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The Effect of Marination Duration using Tamarind Paste (*Tamarindus indica L.*) on pH, Tenderness, Water-holding Capacity and Cooking Loss of Spent Layer Meat

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Abstract: Tamarind fruit (*Tamarindus indica L.*) has a blackish brown colour and emits a sour smell. The organic acids in tamarind can affect the physical properties of meat. The objective of this study was to determine the effect of marination duration for meat from the spent layer using tamarind paste on pH, tenderness, water holding capacity and cooking loss. The research was carried out experimentally using a completely randomized design with 4 treatments and 5 replications. Tamarind paste was made using distilled water and the flesh of tamarind at ratio of 2:1, pasteurized at 80°C for 10 minutes and cooled down. Fresh meat was marinated using tamarind paste at a proportion of 20% of meat weight. The duration of marination using tamarind paste was divided into initial time (0 minute), 15 minutes, 30 minutes and 45 minutes. Data were analysed using one-way analysis of variance and continued by Duncan's multiple range test. The results showed that the duration of marination had a significant effect ($P<0.05$) on the pH and tenderness of the meat without affecting water-holding capacity and cooking loss. It was concluded that the optimal marinating time using tamarind paste for tenderizing spent layer breast meat is 45 minutes.

Keywords: Spent Layer Meat, Tamarind Paste, pH, Water-holding Capacity, Cooking Loss, Tenderness

INTRODUCTION

Spent layer meat is meat cut from laying hens whose egg production is low, between 20-25%, around 96 weeks of age with tough meat characteristics [1]. According to Bulkaini [2], the characteristics of the meat of rejected laying hens are that the texture is rough and the meat is tough. The characteristics of the meat of rejected laying hens are tough because the chicken is slaughtered at an old age. The older the livestock, the tougher the meat will be because there is more connective tissue in the meat [3].

The nutrients contained in the meat of spent layers are protein, fat, carbohydrates, vitamins and minerals, where the meat of spent layers in the breast contains 24.9% protein, 3.6% fat content and 72.86% water content [4]. The nutritional content of meat in chickens will be influenced by feed, chicken age and chicken rearing conditions [5].

The meat of rejected laying hens is less tender, so not many people process or cook the meat of rejected laying hens [6]. Meat tenderness is influenced by the age at which the chicken is slaughtered and the physical activity of the chicken before slaughter, one of which is stress on the livestock before slaughter [7].

In efforts to increase the economic value of the meat of rejected laying hens, several processes need to be carried out, one of which is the marination method. Marinating is the process of soaking meat in marinade ingredients before further processing with the aim of improving the taste, increasing tenderness and increasing the shelf life of the product, where marinating can also make the texture of the meat juicier [8]. Marinating can use the acid contained in fruits in Indonesia such as starfruit, strawberries, lime, gelugur acid and others. One of them can use tamarind.

Tamarind fruit can be used as a marinade ingredient because it contains acid. The use of tamarind as a marinade ingredient has not been widely researched or published. Tamarind is a fruit that is well known to Indonesian people because it has a distinctive sour taste

so that it is used by people as a flavouring agent, spice or flavouring in food and drinks [9]. Tamarind can be a fruit that has many benefits, including as a traditional medicine, antimicrobial, anti-diabetes mellitus, laxative and can help you sleep soundly [10]. Tamarind can be processed into paste form to make it last longer, where tamarind paste can be stored for 90 days (3 months) in the refrigerator [11].

Tamarind can be used as a marinade ingredient because it contains several organic acids which can affect the physical properties of meat. The tamarind content has a pH of 2.50 [12]. The low acid content in tamarind can be used as a marinade ingredient because the acid can damage the protein tissue in meat. The acid content in tamarind includes tartaric acid, malic acid, succinic acid, acetic acid and citric acid. The amount of organic content in tamarind is citric acid, around 4-6%, malic acid around 4.6 - 4.7% and tartaric acid around 5.3-5.8% [13].

The organic acid content in tamarind fruit such as citric acid, tartaric acid and malic acid can become organic acids which affect pH, water holding capacity, cooking losses and meat tenderness. The activity of organic acids hydrolyses protein in muscle fibres, which causes the connective tissue to break down so that the meat becomes more tender [14]. The citric acid content can break down proteins in meat which causes the cathepsin enzyme to be active so that the tenderness of the meat can increase [7]. Citric acid and lactic acid have the ability to prevent oxidation of fat in meat before the cooking process, which contributes to maintaining the sensory quality of meat [15]. Apart from that, acetic acid changes the pH, water content and cooking loss of meat [16]. The higher the use of tamarind solution, the citric acid content and malic acid content in tamarind fruit can cause the value of water holding capacity in the meat of spent layers to decrease [17].

Tamarind fruit (*Tamarindus indica L.*) has bioactive compounds such as saponins, phenols, terpenoids, alkaloids, flavonoids and tannins [18]. The bioactive content of phenols and terpenoids can loosen meat myofibrils when heated, which makes proteins denature and causes intracellular water in the meat to come out, thus affecting meat tenderness. Apart from that, the saponin content in tamarind can soften meat [19]. Tamarind pressure causes damage and changes in the structure of muscle proteins, especially actin and myosin, so that the ability of muscle proteins decreases and the tenderness of meat increases [17].

Apart from the marinating ingredients, you also need to pay attention to the marinating time for the meat. The longer the meat is marinated, the water holding capacity and pH will decrease because the acid will diffuse in the tissue which causes the pH value to drop due to protein denaturation which not only affects the pH and water holding capacity, the length of marinating the meat can affect the tenderness and cooking loss because there is protein denaturation in meat which makes the meat tissue bonds open [20]. The relationship between the length of marination and the use of acid in fruit is that the longer the marination is carried out, the more acid can be absorbed optimally in the meat myofibril tissue so that the peptide bonds in the meat fibre protein are cut [7].

There have not been many studies related to the use of tamarind paste and the duration of marination to tenderize spent layer meat. Using tamarind with a concentration of 15% with a soaking time of 20 minutes has the best effect on the pH and water holding capacity of turkey meat [17]. The acid in fruit can cause a denaturation process because the peptide chain breaks down from polypeptide to dipeptide and each fragment is free of water molecules and dissolves out of the microstructure of the meat [21]. The addition of 20% pineapple extract for 30 minutes provides the best physical quality of the meat of the spent layers, where the bromelain enzyme in pineapple gives a sour taste so that the pH value of the meat of the spent layers decreases. The change in pH is influenced by protein denaturation. Therefore, this study aims to observe the impact of the duration of marination with tamarind paste on pH, water-holding capacity, cooking loss and tenderness of spent layer meat, as well as determine the relationship between meat pH and its tenderness.

MATERIALS AND METHODS

Tamarind Paste Preparation

Tamarind fruit with blackish brown flesh is used as a marinade ingredient. Distilled water is used as an ingredient for soaking tamarind with a ratio of tamarind and water of 1:2 [11]. The tamarind fruit is peeled and the flesh is removed, then soaked in distilled water for 20 minutes at 80 °C to reduce the population of bacteria and yeast. The seeds are separated from the flesh, then the fruit juice is filtered. The tamarind juice is then reheated at 80 °C for 10 minutes to reduce the water content and form a paste. Tamarind paste is cooled and stored in the refrigerator for 24 hours before being used to marinate meat.

Meat Marination

Meat was obtained from 10 culled ISA Brown laying hens aged 80 weeks which were kept under the same management. The average body weight of rejected laying hens is 2.4 kilograms with a coefficient of variation of 8.78. The samples used were the right and left parts of the chest (*Pectoralis major*) with total of 20 samples were obtained. Mechanical tenderizing was performed on meat using fork prior to getting marinated.

Javanese paste as much as 20% of the weight of the meat is smeared on the surface of the meat. The meat was marinated for 15 minutes, 30 minutes and 45 minutes in the refrigerator at 4 °C. After the marination time was reached, the meat samples were immediately rinsed with distilled water, then prepared for testing pH, water-holding capacity, cooking loss and tenderness.

pH value Measurement

Meat pH was measured using a digital pH meter. The pH meter electrode is calibrated using a pH 4 and 7 buffer solution before use. The meat samples were ground using a mortar, transferred to a glass beaker and added with

distilled water in a ratio of 1:10 (w/v), then stirred. The pH value of the meat is recorded when the digital number on the pH meter layer is stable.

Water-holding Capacity Measurement

The water-holding capacity (WHC) of the meat was determined according to Hamm method. Meat sample without visible fat and connective tissue was weighed 0.3 g, put on Whatman No. 42 filter paper, covered with glass plates and pressed using 35 kg load for 5 minutes. After that, the total wet area and meat area were measured. The moisture content of the meat was determined according to AOAC method using oven at 105°C. The weight loss (%) after oven drying was used as moisture content. WHC was measured the following formula:

$$Water\ holding\ capacity = moisture\ content(\%) - moisture\ loss(\%)$$

Cooking Loss Measurement

Meat cooking loss is measured based on the percentage of weight loss during cooking at 80 °C for 30 minutes in a water bath [22]. Meat sample was weighed 30 g and was put into a zipper bag. After 30 minutes, the sample was cooled to room temperature at 27 °C until the weight stabilized. The cooked samples were weighed. Cooking loss was determined using the following formula:

$$Cooking\ loss\ (\%) = \frac{Initial\ weight\ (g) - Final\ weight\ (g)}{Initial\ weight\ (g)} \times 100\%$$

Tenderness Measurement

Meat tenderness is measured using a penetrometer [23]. Cooked meat samples were cut into 5 cm dimensions ×2.5 cm ×1.5 cm with a weight of 30 g. The meat is boiled for 30 minutes at 80 °C, then drained at 27 °C until the weight is stable. The sample is placed on the base of the penetrometer and the position is adjusted to the tip of the awl. Data was recorded 10 times at different points in units of mm/g/10 s.

Statistical Analysis

Data were analysed using one way analysis of variance (ANOVA). If the ANOVA results show a real difference, then the analysis continues with Duncan's multiple range test to separate the means at the 95% confidence level. Simple linear regression analysis was used to determine the correlation between the pH value of meat and its tenderness.

RESULTS AND DISCUSSION

pH Value

The average pH recorded in various marination duration treatments ranged from 4.96-5.93 (Table 1). The results obtained showed that there was a significant effect (P<0.001) between the duration of marination on the pH of spent layer meat. The longer the laying hen meat is marinated, the pH value decreases, where the highest pH is found in untreated laying hen meat with a value of 5.93 and the lowest pH is in the 45-minute marination treatment with a value of 4.97. The pH of the control meat was significantly higher than the marinated meat. The pH value of the breast meat of the rejected laying hens when marinated for 15, 30 and 45 minutes had no significantly different results, presumably because the marination time was not too long.

The pH value in the treatment group was more acidic compared to the control because the pH of the tamarind paste was low (2.5) and the organic acids contained in the tamarind paste were absorbed into the meat and worked optimally. Tamarind fruit contains citric acid, malic acid and tartaric acid which can reduce the pH value of meat so that the quality of the meat becomes good [17]. The high citric acid content can be absorbed into the meat by osmosis, causing the pH of the meat to become acidic, where the citric acid that enters the meat can interact with the water contained in the meat [24].

Apart from the content of tamarind fruit which can cause the pH value of meat to decrease, the length of marination can also affect the pH value. A pH that becomes acidic causes the cathepsin enzyme to become more active [25]. According to Patriani *et al.* [7], a decrease in the pH value of meat can be caused by long periods of marination using acid, which affects the hydrolysis of the substance. Protein hydrolysis can renovate meat protein so that the peptide bonds in meat protein cause more hydrogen ions to be released from the hydrolysis reaction, so the more hydrogen ions contained in the meat, the lower the pH value will be.

Water-Holding Capacity

Table 2 shows that the average water holding capacity produced in various long marination treatments using tamarind paste (*Tamarindus indica L.*) ranged from 42.02 – 47.61%. The results of the analysis of variance showed that the long marinating treatment for the meat of the rejected laying hens using tamarind paste had no significant effect on the water holding capacity of the meat of the rejected laying hens. According to Soeparno [26], meat has a water holding capacity ranging from 20% – 60%. Based on this, the water holding capacity value in this study is classified as normal. The water binding capacity value will be directly proportional to the pH value of the meat. If the pH value of

Table 1: pH values of spent layer meat before and after marination using tamarind paste

Marination duration (minute)	pH value
0	5.93±0.08^{b}
15	5.16±0.16^{a}
30	5.09±0.19^{a}
45	4.97±0.32^{a}

Mean ± Standard Deviation, $^{a, b}$ significant difference (P<0.05)

Table 2: Water-holding capacity of spent layer meat before and after marination using tamarind paste

Marination duration (minute)	pH value
0	5.93±0.08^{b}
15	5.16±0.16^{a}
30	5.09±0.19^{a}
45	4.97±0.32^{a}

Mean ± Standard Deviation

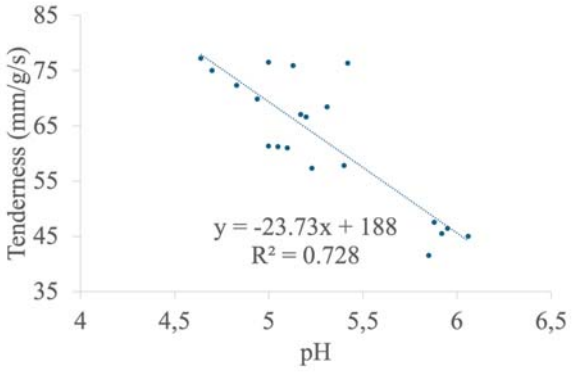


Figure 1: Linear regression between pH value and tenderness of spent layer meat marinated using tamarind paste

Table 3: Cooking loss of spent layer meat before and after marination using tamarind paste

Marination duration (minute)	pH value
0	5.93±0.08[b]
15	5.16±0.16[a]
30	5.09±0.19[a]
45	4.97±0.32[a]

Mean ± Standard Deviation

Table 4: Tenderness of spent layer meat before and after marination using tamarind paste

Marination duration (minute)	pH value
0	5.93±0.08[b]
15	5.16±0.16[a]
30	5.09±0.19[a]
45	4.97±0.32[a]

Mean ± Standard Deviation, [a, b] significant difference (P<0.05)

the meat is low then the water binding capacity value will also be low [20]. However, in this study, water holding capacity was not affected by the marination duration of up to 45 minutes. This is because the marinade ingredient used is tamarind fruit which is processed into a paste so it is thicker. Apart from that, the water holding capacity value did not have an effect because the pH value in this study did not decrease too far so the water holding capacity value was not too influential. According to Zahro *et al.* [27], the pH value is related to the water binding capacity, where the further away the pH isoelectric point of meat (5.0-5.4), the meat will experience an increase in water binding capacity.

Cooking Losses

Table 3 shows that the average cooking losses produced in various long marination treatments using tamarind paste ranged from 27.52 – 31.54%. The results of the analysis of variance showed that the long marinating treatment for the meat of the rejected laying hens using tamarind paste had no significant effect on the cooking loss of the meat of the rejected laying hens. In the range of 15-40%, meat cooking loss is classified as normal [26]. Meat that has a low cooking loss value means the quality of the meat is good because little of the meat's nutrients are lost during the cooking process [7].

Meat that was cooked at the time of the research for 30 minutes resulted in the liquid in the meat having run out due to long cooking so that cooking losses had no real effect. The length of cooking time for meat can affect collagen solubility [20]. Cooking loss is influenced by muscle fibres, size and weight of the meat sample, cooking temperature, cooking time and cross-section of the meat [26]. In this research, the temperature used was 80°C, which is the ideal temperature for cooking meat.

Tenderness

The average R value of tenderness recorded in various marination duration treatments ranged from 45.18-76.18 mm/g/s (Table 4). The results of the analysis of variance showed that the long marination treatment for the meat of the rejected laying hens marinated with tamarind paste had a significant effect (P<0.001) on the tenderness of the meat of the rejected laying hens. The longer the laying hen meat is marinated, the more tender the meat is, where the highest tenderness value is at 45 minutes and the lowest tenderness value is for meat without marinating. The marinated meat starts to become tender after the 15th minute. This is because there is a correlation between pH and meat tenderness (Figure 1). Damage to the muscle protein structure in actin and myosin occurs due to the influence of tamarind which works optimally so that the structure of the muscle protein fibres is damaged and makes the meat tender. In addition, citric acid, which causes the pH in meat to drop, can cause the dark lines of the myofilaments to not be intact, so that the attractive force on the muscle fibres is lost as a result of which the tenderness of the meat increases [24].

The increase in tenderness in this study could be due to the influence of long soaking using tamarind paste, where the acid will make the pH of the meat acidic and the meat protein will break down due to the cathepsin enzyme being activated in an acidic atmosphere [25]. According to Patriani and Hafid [28], the cathepsin enzyme in meat will be active in acidic conditions and break down complex peptide bonds in proteins in meat, resulting in an increase in tenderness.

Tenderness of meat is an important thing that people want. There are three components of meat that determine tenderness, including myofibrillar structure and concentration status, connective tissue content and level of cross-linking and water holding capacity by meat protein and meat juice [1]. Other factors that influence meat tenderness are antemortem factors (before slaughter), including animal age, breed, management, genetics, type and stress and postmortem factors (after slaughter), namely meat processing methods [7].

CONCLUSION

The length of marination using tamarind paste on the meat of spent layers has an effect on tenderness and pH, while water holding capacity and cooking loss have no effect. The effective duration of marination using tamarind paste on spent layer meat is 45 minutes with a pH value of 4.97, water-holding capacity of 42.02%, cooking loss of 27.52% and tenderness of 76.18 mm/g/s.

Key Findings

Marination using tamarind paste at 20% of spent layer breast meat until 45 minutes did not influence meat water-holding capacity and cooking loss, but it decreased meat pH and improved cooked meat tenderness.

Known and New

The flesh of tamarind fruit has been widely used as a food ingredient. Its tangy flavour improves the aroma and taste of food. Its acidic properties have often been used for preserving food. However, its use as a natural tenderizer of spent layer meat has not been published yet. The duration of marination using this ingredient has not been known yet. This study shows that 45 minutes of marination using tamarind paste can tenderize spent layer meat without altering cooking loss.

Implications

The potential use of tamarind paste as natural tenderizer for spent layer meat has been confirmed in this study. Study on the shelf life and convenient use of tamarind paste itself is required to develop a natural meat tenderizer.

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