Microstructural and Proteomic Analysis to Investigate the Effectiveness of Papaya Leaf as a Tenderizer of Beef and Goat’s Meat

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Abstract. Meat quality for the community is determined by the level of tenderizer. The process of meat tenderization could be done by giving papain enzymes derived from papaya fruit or leaves. Meat with the administration of papain enzymes can be analyzed based on protein profiles to see protein and microstructural integrity to see the texture of meat. The research sample was 20 g of beef and goat’s meat with the treatment using the enzyme papain derived from papaya leaves. Time variation of enzyme administration 1 h, 2 h, 4 h, and 8 h, as well as variations in the weight of crude papain extract from papaya leaves as much as 10 g, 15 g, and 20 g. Analysis of protein concentration using Bradford and Kjeldhal methods, and protein profiles using the SDS PAGE method, and microstructural analysis using SEM. The concentration of meat protein with the treatment of crude extract of papaya leaves decreased compared to control meat. Based on protein profile analysis showed that meat given the enzyme papain from papaya leaves experienced protein band loss at a size of 225 kDa, 150 kDa, 96 kDa, 86 kDa. Microstructural analysis showed that beef and goat’s meat treated with damage to collagen fibers, collagen fibers did not stick with muscle fibers, and the arrangement was irregular, in addition, there was a distance between muscle fibers.

Keyword: proteomic analysis, meat microstructural, papain enzyme, meat tenderizer.

1. Introduction

Meat is a source of protein that contains essential amino acids and is needed by the body for various cell activities. Meat that is often consumed by the people and has a tough texture that includes beef and goat’s meat. Meat tenderizer process that is often carried out by the community is by boiling for a long time. The boiling process of meat takes too long cause dissolved meat protein, and meat protein will shrink so that the size of the meat shrinks [29]. The process of processing meat greatly determines the nutrition of meat absorbed by the body.
Meat tenderizer process can be carried out enzymatically. One enzyme that can help the meat tenderizer process is the enzyme papain. The papain enzyme is a hydrolytic enzyme that can degrade meat protein so that the texture becomes soft. Papain enzymes can be obtained from papaya plants. Some studies that use parts of papaya to get papain enzymes include young fruit [5, 7, 23, 32] for the taking of latex. Research conducted by [5] shows that the use of fruit latex has the effectiveness of the enzyme papain which is good for meat tenderizers. Part of papaya tree which has papain content apart from fruit latex, which is yellow papaya leaves [5], and papaya leaves that are still green [36].

The problem is that taking fruit latex by making incisions in fruit which is still thick causes the quality of the fruit to be bad and not sold in the market. This study will use papaya leaves containing the enzyme papain and can be used for meat tenderizers based on microstructural and proteomic analysis.

2. Methods

2.1. Crude papain enzyme from papaya leaves

The crude extraction of the papain enzyme is done by drying the papaya leaves. Papaya leaves are cut to reduce the size so that the surface area becomes wider. The purpose of cutting the papaya leaves becomes small is to speed up the drying process. The drying process uses an oven at 50 °C. The dried papaya leaves are then pureed using a blender machine.

2.2. Soaking Beef and Goat’s Meat

A total of 20 g of beef and goat’s meat are smeared with papaya leaf powder with a variation of the weight of 10 g of leaf powder, 15 g, and 20 g, and variations in the time of administration of 1 h, 2 h, 4 h, and 8 h, were incubated at 4° C.

2.3. Protein Isolation of Beef and Goat’s Meat

Beef and goat that have been given negative treatment and control weighed 3 g, mashed and added 7 ml PBS, then centrifuged 6000 rpm for 10 mins, the supernatant is removed and stored at 4 °C.

2.4. Protein concentration measurements using the Bradford and Kjeldahl methods

Bradford method by making a standard curve using multilevel BSA dilution and reacted with Biorad Protein Assay. Supernatant of beef taken 2µl dissolved in 798µl dH2O then added with 200µl Biorad Protein Assay then homogenized and incubated for 10 mins, measured the absorbance with a spectrophotometer visible wavelength 595 nm.

The Kjeldahl method by means of the sample was weighed 10 mg (requires 3-10ml HCl 0.01N), then transferred into the Kjeldahl flask, adding 1.9 g of K$_2$SO$_4$, 40 mg HgO, 2 ml of H$_2$SO$_4$. The sample was boiled for 1-1.5 h until the liquid is clear. Then cool, add water slowly, then cool again. The contents of the flask are transferred to a distillation device. Place the Erlenmeyer 123 ml containing 5ml of H$_3$BO$_3$ solution and 2-3 drops of the indicator, add 10 ml of NaOH-Na$_2$S$_2$O$_3$ solution, then distillate until 15 ml of distillate is accommodated. Rinse the condenser with water and hold the rinse in the same Erlenmeyer. Dilute the Erlenmeyer contents to about 50 ml then titrate with 0.02 N HCl until the color changes to gray.

3. Results

Beef and goat’s meat which have been treated with papaya leaves based on variations in concentration and time of administration can affect the concentration of meat. Untreated beef and goat’s meat have higher meat concentrations than those using the treatment. The highest concentration of beef and mutton was treated with papaya leaves for 2 h.
Table 1. The result of Bradford method beef and goat’s meat concentrations using time variation treatment with papaya leaves

| Samples and treatment                      | Concentration (X) ng/2µl |
|--------------------------------------------|--------------------------|
| beef 1 h given papaya leaves               | 16,493                   |
| beef 2 h given papaya leaves               | 28,494                   |
| beef 4 h given papaya leaves               | 23,28                    |
| Raw beef                                   | 44,185                   |
| Raw goat’s meat                            | 52,104                   |
| Goat’s meat 1 h given papaya leaves        | 9,796                    |
| Goat’s meat 2 h given papaya leaves        | 11,878                   |
| Goat’s meat 4 h given papaya leaves        | 9,48                     |

Table 2. Protein concentration of beef and goat’s meat using Kjeldahl method

| Samples and treatment                      | Concentration (% protein) |
|--------------------------------------------|---------------------------|
| Raw beef                                   | 32,953                    |
| Beef given papaya leaves                   | 27,454                    |
| Raw goat’s meat                            | 38,13                     |
| Goat’s meat given papaya leaves            | 31,406                    |

Table 2 shows the results of the Kjeldhal test to determine higher protein concentrations using untreated beef and goat’s meat. Protein analysis of beef and goat’s meat that has been treated using papaya leaves can be seen in Figure 1. Figure 1 shows missing bands of protein in beef tenderized with papaya leaves measuring of 225 kDa, 150 kDa, 96 kDa, 86 kDa, 77 kDa. Figure 1 shows that papaya leaves can cause degrade protein bands with molecular weights of 225 kDa, 150 kDa, 96 kDa, 86 kDa, 77 kDa. The time of soaking with leaf powder on 8 h beef and 1 h goat’s meat can cause loss of protein bands.

Figure 1. Beef and goat’s meat protein profile using papaya leaves tenderizer, ((1) raw beef; (M) marker; (2) 20 g of beef in 20 g of papaya leaves for 8 h; (3) 20 g of beef in 15 g of papaya leaves for 8 h; (4) 20 g of beef in 10 g of papaya leaves for 8 h; (5) raw goat’s meat; (6) 20 g of goat’s meat in 20 g of papaya leaves for 1 h; (7) 20 g of goat’s meat in 15 g of papaya leaves for 1 h)
Figure 2. Variation of papaya leaves and amount given to beef and goat’s meat. (1) raw beef; (2) 20 g of beef in 10 g of papaya leaves for 4 h; (3) 20 g of beef in 15 g of papaya leaves for 4 h; (4) 20 g of beef in 20 g of papaya leaves for 4 h; (M) marker; (5) raw goat’s meat; (6) 20 g of goat’s meat in 15 g of papaya leaves for 8 h; (7) 20 g of goat’s meat in 10 g of papaya leaves for 8 h; (8) 20 g of beef in 20 g of papaya leaves for 8 h; (9) 20 g of beef in 15 g of papaya leaves for 8 h; (10) 20 g of beef in 10 g of papaya leaves for 8 h; (11) raw beef

Figure 2 shows that the treatment of papaya leaf powder with variations in concentration and time of immersion affects the number of protein bands formed in both beef and goat’s meat. Treatment given by 20 g of papaya powder caused more damage to goat’s meat protein bands including those measuring 225 kDa, 150 kDa, 96 kDa, 86 kDa, 77 kDa, 67.5 kDa, 63.5 kDa, 58.5 kDa, 56, 51 kDa. Damage to beef protein bands given 20 g of papaya leaf powder occurred at 225 kDa, 150 kDa, 96 kDa, 86 kDa, 77 kDa, 65 kDa, 57.4 kDa.
Figure 3. Scanning Electron Microscopy photographs of collagen fibers for beef muscle of raw (A magnification were 50x, B 200x, C 1000 x, D 1000x); beef muscle effect tenderizer using leaf papaya powder (E. magnification were 50x, F. 200x, G. 1000x, H. 500X), CF collagenous fibers, MF muscle fiber, A fibers arrangements became loose

Figure 3 shows the muscle fiber in control beef densely arranged and covered by collagen fibers. Muscle fibers adhere to each other, and collagen fibers are interconnected. Figure 3 in beef treated with papaya leaf powder has stretchy muscle fiber, irregular muscle fiber and some fibers are damaged and not parallel, besides collagen fibers are also destroyed so that the bonds are separated between other collagen fibers. Muscle fiber in beef with tenderizer treatment is stretched so that there is a space between muscle fibers.
Figure 4. Scanning Electron Microscopy photographs of collagen fibers for goat’s meat muscle of raw (A magnification was 50x, B 200x, C 500 x, D 1000x); goat’s meat muscle effect tenderizer using leaf papaya powder (E. magnification were 50x, F. 200x, G. 500x, H. 1000X), CF collagenous fibers, MF muscle fiber, A fibers arrangements became loose

Figure 4 shows that raw goat’s meat has muscle fibers that are neatly arranged and attached to one another. Collagen fibers that hold muscle fibers neatly arranged and binds strongly to muscle fiber. Goat’s meat with tenderizer treatment uses papaya leaves having denatured collagen fibers, and collagen released from muscle fiber so that the arrangement is irregular and cannot bind muscle fiber. Muscle fiber in goat’s meat which was treated with tenderizer of papaya leaves stretched so that there was space between the fibers.

4. Discussion

Meat is one source of animal protein that is popular with the community. Meat that is commonly consumed by the public is beef and goat’s meat. Goat’s meat has a texture that is harder than beef. Most people processed the meat so that the texture is more tender. However, long boiling can cause loss of meat protein because it dissolves in water so that the nutritional value drops and changes the size of the meat to be smaller. Papain is a proteolytic enzyme that can be found in papaya leaves and fruit. Papain plays a role by damaging the binding tissue in meat. The first mechanism is to damage mucopolysaccharide in the basic matrix substance and then reduce connective tissue fibers so that the shape is irregular [17].

Meat tenderizer process can affect texture and concentration. The concentration of meat given with papaya leaf powder was reduced compared to control meat. Based on the concentration data of beef and goat’s meat using both Bradford and Kjeldahl methods showed a decrease in concentration compared to controls. The decrease in concentration occurs because the protein is hydrolyzed by the enzyme papain which comes from papaya leaves and fruit. Papain plays a role by damaging the binding tissue in meat. The first mechanism is to damage mucopolysaccharide in the basic matrix substance and then reduce connective tissue fibers so that the shape is irregular [17].

Analysis of proteins that are influenced by the treatment of papain enzymes was carried out using the SDS PAGE method. The SDS PAGE method can be used to see protein integrity in both treated and negative control groups of beef and goat’s meat. SDS PAGE results can be used for identification of myofibrillar, sarcoplasmic, myosin and actin muscle proteins, actinin, tropomyosin, and troponin. Protein integrity is very. Raw goat’s meat have protein bands measuring 225 kDa, 150 kDa, 96 kDa, 86 kDa, 77 kDa, 63.5 kDa, 58.5 kDa, 58.5 kDa, 56, 51 kDa, 47.3 kDa, 44 8 kDa, 40.7
kDa, 37.8 kDa, 34 kDa, 32 kDa, 29.2 kDa, 24.9 kDa, 22 kDa, 18.8 kDa, 18 kDa, and 16 kDa. Raw beef has protein bands measuring 225 kDa, 150 kDa, 96 kDa, 86 kDa, 77 kDa, 65 kDa, 57.4 kDa, 47 kDa, 43 kDa, 40.7 kDa, 39, 34 kDa, 31 kDa, 29.2 kDa, 24.9 kDa, 22 kDa, 18.8 kDa, 18 kDa, and 16 kDa. The molecular weight of protein subunits measuring 96 kDa and 86 kDa is thought to be bovine serum albumin, ovalbumin (47 kDa), carbonic anhydrase (34 kDa), lysozyme (22kDa) (Hidayat et al., 2018). Myosin light chain 1 (86 kDa), myosin light chain 2 (77 kDa), Actin (47 kDa) (Casserly et al., 2000; Claeys et al., 2004; Ahhmed et al., 2007), Glysinin A (40 , 7 kDa), glysinin B3 (24.9 kDa), Glycinin B1 (22 kDa) [9, 10, 33]. Actin protein also affects the texture of meat because it is a constituent of muscle fibers [3]. Figure 1 shows that meat that has been treated with the enzyme papain derived from papaya leaves has a loss of protein bands above 75 kDa, which is 225 kDa, 150 kDa, 96 kDa, 86 kDa, 77 kDa. Most of these protein bands are myosin light chains and myosin heavy chains. Myosin light chain is a group of regulator proteins and metabolic enzymes in meat. Myosin is very important in giving texture to meat, meat meatiness [3 – 4]. Based on these results, the administration of the enzyme papain from leaf powder causes the meat to become tender as evidenced by the loss of the myosin light chain protein band that plays a role in giving texture to the meat.

Meat protein can be divided based on its solubility to salt solvents (30-50% sarcoplasmic protein), protein dissolved in salt (50-60% protein myofibrillar), and not dissolved by water or salt (binding tissue protein 15-20%). Myofibrils, myosin has a globular structure and the ability to hold high amounts of water, actin is formed by G-actin and F-actin, actomyosin is not dissolved in water and tropomyosin is thicker and is a subunit in myosin. Sarcoplastic protein, for example myogen, globulin, myoglobin, which gives characteristics of the color of meat and binds to oxygen. Non-soluble proteins are collagen and reticulin [13].

The results of observations using SEM showed that control beef and goat’s meat had collagen fibers that were neatly arranged and attached to muscle fibers. Collagen fibers show complex structures. Beef and goat’s meat treated with soaking papaya leaf powder have different microstructures than controls. After enzymatic treatment, collagen fibers are damaged and the structure is not attached to muscle fibers. Collagen fibers experience denaturation, shrinkage, and damage, irregular participation and visible distance between muscle fibers.

5. Conclusion

Beef and goat’s meat treated with papaya leaf powder decreased in concentration and damage to protein integrity was seen from the loss of protein bands measuring 225 kDa, 150 kDa, 96 kDa, 86 kDa, 77 kDa, 75 kDa. Degraded protein bands are a type of myosin heavy chain, myosin light chain, actin, the three types of proteins play a role in giving texture to the meat, hardness, and meatiness so that the meat becomes tender. Protein damage that causes softness of meat can also be seen from SEM results which show damage to collagen fibers and muscle fibers. Stretchable muscle fibers and collagen begin to escape muscle fibers.

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