Effect of kluwak biomass fermentation and storage time on meat quality

P Patriani1*, H Hafid2, E Mirwandhono1, and T H Wahyuni1

1Department of Animal Husbandry, Faculty of Agriculture, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia
2Department of Animal Science, Faculty of Animal Science, Halu Oleo University, Jl.HEA Mokodompit, Anduonohu, Kendari 93232, South East Sulawesi, Indonesia
Email: penipatriani@usu.ac.id

Abstract. Lamb meat is one of the animal protein sources that contains high protein so it is easily damaged. One easy and cheap method to extend the storage time is by using spices, known as kluwak seeds (Pangium edule). This study aimed to determine the effect of fermented kluwak biomass in the storage time of water holding capacity, cooking loss, drip loss, and texture of lamb meat. This study used a factorial complete design with 3 replications. The results showed that the immersion level of fermented kluwak and storage time had a significant effect on water holding capacity, meat texture and drip loss (P<0.05) but had no effect on cooking meat loss (P>0.05). It is concluded that the use of fermented kluwak from 3% to 9% of meat weight can maintain the physical quality of lamb meat for 36 hours.

1. Introduction
Lamb meat is a fairly high source of protein. High protein content causes microbes to develop rapidly and could affect the quality of meat. The method of extending the storage time to maintain the quality of the meat is necessary. One method to maintain the quality of meat that is often used is by freezing or storing meat using ice cubes [1]. Various constraints such as scarcity or interference with the source of electricity used for cooling machines often occur [2], especially in rural areas. Another method for preserving fresh meat is by adding chemical preservatives such as formalin, borax and sodium nitrate but it can interfere with the health of consumers.

One effort to extend the storage time of lamb meat with easily available and abundant materials available is by using kluwak seed. It is one alternative to overcome the problem. Kluwak is easy to get and cheap, another benefit is its natural content does not endanger the health and kluwak contains various compounds which are thought to extend the storage time of animal food products. Kluwak with the Latin name Pangium edule is one of the herbs in Indonesia. Kluwak is a fermented seed from the Pangium edule tropical tree which is used as a spice in cooking and undergoes a fermentation process for 40 days after maturation [3].

Kluwak contains anti-bacterial compounds and antioxidants such as vitamin C, beta carotene, and flavonoid class compounds as anti-bacterial namely hydrocarbon acid, khaulmograt acid, tannin, and chloric acid [4]. Kluwak contains compounds that are suspected to be antibacterial namely tannin [5]. Tannin is a compound that is toxic to fungi, yeast, and bacteria [6]. Kluwak also contains cyanide compounds and antioxidant activity as free radical scavenger compounds in the body [7]. Several
studies have reported kluwak seeds can preserve fish because of the cyanide compounds contained but have not been further investigated in livestock products, especially lamb meat. Meat quality test is carried out to find out whether meat quality has decreased after cutting. Meat quality can be seen from the physical quality which includes pH, water holding capacity, cooking losses, drip loss, and odor.

1.1 Water Holding Capacity (WHC)
The water holding capacity of meat is the ability of meat protein to bind water to meat. Measurement of water holding capacity as an indicator of protein damage to meat is because meat protein can bind water in the meat. Meat can hold water well if the protein content in meat is high so that free water content decreases. There is a relationship between the holding capacity of water and pH in meat, ie a high final pH will cause a high holding capacity for water. Prior to cutting, pressure on livestock such as fasting and treatment is very influential whereas in the post-cutting period cooling, withering, injecting non-meat ingredients and cooking by heating and cooling methods also greatly affect the holding capacity of the brine [8].

1.2 Cooking loss
Cooking loss is a test of meat quality by calculating the temperature and cooking time. Meat quality is said to be good if it has a low cooking loss, during cooking little nutrients are lost. A cooking loss will increase with increasing heating temperature [9]. Cooking losses in meat generally vary greatly between 15% to 40% [10].

1.3 Drip loss
Water and nutrient that comes out of meat are called drip loss. Drip loss can be influenced by a decrease in postmortem pH in meat [11]. Drip loss can be measured by the EZ-drip loss method and the bag method. The bag method uses a piece of flesh from the longissimus dorsi muscle of about 120 grams suspended in an airtight container while the EZ method - using 2 samples of more than 10 grams is placed in a container to collect the liquid that leaks out [12]. The study of drip loss was conducted by Kapper et al. [13] that the water that comes out dripping is an indicator of the capacity to hold water in fresh meat that can be measured using the bag method and the EZ method. The ability to retain water or moisture in meat that is lost from the beginning of postmortem can also be a cause that the ability to hold water is also useful for predicting drip loss of fresh meat [14].

1.4 Texture
The texture test uses a penetrometer to measure the elasticity of the meat to the extent to which the meat will exert force on the pressure. The texture is usually related to the holding capacity of water to meat. If the water holding capacity is high then the elasticity will be high. The most important nature and quality of food ingredients is tenderness because they affect the perception of consumer acceptance [15]. Meat texture or tenderness can be influenced by the amount and wrinkles of connective tissue, the composition, and contractile muscle fibers and the level of proteolysis in stiff muscles [15]. Tenderness is very important for red meats such as lamb and beef because the composition of muscle tissue and red muscle fiber is higher compared to pork or chicken meat.

2 Materials and Methods

2.1 Materials
The tools and materials used in the study were the male thigh lamb, 40 days fermented kluwak, aquadest, label paper, digital scales, ropes, plastics, hanger wire, millimeter block paper, 2 kg load, glass plate, plastic, and penetrometer. Samples that had been taken from the flesh of the thighs with 10 grams of each then tested with 3 replications. Kluwak used in the research is kluwak that has been fermented for 40 days, mashed using a blender, aerated and sifted. Lamb meat was marinated for 1 hour in a concentration of kluwak that had been mixed with aquadest.
2.2 Trial Design
This research used a 3x4 completely randomized factorial design. As the factor, I was the level of kluwak 0%, 3%, 6%, and 9% and factor II was the storage time for 12, 24, and 36 hours at room temperature. The sample in this study was tested with 3 replications and if there were real differences continued using the Duncan test. The parameters in this study were:

2.2.1 Cooking loss
The cooking loss test had been carried out using the CSIRO method [16] and Hafid et al. [1], ie samples weighing 10 grams were boiled in polyethylene plastic at 80°C for 60 minutes. The meat was then dried using tissue paper and weighed again. Cooking loss was calculated using the formula:

Cooking loss = (meat sample before boiling - meat sample after boiling) x 100%

2.2.2 Water Holding Capacity (WHC)
WHC had been carried out using the Hamm method [16] by preparing a 300 mg lamb/lamb meat sample on Whatman filter paper No. 41. Sapper pressed between 2 glass plates with a load of 35 kg for 5 minutes. The load was then lifted and the image of the meat sample was moved to the millimeter blocks then the moisture content of meat was calculated by means of meat in the oven for 12 hours at a temperature of 105 °C.

Wet area was calculated: MgH₂O = \( \frac{{\text{Wet Area (cm}^2\text{)}} \times 8.0}{{0.0848}} \)

WHC Constants: 8.0 and 0.0848

WHC = Meat moisture content (%) - (MgH₂O / 300 mg x 100%)

2.2.3 Drip loss
Dripp loss had been tested using the bag method that utilized 100-gram pieces of meat [17]. The sample was then hung and the water drop was collected in a plastic bag. After 24 hours the meat was weighed to find out the final weight after hanging. Drip loss was calculated using the formula:

Drip loss (%) = \( \frac{{\text{Meat sample before hanging} - \text{Meat sample after hanging 24 hours}}}{\text{Meat samples before hanging}} \times 100\% \)

2.2.4 Lamb Texture
Meat texture in the study was measured using a penetrometer [18]. Meat weighed 5 grams placed on the standard and needle awl adjusted to the location of the meat. The loaded bar was removed simultaneously by pressing the timer and the load was kept pressed so the needle can penetrate within 10 seconds. The depth of the needle through the flesh can be seen on the scale.

2.3 Research Time and Location
This research was carried out from April to May 2019 in the Laboratory of Food Sciences and MIPA Organic Chemistry Laboratory at the University of North Sumatra.

3 Results and Discussion

3.1 Cooking Loss
Cooking loss is the weight of meat lost during the cooking process and is an indicator of nutrition in meat. Average cooking losses in the study can be seen in Table 1.
Table 1. Cooking loss of lamb meat given with fermented kluwak at the different storage time

| Times       | P0 (0%)  | P1 (3%)  | P2 (6%)  | P3 (9%)  |
|-------------|----------|----------|----------|----------|
| T1 (12 hours)| 43±3.08  | 49±3.57  | 43±11.4  | 49±8.68  |
| T2 (24 hours)| 51±4.09  | 52±1.33  | 50±5.26  | 51±3.56  |
| T3 (36 hours)| 31±14.94 | 33±4.73  | 31±4.45  | 28±5.51  |

Note: Asterix signs show significant differences

The results showed that kluwak immersion had no effect on cooking losses (P>0.05). The interaction between sample and storage time (hours) also did not significantly affect the cooking loss of lamb. Figure 1 shows the lowest percentage of cooking meat shrinkage in 36 hours storage when the levels of kluwak were 6% and 9%. Cooking loss was highest at 24 hours of storage at the level of 0% and 3% kluwak.

![Figure 1](image_url)  
**Figure 1.** Cooking loss of lamb meat by adding kluwak at different storage times

Cooking loss is related to texture or tenderness in meat and has a correlation with cooking duration [19] [20]. The results of this research were in line with Soeparno [16] that the length of soaking time with bay leaves as preservatives on meat did not affect the cooking losses of meat. It can be caused by the content of compounds present in fermented kluwak does not hydrolyze meat protein. This is reinforced by Gardt and Tuomi [21] that soaking can make the size of the meat enlarge and then shrink so as to experience disintegration. The lower percentage of cooking loss in meat was better than the high percentage of cooking loss because meat with high nutritional value in cooking meat comes out more during the boiling process. The best cooking loss results in this research had been done on P3T3. The average cooking loss in this study was good according to Gardt and Tuomi [21] that meat had a cooking loss value between 1.5% to 54.5%.

### 3.2 Texture of Lamb Meat

Meat texture or tenderness is one of the most important factors in testing meat quality and consumer attributes. The texture test can predict the tenderness of the meat from the force required to penetrate the raw meat sample [22]. Average textures in the study can be seen in Table 2 and Figure 2.
Table 2. The thickness of meat given by kluwak fermentation and different storage time

| Times       | Percentage of kluwak |
|-------------|----------------------|
|             | P0 (0%)   | P1 (3%)   | P2 (6%)   | P3 (9%)   |
| T1 (12 hours) | 108±8.7   | 108±14   | 93±13.4   | 80±8.5   |
| T2 (24 hours) | 110±7.3   | 113±11   | 97±14.5   | 102±12.6 |
| T3 (36 hours) | 108±9.5   | 106±8.08 | 105±13.5  | 102±8.6  |

Note: Asterix signs show significant differences

The results showed that immersion in fermented kluwak significantly affects meat texture (P<0.05). This means that soaking lamb in fermented kluwak can change the texture of meat compared to meat without immersion. Duncan test results show that the storage time is significantly different from the texture of meat at 5% level, namely P0T1 with P2T1, P0T1 with P3T1, P0T2 with P2T2. The P3T1 treatment has the lowest texture and the P2T2 treatment interaction gives the greatest texture value, as can be seen in Figure 2.

Figure 2. Lamb meat texture with the addition of kluwak at different storage times

Lawrie [23] stated that some of the most important factors affecting meat texture are collagen content, muscle shortening rate, temperature, livestock species, and enzymes. Hughes et al. [24] revealed that meat texture can also be affected by connective tissue content and muscle size. Pickling meat can also occur by proteolytic enzymes contained in preserved and stored meat.

3.3 Water Holding Capacity (WHC)

The results of the research can be seen in table 3 and figure 3. The ability of meat to bind water is also called water holding capacity. Water holding capacity (WHC) and tenderness of meat are the main determinants of visual and sensory attractiveness [25]. Water content in meat acts as a muscle protein plasma, and water is lost from the myofibril structure as a result of protein denaturation [25]. The results showed that the immersion level of kluwak and the storage time had a significant effect on the water holding capacity (P < 0.05).

Table 3. WHC lamb chops given kluwak levels at different storage time

| Times        | Level of kluwak |
|--------------|-----------------|
|              | P0 (0%) | P1 (3%) | P2 (6%) | P3 (9%) |
| T1 (12 hours)| 19.2±3.5 | 21.3 ±0.4 | 21.5±0.2 | 22.4±1.2 |
| T2 (24 hours)| 21.5±1.0 | 40.5±5.0 | 40.8±4.1 | 30.1±1.1 |
| T3 (36 hours)| 26.0±0.9 | 33.6±12 | 26.3±7.5 | 21.8±1.9 |

Note: Asterix signs show significant differences
This means that the immersion of lamb in fermented kluwak affects lamb meat. Duncan test results showed that the storage time was significantly different from the meat texture at 5% level, namely P0T2 with P1T2, P0T2 with P2T2, P0T2 with P3T2, P0T3 with P1T3. In the P0T1 control had the lowest WHC while the interaction of the P1T2 and P2T2 treatments gave the highest WHC value. It is suspected that prolonged storage time will reduce the water holding capacity so that there is a change in the structure of the protein in meat. Denaturation of meat protein that occurs after slaughtering will cause meat’s WHC to fall. The best WHC in this study were the P1T2 and P2T2 samples as can be seen in Figure 3. The higher WHC value indicated better meat quality because the meat can maintain the water content.

Figure 3. WHC lamb with the addition of kluwak at different storage times

The water holding capacity (WHC) in this study was higher than the research of Patriani et al. [26] that the holding capacity of meat water 12 hours after cutting was 17.89%, added that the change in the holding capacity of meat water during storage was thought because some ions were bound by meat protein.

3.4 Drip Loss
Drip loss is the drop of water during the storage process, causing a heavy shrinkage of meat. Drip loss can cause fluid loss with meat nutrients usually in the refresher of frozen meat and withering meat. Drip loss of meat lamb is usually influenced by the length of hanging after cutting, the location of the muscle of the meat, and temperature. The results showed that the immersion of fermented kluwak had a significant effect on drip loss (P<0.05).

| Times      | Level of Kluwak |
|------------|-----------------|
|            | P0 (0%) | P1 (3%) | P2 (6%) | P3 (9%)   |
| T1 (12 hours) | 3.32±0.68 | 1.79±0.76 | 2.25±1.33 | 3.63±1.91 |
| T2 (24 hours) | 10.24±7.60 | 2.01±0.61 | 4.07±2.80 | 4.22±4.92 |
| T3 (36 hours) | 6.23±6.35 | 1.12±0.63 | 2.80±1.61 | 1.83±1.98 |

Note: Asterix signs show significant differences

Duncan test results showed that the storage time is significantly different from meat drip loss at 5% level, namely P0T1 with P1T1, P0T2 with P1T2, P0T3 with P1T3 and P3T3. The highest average drip loss was P0T2 with 10.24%, while the lowest drip losses were P1T1 at 1.79%, P1T3 at 1.12% and P3T3 at 1.83%. It is suspected that fermented kluwak can inhibit the release of water droplets in the
process of hanging meat so that the drip loss can be maintained. The higher the percentage of drip loss, the more meat loses water and protein from meat so that the meat quality is deteriorated.

![Figure 4. WHC lamb with the addition of kluwak at different storage time](image)

All of these effects, the occurrence of quality deviations such as PSE, DFD, sour meat are also very influential on drip loss. The drip loss depends on the shortening of the sarcomere which is drip loss in meat. Lamb meat marinated in fermented kluwak in this study was lower than the study of Bramblet et al. [27] that lamb meat had a drip loss between 4.04-6.46%. Bramblet et al. [27] in their study stated that sheep given high-stress drip loss were equal to 13.50%. Several factors that influence the occurrence of quality deviations such as PSE, DFD, sour meat, RSE, PFN cooking affect the level of droplet loss as well [28] [29]. Based on this, the percentage of drip loss in research was quite good.

4. Conclusion
Meat immersion in fermented kluwak and storage time significantly affected the water holding capacity, meat texture and drip loss (P<0.05), but did not affect the cooking meat loss (P>0.05). The use of fermented kluwak from 3% to 9% by meat weight can maintain the physical quality of lamb for 36 hours. Further research is needed on the use of fresh kluwak seed extraction to extend the storage time of lamb meat.

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