range of color variations occurred at 60 min. If to represent the Figure 2a as a scale, the color variation is more clearly visible at 30 min (mild and moderate degradation) and 60 min (moderate and complete degradation).

Table 1. The change in protein content for goat meat and beef meat

Sample	0 min	30 min	60 min
B	17.35 ± 0.28^{ax}	17.34 ± 0.21^{dx}	17.35 ± 0.24^{dx}
B-C	17.28 ± 0.22^{az}	13.05 ± 0.25^{ay}	2.59 ± 0.21^{ax}
B-M	17.34 ± 0.23^{az}	16.1 ± 0.26^{cy}	8.02 ± 0.3^{cx}
B-S	17.34 ± 0.22^{az}	15.03 ± 0.23^{by}	2.59 ± 0.21^{ax}
GM	8.56 ± 0.23^{ax}	8.54 ± 0.21^{cx}	8.54 ± 0.26^{dx}
GM–C	8.55 ± 0.11^{az}	6.23 ± 0.32^{ay}	2.43 ± 0.17^{ax}
GM–M	8.56 ± 0.3^{ay}	8.01 ± 0.13^{cy}	4.78 ± 0.17^{cx}
GM-S	8.56 ± 0.12^{az}	7.5 ± 0.25^{by}	3.59 ± 0.28^{bx}

Difference of sample extracts = *a*, *b*, *c*, and *d*; difference of time = *x*, *y*, and *z*. Significant level ($p < 0.05$).

The time variable provided a significant effect ($p < 0.05$) on reducing meat protein content. Although the protease activity of *Medicago sativa* L. was the lowest, optimization can be done by increasing the time duration and extract concentration (> 15% w/w). Extract concentration and time duration significantly affected the degradation of meat protein in the tenderization process. This seems to be the general consensus among the researchers [28,29].

The V_{max} of protein degradation of *Cosmos caudatus* Kunth leaf extract was the highest (0.134 μg/μL/min) with a substrate K_m of 17.05 μg/μL in on beef. Goat meat also showed the highest value (0.087 μg/μL/min). In goat meat, there was almost no difference in the V_{max} of protein degradation of the three sample extracts. The difference in V_{max} was seen in beef but the substrate K_m in the three samples were almost the same. *Medicago sativa* L. sprout extract gave the lowest V_{max} in beef (0.125 μg/μL/min) and goat meat (0.078 μg/μL/min). The effectiveness of this kinetics was seen from the use of the lowest substrate (K_m) but was able to provide a greater V_{max}. The term 'lowest substrate' refers to the ability of the system to reach V_{max} with a lower substrate concentration, which is indicated by a lower K_m. A lower K_m suggests a higher enzyme-substrate affinity, meaning that less substrate is needed to achieve high reaction velocity. In this study, the effectiveness of the kinetics was evaluated based on achieving high V_{max} with a relatively low K_m. In this study, the effectiveness of *Cosmos caudatus* Kunth leaf extract was demonstrated in goat meat but not in beef (Figure 2c and Table 2).

The above series of analysis proved that the extracts of *Cosmos caudatus* Kunth leaves, *Sechium edule* fruit, and *Medicago sativa* L. sprouts can be used in meat processing. Although the V_{max} was relatively low compared to commonly used plant proteases, i. e.bromelain, obtained from *Ananas comosus* has 3,969 U/min (79.38 μg/μL/min) [28]. Papain enzyme from Carica papaya leaves has a K_m value of1.47–8.70 mg/mL

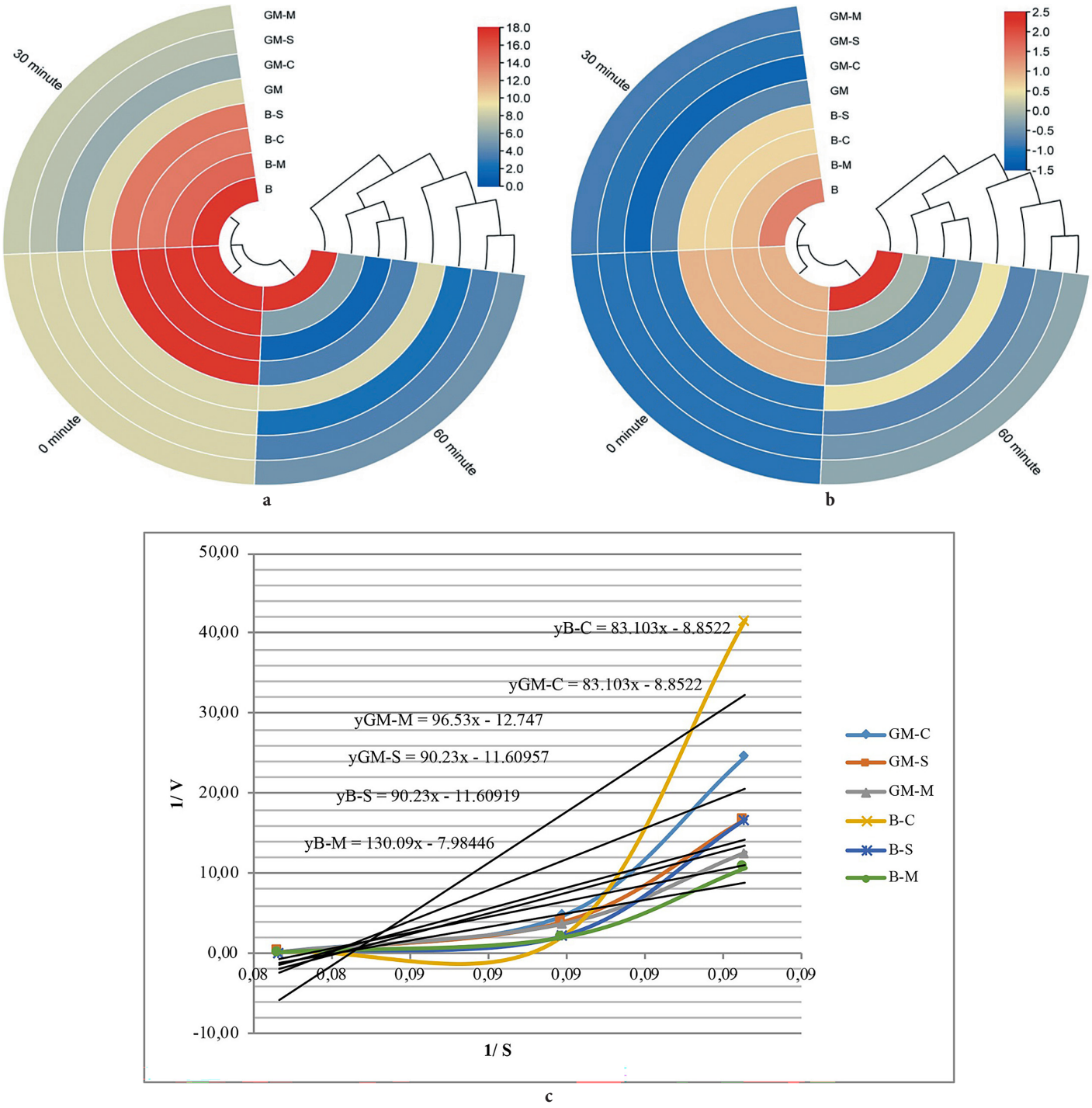

Figure 2. The comparison of the effect of plant protease on protein content of beef and goat meat. Changes in protein content in μg/μL (a). Scale of changes in protein content (b). Degradation reaction kinetics due to plant proteases (c)

Table 2. Degradation reaction kinetics due to plant proteases in a meat of goat and beef

Sample	Item	*Medicago sativa* L.	*Sechium edule*	*Cosmos caudatus* Kunth
Goat meat (GM)	y	96.53x – 12.747	y = 90.23x – 11.609	y = 83.103x – 8.8522
	R^2	0.9598	0.9996	0.9984
	V_{max}	0.078 μg/μl/min	0.086 μg/μl/min	0.087 μg/μl/min
	K_m	7.53 μg/μl	7.76 μg/μl	7.23 μg/μl
Beef (B)	y	130.09x – 7.9844	128.8x – 7.7046	127.2x – 7.4566
	R^2	0.9896	0.9983	0.9984
	V_{max}	0.125 μg/μl/min	0.129 μg/μl/min	0.134 μg/μl/min
	K_m	16.29 μg/μl	16.61 μg/μl	17.05 μg/μl

and a V_{max} value of 0.42–0.4167 μmol/mL/min [30]. In addition, for hydrogen peroxide substrate, ficin has been reported to have a V_{max} of 4.69 μg/mL at 0.35 mMol [31]. However, discovering plant proteases in under-researched plants is equally important.

The impact of plant protease on the degradation of proteins

The results of SDS-PAGE analysis are shown in Figure 3. The effect of plant protease caused degradation of goat meat protein (Figure 3a). All sample extracts were only capable of mild degradation at 30 min, moderate degradation and complete degradation at 60 min. *Cosmos caudatus* Kunth was able to degrade completely in the area of small peptide and troponin C (10–17.5 kDa). Intermediate degradation occurred in the area of α and β tropomyosin (33 kDa) and α actinin to myosin heavy chain (103–223 kDa).

Sechium edule degrades completely (Figure 3a) in the area of small peptide (10–15 kDa) and intermediate scale degradation in the zone of myosin light chain, troponin T, α and β tropomyosin and actin (25–42 kDa). *Medicago sativa* L. was only able to degrade in the narrower area of small peptide (12–14 kDa) but was able to intermediate scale degradation in a fairly wide area of 17–21 kDa, 26–38 kDa and 53–223 kDa).

Degradation of beef protein (Figure 3b) using all three sample extracts for 30 min resulted in only minor degradation. The 60-min aging time also caused minor degradation in the 40–75 kDa area. *Cosmos caudatus* Kunth was able to completely degrade proteins in the area of small peptides (10–17 kDa), troponin C (17.8 kDa), troponin I (22 kDa), and in the narrow area of α and β tropomyosin (33 kDa). There was intermediate-scale degradation in the narrow area of troponin C (17.8 kDa), the broad area including myosin light chain (25 kDa), troponin T (30 kDa), α and β tropomyosin (33 kDa) and the broad area of 75–200 kDa.

Sechium edule degrades completely in the small peptide area (10–17 kDa) and in the narrow area of α and β tropomyosin (33 kDa). Intermediate scale degradation was seen in the 20–33 kDa area (troponin I, myosin light chain, troponin T, αandβ tropomyosin) and the 75–223 kDa area (α actinin, protein C and myosin heavy chain). Small-scale degradation was formed in the coverage area of 40–75 kDa (Figure 3b).

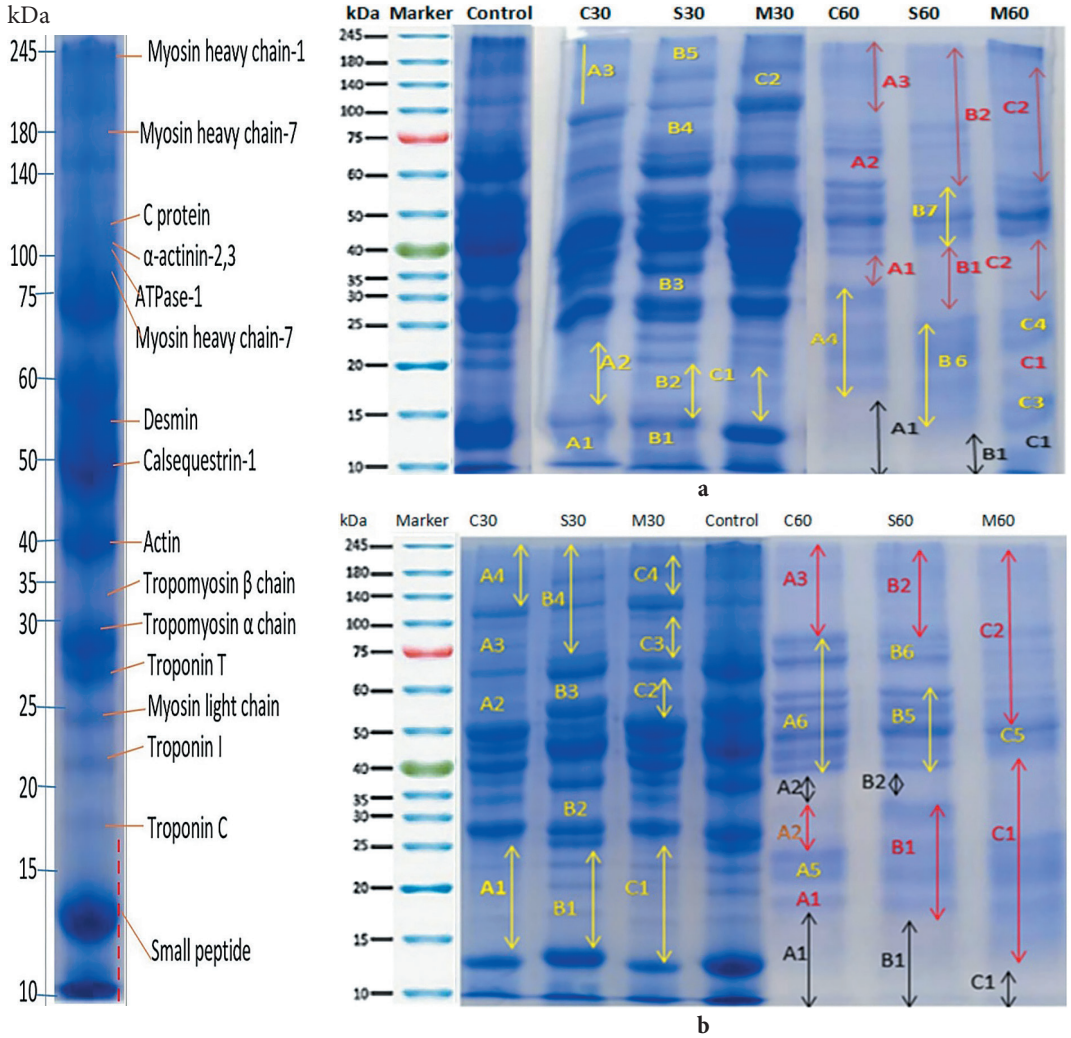

Figure 3. Meat protein degradation zone based on SDS-PAGE analysis. Protein degradation zones of goat meat (a) and beef (b) using *Cosmos caudatus* Kunth extract for 30 and 60 min (C30, C60), with *Medicago sativa* L. extract for 30 and 60 min (M30, M60), with *Sechium edule* extract for 30 and 60 min (M30, M60). The effects of *Cosmos caudatus* Kunth (A), *Sechium edule* (B) and *Medicago sativa* L. (C) extracts resulted in mild (yellow), moderate (red) and complete (black) degradation

Medicago sativa L. degrades completely (Figure 3b) in the area of 10–12 kDa (small peptide). Medium-scale extensive degradation areas were seen in the 13–40 kDa (small peptide, troponin C, troponin I, myosin light chain, troponin T, and α and β tropomyosin) and 75–223 kDa areas (α actinin, protein C and myosin heavy chain).

Different results of *Sechium edule*, shown by Darmawati et al. [32], with degradation observed in buffalo meat (10–26 kDa), beef (10–43 kDa), and goat meat (10–17 kDa). Unfortunately, we found no information on *Cosmos caudatus* and *Medicago sativa* L. used directly in meat tenderization.

The effects of plant proteases on meat connective tissue

In muscle tissue, collagen will form the *perymisium* and *endomysium*. Collagen is the most abundant component in muscle tissue. *Perymisium* will separate muscle fibers, and endomysium serves as a coating on muscle fibers. The tissue (*Perymisium* and *endomysium*) will be visible if there is a tear in the meat fiber.

Goat meat

Goat meat's connective tissue was exposed to plant protease treatment (Figure 4), with untreated meat serving as the control sample (Figure 4a). The muscle tissue was still tightly packed and collagen dominated in appearance. *Endomysium* tissue was also found (*endomysium* may be formed due to cutting factors).

The protease in *Cosmos caudatus* Kunth affected the goat meat tissue (Figure 4b). The muscle tissue was separated when compared to the control sample of meat (Figure 4a). The dominance of collagen was reduced as the *perymisium* and *endomysium* tissues increased. *Endomysium* dominates which is characterized by broken cross-links so that tears are formed in the meat fibers and *perimysium* tissue is also visible at some points but does not dominate. The number of broken cross-links will affect the tenderness of the meat. In Figure 4b, the tear in the meat is long and wide and small cracks are visible at some points.

The protease in *Sechium edule* also showed its effect on meat connective tissue (Figure 4c). The changes in connective tissue were similar to those of *Cosmos caudatus* Kunth extract, where muscle tissue was seen to have separated at some points. Collagen content intissue was reduced. *Endomysium* tissue dominated in the meat and *perimysium* tissue was seen at some points.

Changes in the meat connective tissue due to the effect of proteases in *Medicago sativa* L. (Figure 4d) did not show long cracks or tears like the extracts of *Cosmos caudatus* Kunth and *Sechium edule* did. Muscle tissue appeared to have separated, while collagen bundles were also reduced along with the prevalence of *endomysium* bundles. *Perimysium* tissue was visible at some points.

Beef

The impact of plant protease on cattle connective tissue is shown here (Figure 5). and control sample of meat (Figure 5a). The muscle tissue was still tight and collagen dominates in appearance. No *endomysium* and *perimysium* tissues were found. This is because the cross-linking tissue is still strong. In the control sample, fat was found, which is almost similar to the appearance of collagen.

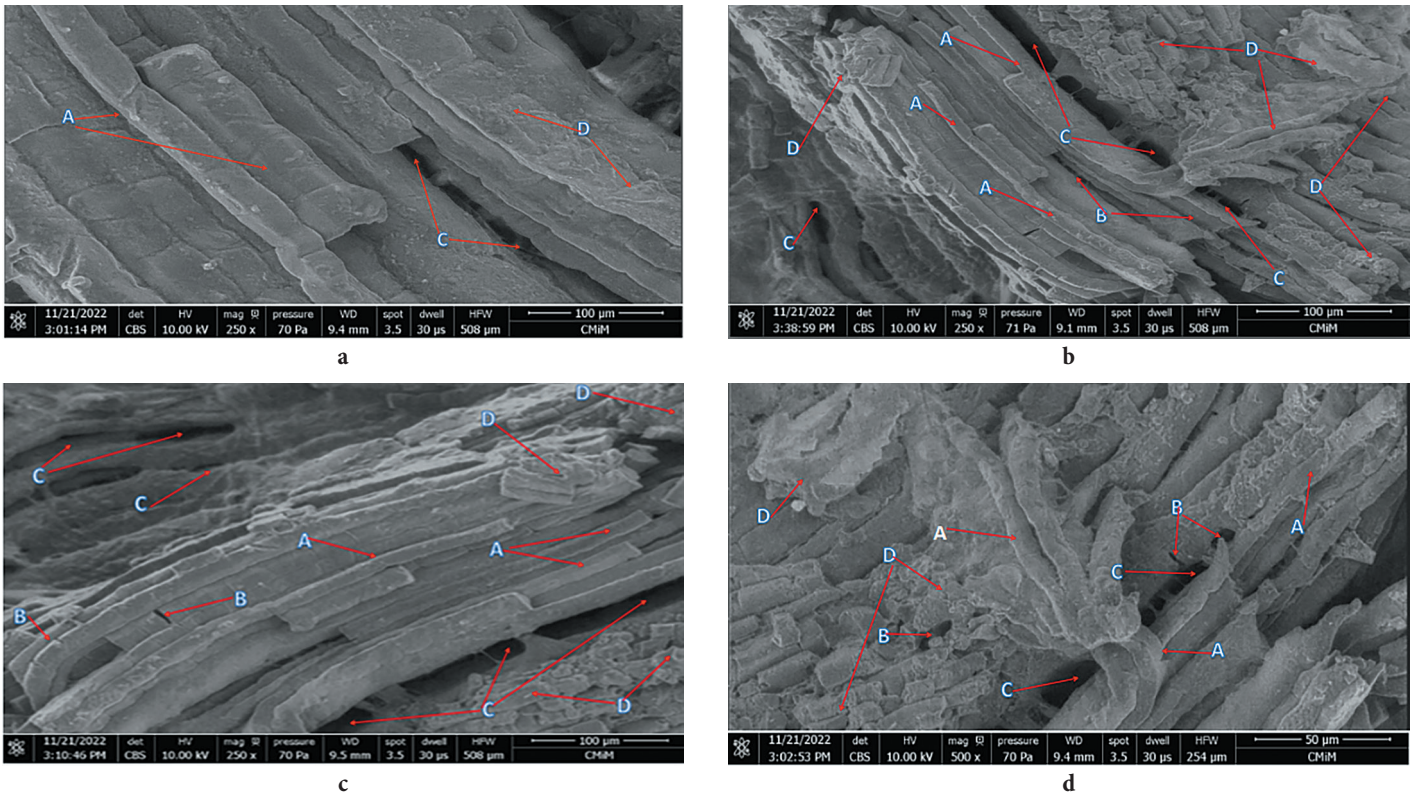

Figure 4. The effects of plant proteases on connective tissue of goat meat. The shape of connective tissue in control sample of meat (a) degraded by *Cosmos caudatus* Kunth leaf extract (b), *Sechium edule* fruit extract (c), and *Medicago sativa* L. sprout extract (d) will result in changes in muscle tissue (A), perimysium (B), endomysium (C), and collagen (D)

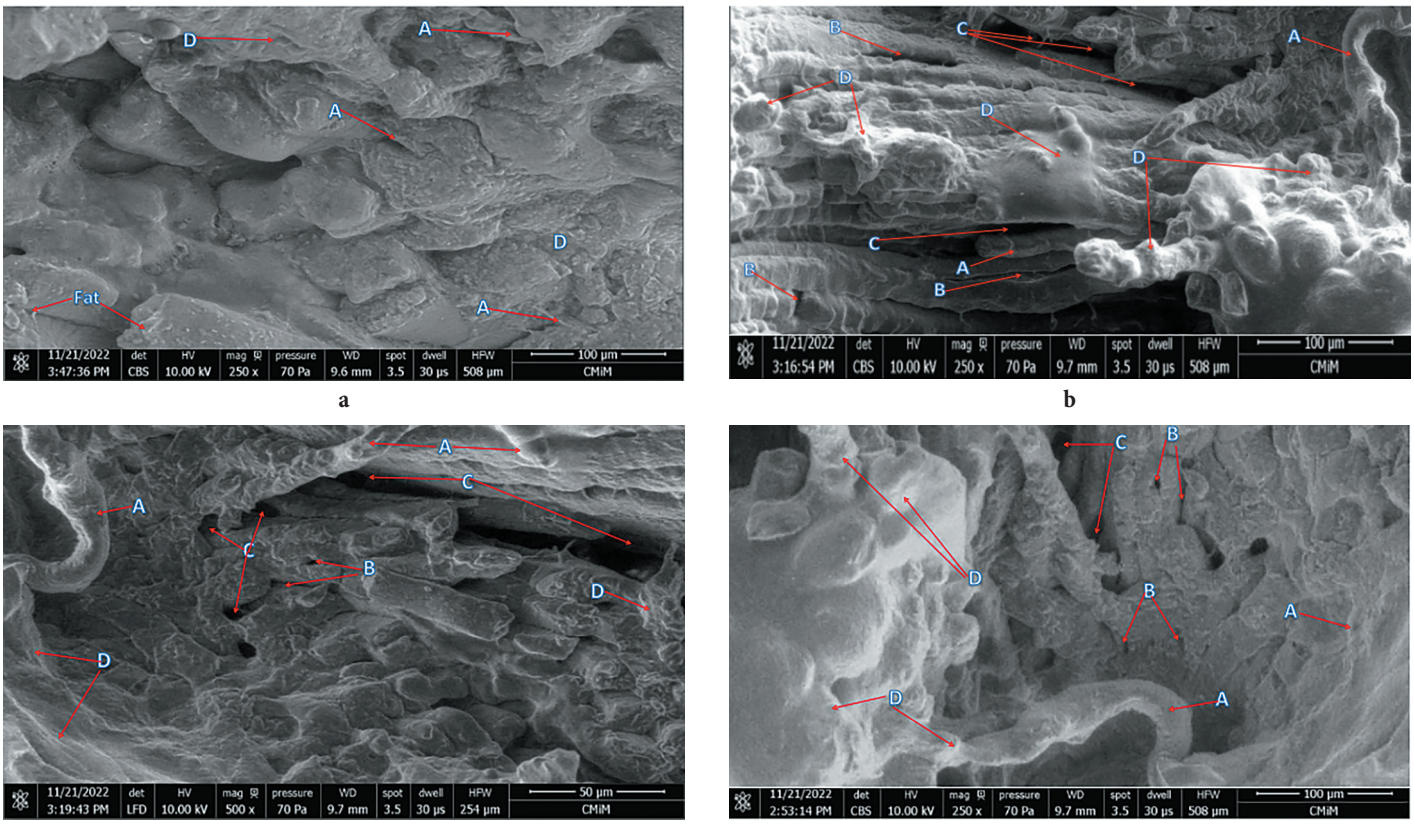

Figure 5. The effects of plant proteases on beef connective tissue. The shape of connective tissue in control sample of meat (5a) degraded by *Cosmos caudatus* Kunth leaf extract (5b), *Sechium edule* fruit extract (5c), and *Medicago sativa* L. sprout extract (5d) will result in changes in muscle tissue (A), perimysium (B), endomysium (C), and collagen (D)

The protease in *Cosmos caudatus* Kunth affected the beef tissue (Figure5b). Muscle tissue appeared separated when compared to control sample of meat (Figure5a). Collagen did not prevail as perymisium and endomysium tissues increased. Endomysium prevails, which is characterized by the breaking of cross-links resulting in tears in the meat and perimysium tissue is also visible at some points. In Figure 5b, the tears are narrow and short but evenly distributed on all sides.

The protease in *Sechium edule* showed its effect on meat connective tissue (Figure 5c). Muscle tissue was seen as having separated at some points. Collagen tissue was reduced, the dominance of perimysium endomysium bonds was seen as clustered in the center or unevenly distributed on all sides of the meat. Meat fibers tears are shorter but deeper.

The effect of proteases in *Medicago sativa* L. on beef connective tissue (Figure 5d) did not show long cracks or tears like the extracts of *Cosmos caudatus* Kunth and *Sechium edule* did. Muscle tissue appeared to have separated; collagen bundles were also reduced with the dominance of endomysium bundles. Perimysium tissue was visible at some points.

Protein degradation kinetics shows the speed at which collagen molecules change from helical to coiled structure and fibrous collagen changes to fibrillar. The breaking of hydrogen bonds will be followed by the shrinkage of collagen fibers (becoming short). During the degradation process, collagen contraction will occur; the more often it contracts, the lower its mechanical strength. This is in line with Astruc [33] statement about collagen damage due to thermal factors.

The three plant proteases are also able to separate myofibers from the perimysium. Perimysium tissue is the most susceptible tissue to damage. Before the degradation process sometimes the damage can be caused by meat cutting factors. Intramuscular isometric tension can decrease due to storage conditions as well [34]. Plant proteases *(Cosmos caudatus Kunth, Sechium edule,* and *Medicago sativa L.)* in this study were able to activate perimysium and separate muscle fibers in muscle connective tissue. Perimysium is a large circular fascicle that has an order based on the size of the diameter, namely primary, secondary and tertiary fascicles [33].

The prevalence of *endomysium* appearance (Figure 4 and Figure 5) in goat meat and beef due to the degradation of plant protease *(Cosmos caudatus* Kunth, *Sechium edule,* and *Medicago sativa* L.*)* is the first step of meat tenderization. This condition indicates that the endomysium has been detached from the sarcomere. *Endomysium* surrounds muscle fibers which include basal lamina, proteoglycans, collagen and laminin [35], so *endomysium* will be visible if a tear is formed in the meat fiber.

Conclusion

The extracts of *Cosmos caudatus* Kunth leaves, *Sechium edule* fruit, and *Medicago sativa* L sprouts demonstrated their ability to degrade meat proteins, so their using in meat processing should be considered. Based on protease enzyme

kinetics, *Cosmos caudatus* Kunth leaf extract showed higher effectiveness on goat meat (V_{max} = 0.087 μg/μL/min) and beef (V_{max} = 0.134 μg/μL/min) compared to *Sechium edule* fruit and *Medicago sativa* L sprouts, while the lowest V_{max} was shown by *Medicago sativa* L sprouts extract.

In goat meat, *Sechium edule* fruit extract and *Medicago sativa* L sprouts were only able to degrade in the small peptide zone area (10–15 kDa). Meanwhile, *Cosmos caudatus* Kunth leaf extract could only affect the area of small peptide (10–17 kDa), and troponin C (17.8 kDa). In beef, the affected area of *Cosmos caudatus* Kunth and *Sechium edule* degradation covers a wider area in the range of 10–33 kDa. While *Medicago sativa* L was only able to degrade in the small peptide zone (10–15 kDa).

The prevalence of endomysium content in mutton and beef due to the degradation of plant protease (*Cosmos caudatus* Kunth, *Sechium edule*, and *Medicago sativa* L.) is the first step of meat tenderization. The three plant proteases were able to separate the myofibers from the perimysium, change the collagen molecules from a helical state to a circular structure, and transform fibrous collagen into fibrillar collagen.

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