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Enhancing Physicochemical Properties of Spent Hen Sausages Using Banana Peel Paste and Microbial Transglutaminase

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Abstract

This study aimed to evaluate the synergistic effect of banana peel paste (BPP) and microbial transglutaminase (MTG) on the physical and chemical quality of spent hen sausages. An experimental method using a Completely Randomized Design (CRD) was employed with four treatments: T0 (Control), T1 (60% BPP), T2 (1% MTG), and T3 (60% BPP + 1% MTG) with five replicates. Parameters measured included product yield, total solids, and hardness. The results indicated that the addition of BPP and MTG did not significantly affect product yield ($P>0.05$). However, the treatments had a highly significant effect ($P<0.01$) on total solids and hardness. The range of values obtained was 99.22%–99.75% for yield; 34.21%–37.08% for total solids; and 2864.24–4150.15 g/s for hardness. The addition of 60% BPP significantly reduced both total solids and hardness due to the degradation of connective tissues by metalloprotease enzymes, while 1% MTG contributed to structural restructuring through protein cross-linking. The combination of BPP and MTG produced a spent hen sausage with favorable textural characteristics without compromising its nutritional profile, as total solids remained above 33%. These findings demonstrate that utilizing spent hen meat with BPP and MTG is an effective strategy for developing high-value, processed meat products.

Keywords: *Banana peel paste, Microbial transglutaminase, Physicochemical characteristics, Sausage, Spent hen*

1. INTRODUCTION

Spent hen meat is derived from laying hens that have concluded their productive period. This type of meat possesses several disadvantages, including a tough texture, a fishy odor, and a darker color. Conversely, the advantages of spent hen meat include its abundant availability and competitive pricing. In 2024, the spent hen population in Indonesia reached 414,758,411 heads [1]. Furthermore, spent hen meat is characterized by a high protein content (21.7–22.9%) and the presence of functional fatty acids [2]. These nutritional attributes render spent hen meat a viable candidate for development into high-value food products. Such optimization can be achieved through innovative processing techniques, such as the formulation of sausage products.

Sausages can be manufactured from various meat sources, including lower-quality materials like spent hen meat. The manufacturing process typically involves mixing, grinding, and the incorporation of additional functional ingredients [3]. However, processing spent hen meat into sausages often results in a texture that is excessively compact and dense, necessitating a pre-treatment tenderization step. This tenderization can be effectively achieved through the application of protease enzymes [4].

Protease enzymes serve as effective natural tenderizers and can be derived from various sources, including plants, animals, and microorganisms [5]. Banana peels, for instance, contain metalloprotease enzymes capable of enhancing meat tenderness. These enzymes operate by hydrolyzing peptide bonds in meat proteins, specifically targeting the amino acids lysine and arginine. In this study, banana peel paste was utilized as a pre-treatment at a concentration of 60% relative to the total meat mass, a level

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previously shown to improve the tenderness of free-range chicken meat [6]. While this tenderization process effectively softens the meat fibers, it may also cause them to become fragile. Consequently, an additional ingredient is required to facilitate protein cross-linking, thereby maintaining the desired compact and chewy sausage texture. Microbial Transglutaminase (MTG) is a suitable enzyme for this purpose.

MTG is widely utilized to enhance the functional properties of restructured meat products. It functions via a protein cross-linking mechanism, specifically catalyzing the bond between the γ-carboxamide group of L-glutaminyl and the ε-amino group of L-lysyl residues, which increases sausage elasticity and cohesiveness. Previous research indicated that the addition of 1% MTG significantly improved the texture, product yield, and water retention of spent hen nuggets [7]. Building upon these findings, this study aims to examine the synergistic effects of protease-based tenderization using banana peel paste and protein restructuring using MTG. The experimental treatments included a control (no enzyme), 60% banana peel paste, 1% MTG, and a combination of 60% banana peel paste and 1% MTG. This combination is hypothesized to balance texture softness with structural compactness, ultimately enhancing the product yield, total solids, and hardness of the spent hen sausages.

2. METHOD

2.1. Materials

The tools utilized in this study included digital scales (Sartorius BSA224S-CW, Germany), blenders (Miyako, Indonesia), choppers (New Style, China), sausage stuffer, plastic casings (18 mm), thread, steaming pans, desiccator, oven (Memmert, Germany) porcelain crucibles, and Food Texture Analyzer (TA-XT plus, Stable micro-systems, UK). The materials used consisted of breast meat from spent hens (Fajar's RPU, Kembaran, Banyumas), ambon banana peels (*Musa paradisiaca*), microbial transglutaminase (MTG) enzyme (ACTIVA® GS Ajinomoto), tapioca flour (Cap Pak Tani Gunung), bratwurst sausage seasoning (Sumber Lezat), ice cubes, vegetable oil (Tropical), distilled water, salt (Refina), garlic powder (Koepoe-Koepoe), pepper (Ladaku), paprika powder (La Fancy), chili powder (La Fancy), and refined sugar (Claris).

2.2. Banana Peel Paste Preparation

The procedure for preparing banana peel paste in this study was based on the method described by [8], with modifications to the ingredient quantities. Banana peel waste was collected, washed thoroughly to remove impurities, and manually cut into 1–2 cm pieces. Approximately 96 g of banana peel pieces were mixed with 96 mL of distilled water (1:1 w/w ratio). The mixture was then processed in a blender until a smooth consistency was achieved. The resulting paste was weighed according to the specific treatment requirements and applied directly as a marinade for the spent hen meat. The flowchart for the banana peel paste production is illustrated in Figure 1.

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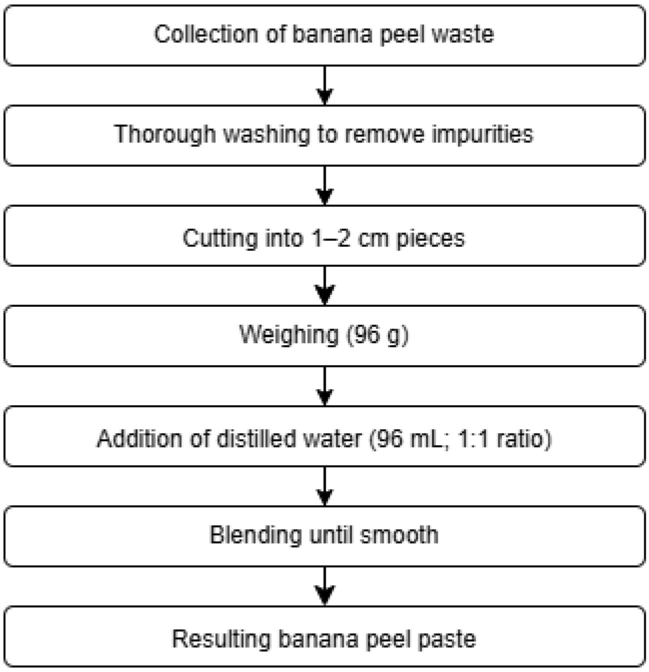

Figure 1. Flowchart of banana peel paste preparation.

2.3. Spent hen Meat Marination

The marinating procedure followed the method of [9] with modifications to the concentration of banana peel paste and the duration of marination. Breast meat (320 g) was cut into cubes to increase the surface area and divided into four equal portions (80 g each). Each portion was treated with the designated dosage of banana peel paste. Marination was conducted for 2 hours to allow the protease enzymes in the banana peel paste to effectively hydrolyze the connective tissues. The flow diagram of the marination process is presented in Figure 2.

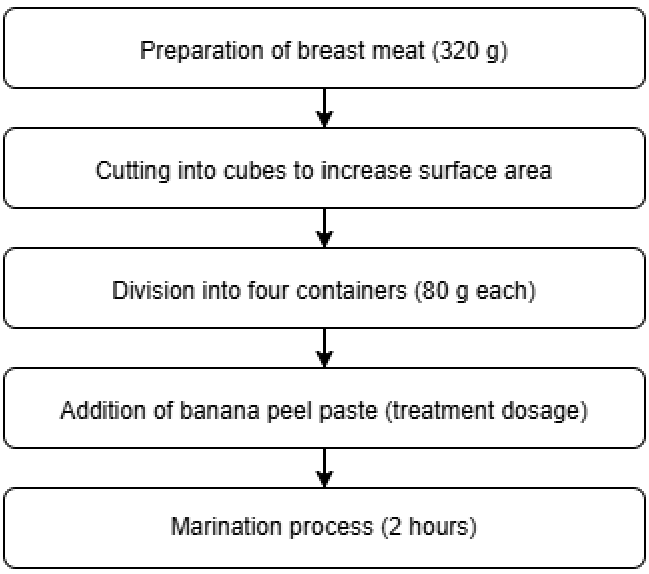

Figure 2. Flowchart of spent hen meat marination.

2.4. Spent hen Sausage Manufacturing

The sausage manufacturing process was based on the method of [10] with modifications to the ingredients. Each 80 g portion of marinated meat was placed in a mixing bowl and ground with the

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addition of ice cubes to maintain a low temperature. Spices were added according to the experimental design and mixed until homogeneous. Vegetable oil and the MTG enzyme were incorporated based on the respective treatment doses. The mixture was then conditioned for 1 hour to allow the MTG enzyme to facilitate protein cross-linking. Following conditioning, the mixture was stuffed into casings (10 cm length) and steamed for 20 minutes over low heat. The flowchart of the sausage production stages is presented in Figure 3.

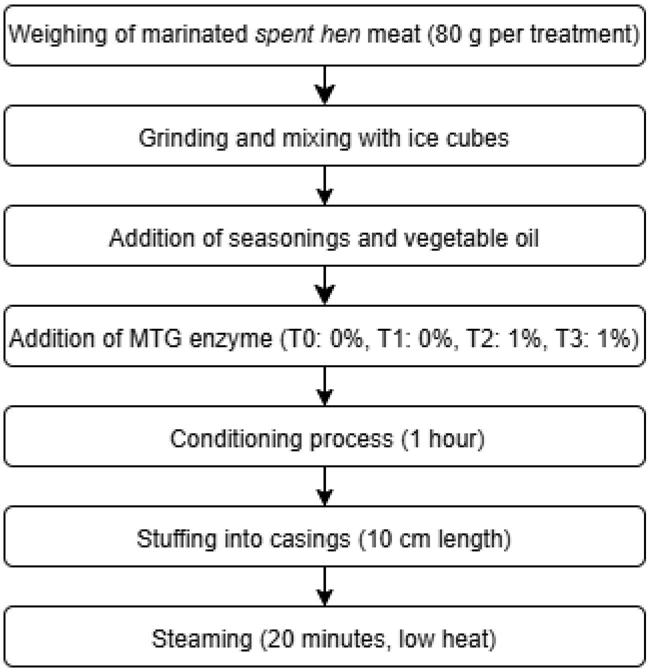

Figure 3. Flowchart of spent hen sausage manufacturing.

2.5. Yield

Product percentage (yield) was determined using the procedure established by [11]. The weights of the sausages were measured both before and after the cooking process. The percentage was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{weight after cooking (g)}}{\text{weight before cooking (g)}} \times 100\%$$

2.6. Total Solids

Total solids were measured using the gravimetric method [12]. A porcelain crucible was heated at 105°C for 30 minutes, cooled in a desiccator, and weighed (x). A 5 g sausage sample (y) was placed in the crucible and dried in an oven at 105°C for 24 hours. The dried sample was cooled in a desiccator and weighed (z). Water content and total solids were calculated as follows:

$$\text{Water Content (\%)} = \frac{x+y-z}{y} \times 100\%$$

$$Total\ Solids\ (\%) = 100 - Water\ Content$$

(Where x = weight of empty crucible, y = weight of sample, and z = final weight of crucible + dried sample).

2.7. Hardness

Hardness was measured using a Texture Analyzer (TA-XT32) following the procedures of [13]. Samples were prepared into 1×1×1 cm^3. The measurement was performed using a cylindrical probe with the following settings: test speed 1.50 mm/s, distance 10.00 mm, trigger force 1 g, and duration 5 seconds. The hardness value was recorded in g/s.

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The collected data were subjected to Analysis of Variance (ANOVA) using a Completely Randomized Design (CRD) to determine the effect of the treatments. If the ANOVA results indicated a significant effect ($P<0.05$) or highly significant effect ($P<0.01$), further analysis was performed using the Honestly Significant Difference (HSD) test.

3. RESULT

The physical and chemical quality characteristics of spent hen sausages treated with banana peel paste (BPP) microbial transglutaminase (MTG) are presented in Table 1.

Table 1. Mean values of physical and chemical quality of spent hen sausages

Treatments	Variables		
	Total Solids (%)	Yield (%)	Hardness (g/s)
P0 (0% BPP + 0% MTG)	$36.81^{b} \pm 1.28$	99.22 ± 0.67	$4150.15^{b} \pm 371.72$
P1 (60% BPP + 0% MTG)	$34.28^{a} \pm 1.09$	99.34 ± 0.09	$2864.24^{a} \pm 313.30$
P2 (0% BPP + 1% MTG)	$37.08^{b} \pm 1.19$	99.60 ± 0.05	$3911.63^{b} \pm 751.44$
P3 (60% BPP + 1% MTG)	$34.21^{a} \pm 0.90$	99.75 ± 0.08	$2867.77^{a} \pm 346.72$
Mean	35.60 ± 1.11	99.48 ± 0.22	3448.44 ± 445.79
Significance	**	ns	**

*Note: *: Significant effect (P<0.05); **: Highly significant effect (P<0.01); ns: Not significant (P>0.05); BPP: Banana Peel Paste; MTG: Microbial Transglutaminase. Values in the same column followed by different superscripts (a, b) indicate a highly significant difference (P<0.01)*

As shown in Table 1, the combination of BPP and MTG had a highly significant effect on the total solids and hardness of the spent hen sausages, whereas its effect on yield was not statistically significant.

3.1. Yield

Yield parameter remained relatively stable across all treatments, with statistical analysis indicating no significant differences (Figure 4). Although Figure 4 shows an increasing trend in values, this observation was not statistically significant. Consequently, varying the concentrations of banana peel paste (BPP) and microbial transglutaminase (MTG) did not significantly impact the yield of the spent hen sausages.

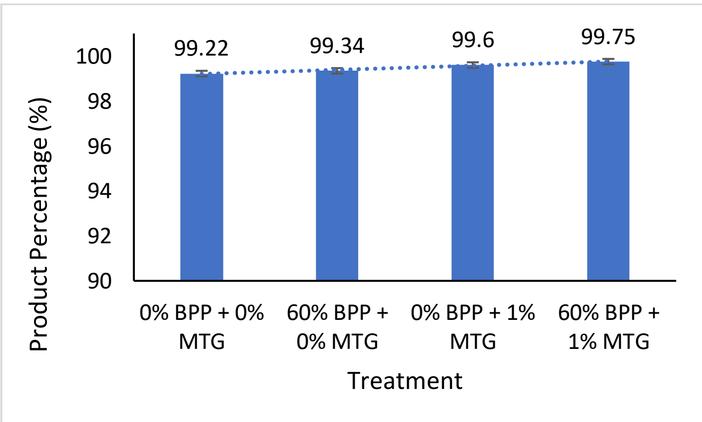

Figure 4. The relationship between the addition of BPP and MTG on the product yield of spent hen sausages.

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3.2. Total Solids

As illustrated in Figure 5, the incorporation of BPP and MTG had a significant effect on the total solids content of the sausages. The addition of 60% BPP significantly reduced the total solids content from 37.08 ± 1.19% to 34.21 ± 0.90%. This reduction demonstrates that the inclusion of BPP significantly influences the total solids profile of spent hen sausages.

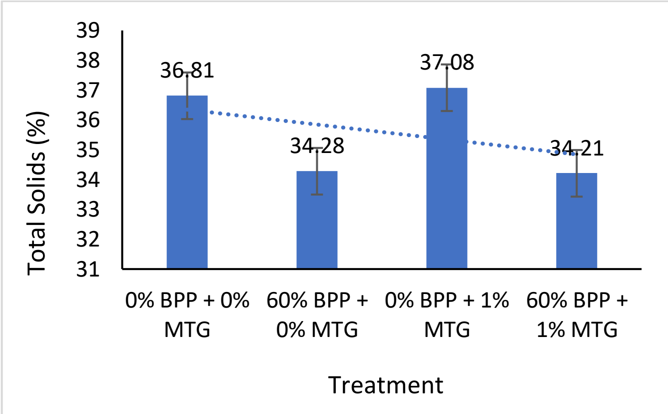

Figure 5. The relationship between the addition of BPP and MTG on the total solids content of spent hen sausages.

3.3. Hardness

Figure 6 indicates that the addition of BPP and MTG significantly affected the hardness of the sausage. A significant decrease in hardness was observed, with values ranging from 4150.15 ± 371.72 g/s to 2864.24 ± 313.30 g/s. This decline suggests that the addition of BPP plays a dominant role in reducing the hardness of sausages derived from spent hens.

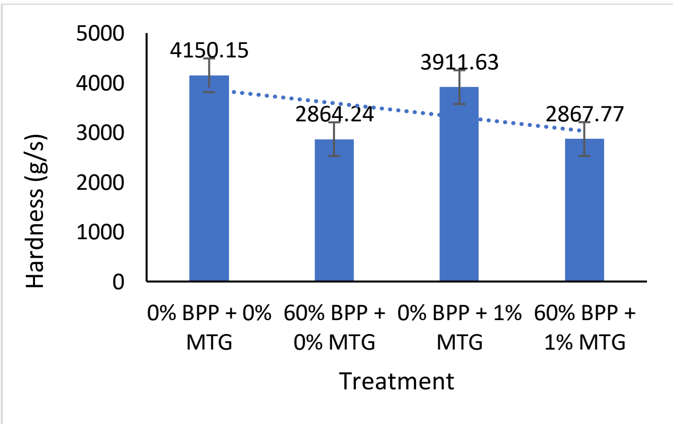

Figure 6. The relationship between the addition of BPP and MTG on the hardness of spent hen sausages.

4. DISCUSSIONS

4.1. Yield

The product yield of spent hen sausages was not significantly affected by the addition of banana peel paste (BPP) and microbial transglutaminase (MTG) (Figure 4). Yield stability can be attributed to the inherent characteristics of spent hen meat, which naturally contains sufficient myofibrillar protein to form a stable gel matrix, even in the absence of MTG. These findings diverge from those reported by [14], where the addition of MTG (0.25% and 0.5%) to hybrid beef sausages supplemented with soy and

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rice proteins significantly influenced product yield. This discrepancy may be attributed to differences in meat source protein profile and the specific binding capacity of the fillers used [15].

The yield of sausage products is fundamentally linked to the homogeneity of the emulsion. As sausages are emulsion-based products, the interaction between fillers and binders plays a critical role in stability. The use of binders, such as wheat flour and tapioca, during the mixing process ensures effective emulsion stability [16]. This stable emulsion facilitates the trapping of water and fat, thereby minimizing liquid loss during cooking and resulting in a consistent yield across all treatments.

The product yield of spent hen sausages is a key indicator of moisture retention during the cooking process [17]. The product yield of spent hen sausages is a key indicator of moisture retention during the cooking process [18], while MTG catalyzes the formation of covalent cross-links between the glutamine and lysine residues of meat proteins. These covalent bonds construct a stable, dense protein matrix with an enhanced capacity to retain water and fat molecules within the sausage gel [19]. Consequently, the balanced efficacy of both ingredients in preserving the product's internal moisture accounts for the consistent product yield observed across all treatments.

4.2. Total Solids

The total solids value reflects the concentration of non-water components, including protein, fat, carbohydrates, and minerals. According to Figure 5, the addition of 1% MTG resulted in the highest total solids (37.08 ± 1.19%). This high value is attributed to the formation of a dense gel matrix induced by MTG, which reconstructs the protein network into a more stable structure [19]. These covalent bonds effectively entrap dissolved components, such as proteins, fats, and minerals, preventing their loss during steaming and thereby increasing the solids content of the final product [20].

Conversely, the use of BPP for marination reduced the total solids content. This decrease is caused by the metalloprotease enzymes in the BPP, which degrade proteins and loosen the connective tissue structure [21]. A more open connective tissue architecture increases the availability of hydrophilic groups within the protein chains [22], thereby enhancing the ability of the protein matrix to bind free water. As total solids are calculated by subtracting the moisture content (100% – moisture %), an increase in moisture retention leads to a lower total solids percentage [23]. Additionally, the leaching of sarcoplasmic proteins during the washing process after marination may further contribute to a reduction in the proportion of solids.

The combination of BPP and MTG resulted in the lowest average total solids content compared to the other treatments. This indicates that the water-retention capacity induced by BPP marination is more dominant than the gel-reconstructing effect of MTG. Although this leads to lower total solids, the value remains well above the minimum standard of 33% [24]. These results demonstrate that the combination of BPP and MTG can produce a juicier and more tender spent hen sausage without compromising its nutritional profile.

4.3. Hardness

The addition of BPP decreased the hardness of spent hen sausages (Figure 6). The decrease in hardness is due to the degradation of connective tissue by the protease enzyme in the banana peel paste. Banana peels contain metalloprotease enzymes with an activity of 42.63 ± 0.05 CDU/mg, which can increase meat tenderness [21]. This enzyme works by hydrolyzing peptide bonds and degrading collagen, the main component causing the tough texture of spent hen meat. The protein degradation process causes complex protein chains to break down into simpler ones, resulting in lower hardness and breaking strength [5]. Consequently, sausages with the addition of banana peel paste have lower hardness values.

Conversely, Figure 6 shows that the addition of MTG can increase sausage hardness by promoting protein cross-linking. As a biocatalyst, MTG facilitates an acyl-transfer reaction between the γ-

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carboxamide group of glutamine and the ε-amino group of lysine. This mechanism produces highly stable covalent cross-links that are resistant to proteolysis and heat treatment [25]. Interestingly, sausages treated with a combination of BPP and MTG exhibited hardness values similar to those treated with BPP alone. This suggests that the protease activity of BPP is dominant over the restructuring capability of MTG. The degradation of the protein structure by BPP prevents MTG from optimally forming the cross-links required to enhance gel strength. Nevertheless, despite the reduction in hardness, the sausages maintained a desirable, chewy, and solid texture that does not crumble easily upon consumption [19].

The hardness value is also influenced by the type of raw material used. Based on the results of research by [14] regarding the production of beef sausages formulated with soy and rice proteins with the addition of MTG enzyme (0.25 and 0.5%), the average hardness obtained was 77.5 N or 7902.67 g/s. The value obtained was much higher than the hardness value of sausages from spent hen. This is likely due to differences in the raw materials used. Beef has denser and stronger muscle fibers than spent hen meat, so the resulting sausages also have a higher hardness value than those from spent hen.

5. CONCLUSION

The addition of banana peel paste (BPP) and microbial transglutaminase (MTG) did not affect the percentage of sausage produced from spent hens, but it did reduce the hardness and total solids of the sausage. The addition of BPP and MTG to the sausage improved its quality, as the resulting sausage had a stable yield, a chewy, and compact texture without compromising its nutritional content.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with any financial or personal relationships that could have appeared to influence the work reported in this paper.

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