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Microbial changes in vacuum-packed beef dendeng added with black soldier fly maggot meal during storage

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Abstract
Beef dendeng is a traditional Indonesian food made from beef that is seasoned and dried, rich in nutrients, particularly protein. Innovations in beef dendeng processing include the addition of maggot meal from the larvae of the black soldier fly (*Hermetia illucens*), which contains high protein levels, reaching 40-50%. Insect meal can be easily introduced to market in the form of dried products. This maggot meal aims to enrich the nutritional content and provide an alternative, more diverse and sustainable food product. Storing dendeng at ambient temperature might affect the growth of bacteria and fungi. This study examined the microbial changes in vacuum-packed beef dendeng added with black soldier fly maggot meal (BSFMM 5% w/w) during 30 days of storage at ambient temperature compared to control (without BSFMM addition). The results show that both bacteria and fungi grew until day 30 at acceptable count. The addition of BSFMM did not influence both bacteria and fungi growth. The moisture content, which reached 50,78% on day 30, also influenced the growth of bacteria and fungi during storage. This study provides valuable insights into the impact of storage and added with maggot meal on the quality of beef dendeng.

Keywords: Beef dendeng, BSF maggot meal, microbiological quality, storage

Introduction

Beef is a source of animal protein that is rich in nutrients, but is also susceptible to the growth of microorganisms that can damage its quality if not processed properly. To extend the shelf life and maintain the quality of beef, one of the processing methods used in making dendeng. Dendeng is produced through a drying process that reduces the moisture content in the meat, as well as adding spices that function to prevent microbial growth (Soesetyaningsih *et al*., 2020) [10]. Seasonings such as salt, coriander, pepper, and garlic not only provide a distinctive taste but also function as natural preservatives. This drying process allows dendeng to be stored longer without the need for additional preservatives. In making dendeng, there is now an innovation by adding maggot meal from black soldier fly larvae (*Hermetia illucens*). This maggot meal is rich in protein, which aims to increase the nutritional content of dendeng, as well as provide an alternative source of more affordable protein. Although dendeng has gone through a preservation process, microbiological quality must still be maintained. Storing dendeng at ambient temperature can affect the growth of bacteria and fungi, which has the potential to reduce product quality. Therefore, attention to microbiological quality is very important, especially in terms of product durability during storage (Cahyani *et al*., 2020) [3].

The maggot meal used in making dendeng comes from maggot larvae that are raised with natural food in the form of fresh fruit that has a high moisture content. This natural food supports optimal maggot growth, unlike other maggots that are often raised using waste. These maggots grow on fine paper media that are very clean, ensuring a hygienic environment and free from contamination of hazardous materials. After going through a thorough cleaning and processing process, these maggots are converted into meal that is clean, healthy, and rich in various essential nutrients, such as protein and amino acids, which are beneficial for the body.

Environmental factors such as moisture content, temperature, and humidity during storage greatly affect the quality and safety of dendeng. Therefore, proper packaging is very important to maintain optimal dendeng quality. One method that is often used is to pack dendeng in vacuum packaging, which helps reduce air exposure and maintain product stability. The study aimed to evaluate the effect of storage duration and maggot meal added on the microbiological quality of beef dendeng stored in vacuum packaging for 30 days at ambient temperature. This study also includes analysis of the number of bacteria and fungi, moisture content, and changes in product quality over time (Nauman *et al*., 2022) [8]. Through this study, it is hoped that we can find out about the microbiological resistance of dendeng-containing maggot meal, as well as the effect of storage duration on product quality. The results of this study will provide a clearer picture of product resistance to microbes, and help optimize the packaging and storage process of dendeng to improve overall product safety and quality.

Materials and Methods

1. Maggot Meal and Beef Dendeng Preparation

This study used 4 treatments and each treatment was repeated 3 times, using 25 different concentrations of materials, so that 24 experimental units were obtained in this study. The treatments were differences in storage time (0 days, 10 days, 20 days, 30 days) and black soldier fly maggot meal (BSFMM) addition level (0% and 5%, w/w). The variables observed were the amount of bacterial growth, the number of yeast molds, and the amount of moisture content.

Preparation of maggot samples was carried out by harvesting maggots after 25 days, separating them from food waste (leftover fruits and vegetables), and then blanching them in boiling water for one minute. After that, the maggots were sun dried for two days, dried in an oven at 50°C for 24 hours, ground into meal, and stored in the freezer. For making dendeng, beef was ground, mixed with spices, and maggot meal (0% and 5%) was added. The dough was molded, baked in a two-stage oven at 80°C for 30 and 15 minute, then cooled and packed in vacuum pack. The beef dendeng samples were stored for 0, 10, 20, and 30 days and observations were made on the number of bacteria, fungi, and moisture content.

2. Bacteria Enumeration

The preparation of Nutrient Agar (NA) for bacterial testing begins by dissolving 28 grams of NA in 1000 ml of aquadest, heating until the solution is clear, and then cooling. Furthermore, the solution is sterilized using an autoclave at 121°C for 15 minutes, then poured into a petri dish and left to harden. For sample dilution, 10 grams of sample is crushed and dissolved in 90 ml of NaCl, then successive dilutions are carried out up to 10^{-5}. Each dilution was put into a petri dish containing NA media and then incubated at 37°C for 24 - 48 hours. After incubation, the number of colonies was counted to determine the number of microorganisms in the sample using the cfu/gram formula. The total plate count of bacteria was calculated using the formula:

$$N = \frac{\Sigma C}{[(1 \, x \, n_1) + (0{,}1 \, x \, n_2)] \, x \, (d)}$$

Description:
N: number of product colonies, expressed in colonies per ml or colonies per gram
ΣC: number of colonies on all plates counted
n1: number of plates in the first dilution counted
n2: number of plates in the second dilution counted
D: the first dilution counted

3. Fungi Enumeration

A total of 39 grams of Potato Dextrose Agar (PDA) was dissolved in 1000 ml of distilled moisture, heated until the solution was clear, then added the antibiotic cefadroxil monohydrate to prevent bacterial growth. The solution was sterilized by autoclaving at 121°C for 15 minutes and poured into a petri dish. A 10-gram sample was crushed and dissolved in 90 ml of NaCl, then dilutions were carried out successively up to 10^{-5}. Samples from dilutions 10^{-2}, 10^{-3}, and 10^{-4} were put into two petri dishes containing PDA and incubated at 27°C for 3 - 5 days. After incubation, the number of colonies was calculated using the cfu/gram formula to determine the concentration of microorganisms in the sample. The number of yeast molds can be calculated using the formula:

$$N = \frac{\Sigma C}{[(1 \, x \, n_1) + (0{,}1 \, x \, n_2)] \, x \, (d)}$$

Description:
N: number of product colonies, expressed in colonies per ml or colonies per gram
ΣC: number of colonies on all plates counted
n1: number of plates in the first dilution counted
n2: number of plates in the second dilution counted
D: the first dilution counted

4. Moisture Content Measurement

Testing the moisture content of the dendeng samples began by preparing an empty cup that was heated in an oven at 105°C for 2 hours, then cooled in a desiccator for 30 minutes and weighed to obtain the initial weight. A sample of dendeng of about 3 grams was placed in a cup and weighed before going oven. The ovening process was carried out at 105°C for 20 hours to evaporate the moisture content. After ovening, the cup was placed in a desiccator for 15 - 30 minutes to prevent absorption of moisture vapor, then reweighed to obtain the final weight. The percentage of moisture content was calculated based on the difference between the initial and final weights, ensuring accurate results. Moisture content can be calculated using the formula:

$$\text{Moisture content (\%bb)} = x = \frac{W2-W3}{W2-W1}$$

Description:
W1: Weight of cup (g)
W2: Weight of cup and sample before drying (g)
W3: Weight of cup and sample before drying (g)

5. Statistical analysis

Data on the number of bacteria, fungi, and moisture content obtained were analyzed quantitatively using the analysis of variance test of the Completely Randomized Design (CRD) Nested Pattern with Tukey's further test.

Result and Discussion

1. Bacteria Enumeration

Table 1: Bacterial Contamination Level in Beef Dendeng Added with BSF Maggot Meal Over 30 Days

Treatment	Storage Time (Days)			
	Day 0	Day 10	Day 20	Day 30
0%	0,96 ± 0,25	0,93 ± 0,23	1,46 ± 0,25	1,63 ± 0,05
5%	1,23 ± 0,15	1,30 ± 0,43	1,40 ± 0,26	1,63 ± 0,55

Description: There was no effect from treatment (P > 0,05).

The treatment that resulted in the lowest average Total Plate Count (TPC) value was the 0% treatment on days 0 and 10, while the highest average value was observed in the 0% and 5% treatments on day 30. The research results showed that the total bacteria in beef dendeng with added BSF maggot *meal* stored for 30 days at room temperature in vacuum packaging were not significantly different (P > 0,05). According to SNI number 2908:2020, the bacterial count was still below the maximum allowable microbial contamination limit for beef dendeng or other meat products. However, it was above the acceptable microbial limit, indicating that the beef dendeng processing met the Good Manufacturing Practices standards.

Table 1 shows that storage at room temperature for 30 days caused no change in the total bacteria of beef dendeng with a 0% maggot *meal* concentration. On day 0, it was 0,96 × 10^5 cfu/g, on day 10 it was 0,93 × 10^5 cfu/g, on day 20 it was 1,46 × 10^5 cfu/g, and on day 30, it reached 1,63 × 10^5 cfu/g. For the 5% maggot *meal* concentration, on day 0, it was 1,23 × 10^5 cfu/g, on day 10 it was 1,30 × 10^5 cfu/g, on day 20 it was 1,40 × 10^5cfu/g, and on day 30, it was 1,63 × 10^5 cfu/g.

According to Indonesian National Standard (SNI), the maximum allowable bacterial count in processed meat products, such as dendeng, is 1 × 10^5 cfu/g. If the total bacteria in the product is still below this limit, the product is considered safe and suitable for consumption. The research findings show that beef dendeng with added Black Soldier Fly (BSF) maggot *meal*, stored in vacuum packaging for 30 days, maintained bacterial levels below the maximum threshold set by SNI. Therefore, this product still meets microbiological quality standards and is safe for consumption.

Based on previous research by Michaud *et al.* (2020), it was stated that after 30 days of storage, the bacterial content in beef dendeng packaged in vacuum packaging was lower and not significantly different when compared to dendeng packaged in regular plastic bags. Vacuum packaging is beneficial because it prevents contamination from external sources, inhibits the growth of anaerobic bacteria, and helps maintain the quality of the product by preventing oxidation, moisture loss, and preserving the dendeng's quality (Sembiring *et al*., 2019) [9].

2. Fungi Enumeration

Table 2: Fungal Contamination Level in Beef Dendeng Added with BSF Maggot Meal Over 30 Days

Treatment	Storage Time (Days)			
	Day 0	Day 10	Day 20	Day 30
0%	n. d	n. d	n. d	n. d
5%	n. d	n. d	n. d	n. d

Description: n.d shows not detected.

The amount of fungal contamination in the tested product from day 0 to day 30 was not detected, which means that the product remains safe from fungal contamination during the test period. The amount of fungal contamination in the beef dendeng study was not detected because it was still below the amount of fungal contamination according to the Bacteriological Analytical Manual (FDA BAM) 2001. The number of fungi found in food samples is generally in the range of 10 to 150 colonies per gram/ml, but the number of fungi that appeared in beef dendeng products was still below that number.

Fungi generally thrive in humid and oxygen-rich conditions, such as mold and yeast. Therefore, by reducing the oxygen content in vacuum packaging, the ability of fungi to thrive is very limited (Kaban *et al*., 2019) [4]. Vacuum packaging is a very effective packaging method for extending the shelf life of food products, including processed meat products such as dendeng (Rohmah *et al*., 2022). The results of the microbiological test of fungi in dendeng products showed that no fungal population was detected, which means that there was no fungus. This may indicate that vacuum packaging is successful in suppressing microorganisms in the product.

Based on research conducted by Azaka *et al*. (2019), vacuum packaging is effective in suppressing the number of microorganisms in beef dendeng, with microbiological test results showing very low levels of contamination after 30 days of storage. Vacuum packaging inhibits the growth of fungi in processed meat products, including dendeng, and extends the shelf life of the product by maintaining its microbiological quality (Sukmawati, 2018) [11].

Thus, when microbiological tests show that the microorganism count in vacuum-packed dendeng products is undetectable or very low, it indicates that vacuum packaging has successfully prevented microbial contamination and maintained the product's quality, ensuring it remains safe for consumption for a longer period.

3. Moisture Content Measurement

Table 3: Moisture Content Value of Beef Dendeng Added with BSF Maggot Meal

Treatment	Storage Time (Days)			
	Day 0	Day 10	Day 20	Day 30
0%	44,44 ± 1,60[a]	45,89 ± 2,15[ab]	48,45 ± 0,73[bc]	50,77 ± 1,57[c]
5%	38,90 ± 38,90[a]	40,92 ± 2,09[b]	43,36 ± 1,10[c]	42,10 ± 1,61[c]

Description: The addition of BSF maggot *meal* affected the moisture content of beef dendeng very significantly (P < 0,01).

The moisture content value ranges from 38,90-50,78%. It can be seen that the average moisture content value in the 0% treatment on day 0 was 44,45%, then on the 10th day the moisture content of the dendeng was 45,89%, on the 20th day the moisture content of the dendeng was 48,45%, and on the 30th day it was 50,78%. The average moisture content value in the 5% treatment on day 0 was 38,90%, on the 10th day it was 40,93%, on the 20th day it was 43,37%, and on the last day or the 30th day it was 42,10%.

The results of the analysis of variance showed that the moisture content with storage for 30 days which was given the treatment of 0% and 5% maggot meal showed a significant difference (P < 0,05) resulting in a moisture content that tended to increase. This is because the vacuum packaging used to package the dendeng product. Different vacuum packaging hole spacing results in different dendeng moisture content. Based on research conducted by Wahyuningsih *et al*. (2019) [13], the type of vacuum packaging material used affects the distance of the air holes in the packaging. Air holes with different spacing will result in air transfer from outside the packaging, because the packaging can still be affected by humidity from outside the packaging.

Vacuum packaging is generally made from LLDPE and nylon with varying thicknesses. These varying thicknesses affect the transfer of air and moisture vapor in the packaging. The thickness of vacuum packaging ranges from 75 to 160 microns. The selection of the thickness for each layer considers barrier properties, air transfer capability, and the desired flexibility. Generally, vacuum packaging of 80 microns is used for food products. However, in this study, the 80-micron vacuum packaging was not sufficient to block the transfer of air and moisture vapor in the dendeng stored at room temperature for 30 days.

Moisture content is a key determinant of the shelf life of a product, as higher moisture content shortens the storage time of a product (Delviani *et al*., 2021). As seen in Table 3, the moisture content obtained in this study is still considered high. Therefore, beef dendeng is classified as a semi-moist food product due to its high moisture content. According to SNI number 2908:2020, the moisture content of dendeng that is classified as a semi-moist food is between 10% and 42%. As shown in Table 3, only the dendeng with a 5% maggot *meal* concentration still meets the SNI standard, while the dendeng without BSF maggot *meal* exceeded the SNI standard on day 10.

The addition of insect *meal* to the product can affect the moisture content in the dendeng. In general, insect *meal* made from dried insects tends to have a lower moisture content compared to fresh food ingredients. According to Kaban *et al*. (2019) [4], the drying and processing of insect *meal* generally aims to reduce moisture content and extend the product's shelf life.

Measuring moisture content is crucial as water serves as an indicator to determine the shelf life and freshness of a product. Environmental conditions, such as temperature and humidity, can affect the moisture content in food products. High temperatures and humidity can cause moisture absorption by the product, which accelerates the growth of microorganisms and shortens the shelf life (Cahyani *et al*., 2020) [3].

Conclusion

The addition of Black Soldier Fly maggot meal to beef dendeng stored in vacuum packaging for 30 days resulted in no significant effect on bacterial and fungal growth. There is a change in water content during storage, so it is necessary to use thicker vacuum packaging. The addition of Black Soldier Fly maggot meal does not increase the food safety risk of beef dendeng.

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