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Effect of papaya leaves, noni fruits, and extracts formulation pineapple by-products on the meat quality of culled laying hens

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Abstract
The advancement of livestock food items, particularly chicken meat, relies on innovations in the functional characteristics of food ingredients. Meat from culled laying hens (aged 18-20 months) has a tough consistency that diminishes its appeal to consumers and its market value. The study aimed to assess a marinade mixture's impact on bacterial content and physical quality of chicken meat using extracts from papaya leaf (EDP), noni fruit (EBM), and pineapple by-products (ELN) from culled laying hens. Twelve hens were divided into a control group (P0) and three treatment groups (P1, P2, P3) with varying extract concentrations, pH, cooking loss, total plate count (TPC), and *Salmonella* sp. presence were measured. Results indicated acceptable pH and cooking loss values, but TPC exceeded limits with no *Salmonella* sp. detected. ANOVA and Duncan tests were used for analysis. The study suggests the marinade treatment did not adversely affect pH or cooking loss but increased TPC due to prolonged storage at refrigerator temperatures that result in an increase in microbial activity. This research sheds light on using natural marinade extracts, showing both positive and concerning outcomes for chicken meat quality. Insights are highlighted into enhancing meat quality with marinades using the extracts of pineapple by-products, papaya leaves, and noni fruit.

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INTRODUCTION

The swift progress of edible products, particularly individuals from the animal husbandry industry, is closely related to cutting-edge understanding of the functional characteristics of food ingredients. These properties become evident when these elements engage within a multi-component food system, as described by Komansilan (2015). For instance, culled laying hens have weaknesses in their meat texture, resulting in low consumer appeal. The age of cull chickens, which ranges from 18 to 20 months, causes the meat to be tough, leading to a low sales value and infrequent consumption (Ismanto & Basuki, 2017). Consequently, a technique is needed to improve the tenderness of culled laying hen's meat (Biyatmoko et al., 2018). A single method to enhance the quality of chicken meat from discarded laying hens is to use natural ingredients for

marination, which has the advantage of increasing meat softness and diminishing cooking losses. The process of marinating involves the utilization of acid from natural ingredients, such as yellow passion fruit juice, to alter the meat's pH, resulting in improved capacity to retain water, minimized cooking loss, and increased meat softness (Kamila et al., 2015). In addition, the immersing process can prolong the shelf life of the meat when using *Psidium guajava* extract (Haikal et al., 2021).

The meat of culled laying hens is tougher and less tasty than that of broiler chickens. Research has shown that spent hen meat has a lower taste value than broiler meat, and it is considered to require additional processing or flavor enhancers to improve its palatability (Lee & Kim, 2021). Additionally, the meat of old laying hens is generally tough and chewy, which negatively impacts its cooking quality and technological properties (Liu et al., 2023). According to Khairisa et al. (2023), the average taste of meat from culled laying hens did not exhibit a significant difference across all treatments. However, the treatment which involved a 30% combination of three mixtures of pineapple 10% by-products extract (ELN), 10% papaya leaf (EDP), and 10% noni fruit (EBM) extracts were identified as the best treatment in terms of taste, with a value of 2.62. This suggests that the specific combination of extracts in 30% extract group contributed to an improved taste of the culled laying hen meat.

The marination process utilizes cost-effective and readily available natural ingredients, specifically fruit extracts and by-products from pineapple, papaya, and noni fruit. Noni fruit extract is known for its meat-tenderizing enzymes. The protease enzymes are found in papaya (papain and chymopapain). Pineapple (bromelain) is used to tenderize the meat (Ismanto & Basuki, 2017; Rismawati. R et al., 2016). To determine the novelty of this research compared to previous studies, the researchers sought to evaluate the impact of three combined extract formulations from pineapple waste, papaya leaf, and noni fruit extracts at different concentration levels on the quality of meat from culled laying hens. This involved utilizing a blend of these extracts for seasoning to evaluate pH and cooking loss, followed by testing for Total Plate Count (TPC) and *Salmonella* sp. bacteria in every extract concentration level.

This comprehensive evaluation of the marination process using these specific fruit extracts and its effect on the meat of culled laying hens is a novel approach that has not been previously explored. The researchers aimed to assess the impact of marination on the meat of rejected laying hens by using a combination ELN, EDP, and EBM on pH and cooking loss to determine the preferred extract formulation, toward the quality of culled laying hens' meat, and the results of this research are expected to provide better insights into safe and effective meat processing practices, as well as to enhance the added value of culled laying hens'meat. The combination of these ingredients aims to leverage their proteolytic properties to enhance the quality of meat from spent hens, making it more tender and palatable (Khairisa et al., 2023; O'Quinn et al., 2018). Subsequently, they conducted microbiological testing for TPC and *Salmonella* sp. bacteria. The blend of pineapple by-products, papaya leaves, and noni fruit extracts not only serves to normalize pH and reduce cooking loss but is also effective in inhibiting microbial growth due to the presence of flavonoids in all three ingredients (Abdel-Naeem & Mohamed, 2016).

The synergistic effects of pineapple waste extract, noni fruit, and papaya leaves positively influence the quality of spent hen meat by normalizing pH, reducing cooking loss, inhibiting *Salmonella* growth, and lowering TPC levels, as a balanced pH is crucial for maintaining meat quality and preventing spoilage. The proteolytic enzymes present in papaya leaves and pineapple waste help retain moisture by breaking down tough muscle fibers, thus minimizing cooking loss (Fernández-Lucas et al., 2017). Some studies have shown that extracts from these sources can effectively reduce *Salmonella enterica* and *Salmonella typhi* populations in food

products, enhancing food safety. The antimicrobial properties of flavonoids and other compounds found in pineapple waste, papaya leaves, and noni fruit contribute to lowering TPC levels in meat products, thereby improving their safety and shelf life (Firdaus et al., 2022). There is a complex interplay between pH, cooking loss, *Salmonella* detection, and TPC in determining the quality and safety of meat products. Maintaining an optimal pH through various processing methods not only enhances tenderness but also reduces microbial risks and cooking losses. The aim of this study is to explore the impact of different fruit extract formulations on the meat quality of discarded laying hens.

METHOD

The components utilized for preparing the extract included pineapple peels, papaya foliage, noni fruit, and distilled water. The equipment utilized in producing the extract included a blender, filter cloth, scale, and measuring cup. The study utilized a fully randomized design (FRD) with a factorial layout derived from adaptations by (Falahudin et al., 2022; Rismawati. R et al., 2016; Supiatun, 2018). The independent variables included pineapple waste extract (ELN), papaya leaf extract (EDP), and noni fruit extract (EBM) at concentrations of 15%, 22.5%, and 30%. The research assessed the impact of these extracts on pH, cooking loss in meat, total plate count (TPC), and *Salmonella* sp. Every treatment was conducted five times, leading to 20 experimental units. The data obtained from the tests were analyzed using analysis of variance (ANOVA) with a significance level of 5%. The test data obtained from the study will be analyzed using analysis of variance (ANOVA) with a significance level of 5%. If the treatment has a significant effect, the Duncan test will be conducted using IBM SPSS Statistics 27 for Windows application. The study will evaluate several treatments for culled laying hens, reaching an average age of 80-90 weeks. which will be soaked, and incubated for 30 minutes in each treatment with manual stirring, and storage in the refrigerator at 4°C

The concentrations given are as follows (Kamila et al., 2025; Haikal et al., 2021):
P0: No extract (control group)
P1: 15% extract group (5% ELN + 5% EDP + 5% EBM),
P2: 22.5% extract group (7.5% ELN + 7.5% EDP + 7.5% EBM)
P3: 30% extract group (10% ELN + 10% EDP + 10% EBM)

Meat-Quality

Acidity level (pH) of meat

The pH test involves calibrating the pH meter using pH 4.01 and 6.86 buffers, preparing the meat sample with distilled water, immersing the pH meter into the sample, and averaging the results after five measurements.

Cooking Loss

The meat sample was placed in a plastic clip and tightly sealed. The meat was boiled using hot water in a pan on the stove at 70°C for 30 minutes, followed by cooling while still covered using running water. The meat was then removed from the plastic clip and dried with tissue. The test was conducted three times, and the results were average. The meat was weighed again and used for the final weight determination. The cooking loss of the meat was calculated based on the results (Pang et al., 2020).

$$\text{Cooking Loss} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Final Weight}} \times 100\% \quad (1)$$

Microbiological Tests of Total Plate Count (TPC) and *Salmonella* sp.

The microbiological testing of Total Plate Count (TPC) and *Salmonella* sp. was conducted at the Microbiology Laboratory of PT. Saraswanti Indo Genetech. TPC assessed via pour plate technique utilizing PCA (Merck), in accordance with SNI 2897:2015. Samples diluted in series, incubated at 35°C for 48 hours, counted colonies ranging from 30 to 300, which has been accredited by the National Accreditation Committee (KAN) based on ISO/IEC 17025:2005 with respective SNI ISO 4833-1: 2015 and ISO 6579-1:2017/Amd 1:2020 method.

RESULTS AND DISCUSSION

The pH values of culled laying hens fell within this range, suggesting that their quality met the Indonesian National Standardization criteria (Table 1). Furthermore, the pH values were above the isoelectric point (pH > 5.2-5.4), which allows meat to have high water binding.

Table 1. The pH values for each level of the soaking treatment

Treatments	pH Measurements
P0	6.970[a]
P1	6.835[a]
P2	6.992[a]
P3	6.918[a]

Note: The lowercase letter "a" in the diagram indicates a lack of significance (P > 0.05).

After cooking, the meat is weighed again to determine the final weight, and the cooking loss percentage is calculated based on the difference between the initial and final weights. This calculation is used to evaluate the quality of the meat, as cooking loss can be an indicator of tenderness and juiciness. Figure 1 shows the average cooking loss in each treatment for the cooking loss of discarded laying hens.

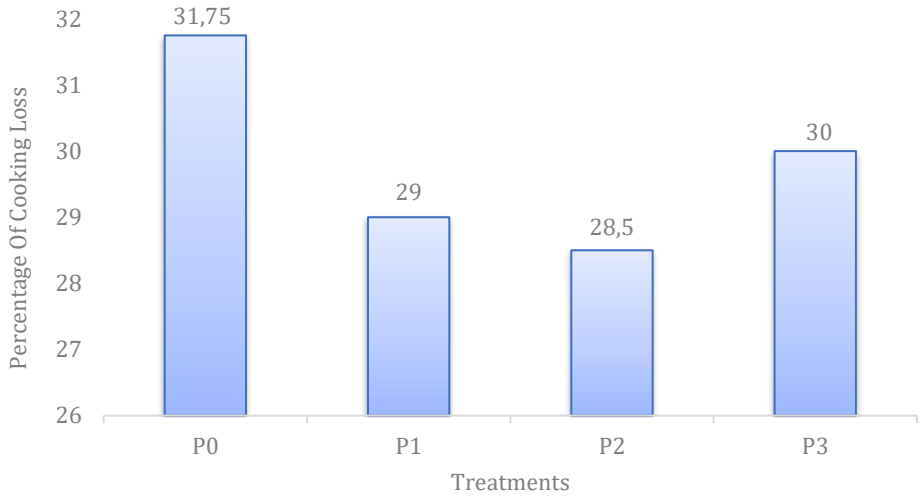


Figure 1. Percentage of cooking loss for each soaking treatment level

Note: The Lowercase letter “a “in the diagram indicates an insignificant difference (P>0.05).

The cooking loss for the meat falls within the range of 28.5% to 31.75%. The impact of the immersion concentration level treatment on the cooking loss of culled laying hens remains within the normal category. The TPC test results for the four treatment samples indicated a range of $7.8x10^6$ to $5.8x10^7$ colonies/gram. These findings suggest that the meat-soaking process leads to significant water retention, creating an environment conducive to bacterial proliferation. All four samples exhibited high total plate counts (TPC), exceeding the SNI ($1x10^6$) standard Table 2. shows the results of microbiological tests, namely TPC and *Salmonella* sp. Using each concentration level of the extract combination (ELN + EDP + EBM). Table 2. shows the results of microbiological tests, namely TPC and *Salmonella* sp. Using each concentration level of the extract combination (ELN + EDP + EBM).

Table 2. TPC and *Salmonella* sp. test results for each marination concentration

Marination Concentration Levels	TPC (CFU/g)	*Salmonella* sp. (/ 25 g)
P0	$1.4x10^7$	Negative
P1	$5.8x10^7$	Negative
P2	$7.8x10^6$	Negative
P3	$1.1x10^7$	Negative

Note: The TPC and *Salmonella* sp. tests were conducted at PT. Saraswanti Indo Genetech, which is accredited by the National Accreditation Committee (KAN) based on ISO/IEC 17025:2005, using the respective SNI ISO 4833-1:2015 and ISO 6579-1:2017/Amd 1:2020 methods.

The TPC test results for the four treatment samples revealed a range of $7.8x10^6$ to $5.8x10^7$ CFU/g. These findings indicate that the meat-soaking process leads to significant water retention, creating an environment conducive to bacterial proliferation. All four samples exhibited high total plate counts (TPC), surpassing the SNI ($1x10^6$) standard.

pH Values

The combined effect of different extracts, including ELN, EDP, and EBM, was studied to understand their influence on pH values. According to the Indonesian National Standardization, food pH values range from 6.0 to 7.0, as stated by Rakhmawati & Handayani (2020). As reported by Ramadhani et al. (2020). Table 1 presents the average impact of varying levels of extract concentration used as a marinade for the meat of culled laying hens on the meat's pH. The pH value serves as an indicator of the quality of culled laying hens. Prayogo et al. (2020) stated that there is no synergistic combination of acids and bases that does not affect the value of cull-laying hens. A decrease in the pH value of meat can occur due to the presence of citric acid in pineapple and ascorbic acid in noni, but it is hindered by alkaloid compounds in papaya leaf extract, which can increase the pH value of meat. Zainal et al. (2021) stated that meatballs can have their pH lowered and hence improved by using organic acids like ascorbic acid and citric acid.

The inclusion of organic acids such as lactic and acetic acid in some fruits, which can limit microbial development and so impact the pH level of meatballs, is another component, according to Majid et al. (2024), that can assist in maintaining the pH value at its ideal levels. A pH of 6.5 to 7.5 can prevent the development of microorganisms. This is in line with what Braïek & Smaoui (2021) had to say. Soeparno (2015) explained that a meat pH higher or lower than the isoelectric point of meat protein will cause an increase in water-holding capacity. Additionally, the reactive groups of meat proteins will increase, resulting in more meat water being bound, and thus increasing the water holding capacity.

The impact of the combination level of ELN, EDP, and EBM extracts on the percentage of cooking loss

The typical cooking loss for meat ranges from 1.5% to 54.5%. Meat with lower cooking losses is of higher quality as it experiences less nutrient loss during the cooking process (Soeparno, 2015). The degradation of meat proteins during grinding and the hydrolysis of proteins during heating can both lead to an increase in cooking loss by causing the proteins to lose their capacity to bind to water. This is consistent with the findings of Suryanadi et al. (2022), which suggested that a rise in cooking loss may be brought on by myofibrillar protein degradation that impairs the proteins' capacity to bind water, which indicates that meat fat may be dissolved by marinating it in natural fruit acids without significantly affecting the increase in cooking loss (Hafid et al., 2021).

The variations in cooking loss observed in the data acquired may be attributed to the presence of certain fruit chemicals, including flavonoids, polyphenols, and tannins, which are soluble in water and so cannot preserve the protein-water bonding of myofibrils in the meat while it is heating. This supports the view of Rahayu et al. (2020), who claim that the presence of polyphenol/tannin, flavonoids (which are readily soluble in water), calcium, and magnesium in some fruit extracts—which have a high melting point—that prevent the protein-water binding of myofibrils in meat during heating is what causes the increase in cooking loss. According to Indi et al. (2023), boiling meat with pineapple peel extract increased the cooking loss value. This result can be explained by the meat's incapacity to hold onto its natural water content when cooking, which causes a large amount of water to be expelled.

Similarly, Suradi (2006) also noted that meat with lower cooking loss values is of better quality than meat with higher cooking loss values due to reduced nutrient loss during the cooking process. According to Soeparno (2015), the typical cooking loss for meat ranges from 1.5% to 54.5%. Meat with lower cooking losses is of higher quality as it experiences less nutrient loss during the cooking process. Similarly, Suradi (2006) also noted that meat with lower cooking loss values is of better quality than meat with higher cooking loss values due to reduced nutrient loss during the cooking process.

The microbiological test of the extract combination level (ELN + EDP + EBM)

The growth of microorganisms in meat was influenced by water content and moisture levels, making meat highly favorable for microbial growth due to its abundant nutrients and water (Mutmainna et al., 2023). Additionally, the increase in TPC in the meat of culled laying hens was attributed to prolonged storage at refrigerator temperatures, leading to increased microbial activity, as supported by research conducted by Windyasmara & Sariri (2021). According to Septianty (2016), contamination of meat from culled laying hens, caused by several factors, including non-sterile carcass cutting, environmental contamination, and contamination from chicken slaughter workers. According to Patriani & Hafid (2023), there was a significant ($P<0.05$) difference in the meet samples' storage time at 24 hours (W1) compared to 48 hours (W2).

The concentration of Sihala acidic fruit juice as a marinade component and the storage time factor interacted, showing that the total bacteria reduced with increasing Sihala concentration and shorter storage periods. On the other hand, the total bacteria rose with longer storage periods and decreased Sihala acidic fruit juice concentrations. The *Salmonella* sp. test results for the four samples at each marination concentration level were negative. Lytou et al. (2020) stated that several variables, including the kind of acid, acid concentration, temperature, marinating duration, storage, and starting bacterial population, might affect the growth, survival, and inactivation of bacteria on chicken marinated with acid-based spices. When compared to the control, the

microbiological quality of chicken marinated in Sihala acid at varying doses and stored for 24 to 48 hours showed a total count value below the SNI standard, indicating that it was safe to consume

CONCLUSION

Marinating with a blend of extracts notably lowered cooking loss (P3: 28.5 ± 0.2% vs P0: 31.7 ± 0.0%; $p<0.05$, Table 1), aligning with a reduction in meat pH from organic acids that enhance water retention. The ultimate pH level remained compliant with SNI 7388:2009 (5.4-6.5 for fresh chicken meat), and Salmonella was absent/25g (zero tolerance, SNI 7388:2009). Nonetheless, TPC surpassed the SNI 3924:2009 threshold (maximum 1×10^6 CFU/g) after 24 hours of cold storage, likely because of the growth of psychotropic microbes despite the presence of antimicrobial agents from the combined extract. The findings suggest that the formulation successfully enhances quality (reducing cooking loss) but needs shelf-life optimization (more preservatives/vacuum sealing).

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