Basil Leaves as a Natural Pathogenic Bacterial Inhibitor in Beef

Yurliasni Yurliasni*, Zuraida Hanum, Halimatus Sakdiah Hasibuan

Laboratory of Meat Science and Processing Technology, Faculty of Agriculture, Syiah Kuala University, Aceh, Indonesia

*Corresponding author: yurliasni@unsyiah.ac.id

Received April 08, 2023; Revised May 13, 2023; Accepted May 21, 2023

Abstract
Meat is a protein-rich diet vulnerable to physical, chemical, and microbiological alterations. Basil (Ocimum basilicum L.) is an herb often consumed with active chemicals such as flavonoids and eugenol. This research aimed to see if basil leaves could be a natural inhibitor of pathogenic microorganisms in beef during storage. The study used a completely randomized design that treated beef by soaking it in basil leaf juice. The results showed that soaking beef in basil leaf extract had a substantial influence (P<0.05) on TPC, a very significant effect (P<0.01) on total *E. coli* bacteria growth and meat pH, but no effect (P > 0.05) on total *S. aureus* bacteria growth. The results indicate that soaking beef in basil leaf extract for 8 hours reduced TPC, suppressed the growth of *E. coli* bacteria, and maintained the pH of the meat but had no influence on the growth of *S. aureus* bacteria.

Keywords: basil leaves, beef, herb, natural pathogen inhibitor, meat


1. Introduction

Currently, meat consumption is rising worldwide, along with population expansion. Beef improves people's diets considerably; it is a high-nutritional-value animal food with an average protein content of 18.26%, an average fat content of 14.7%, and an average water content of 77.65% [1]. As a perishable food meat is very easily contaminated by pathogenic bacteria, according to Suardana and Swacita (2009), since the nutrient and water content are relatively high, and the pH is favorable for pathogenic bacteria to grow [2]. Bacterial infection in meat will reduce meat quality by generating physical, chemical, and microbiological changes in meat.

*Staphylococcus aureus*, a round gram-positive bacterium, is one indicator of microbiological alterations in meat [3]. This bacterium is harmful in beef because it can create enterotoxin, which can induce poisoning symptoms in humans. This bacterial infection can happen when butchering meat or slaughtering livestock. *S. aureus* can also spread through livestock slaughter equipment and by slaughterhouse staff. In addition to *S. aureus*, *Escherichia coli*, a gram-negative bacterium, is frequently discovered. *E. coli* is a facultative anaerobe with a short rod shape, a length of 2 μm, a width of 0.4–0.7 μm, and a diameter of 0.7 μm. These bacteria can induce bloody diarrhea, nausea, and vomiting in humans. *E. coli* contamination in beef can arise from unsanitary handling of food ingredients, such as inadequate equipment cleanliness and seller hand hygiene.

One indicator of the freshness of the meat is the pH value which is one of the conditions that determine the quality of the meat. Beef has a relatively low acid pH value of 5.5 to 5.8 [4]. The rate of glycolysis has a large influence on the pH of meat after slaughter. The longer the beef remains at room temperature, the more likely bacterial activity and the occurrence of a spoilage process, followed by an increase in pH and bacterial growth.

There are several simple ways to prevent the physical, chemical, and microbiological changes that occur, one of which is to soak the meat in basil (*Ocimum basilicum* L.) leaf juice. Basil is an annual wild plant that is widely cultivated in tropical and subtropical regions such as Asia and Africa [5]. Basil leaves are one of the plants with the potential and bioactive compounds that act as antibacterial and antioxidants. There are also 11.8% alkaloids, 11.5% flavonoids, 3.55% tannins, and 0.28% saponins in them [6]. Basil leaves contain antifungal, antioxidant, and antibacterial compounds that can inhibit the growth of *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and fungi, as well as pathogenic bacteria. However, there has been no research on the benefits of basil leaves in overcoming beef contamination. As a result, this study aimed to examine the ability of basil leaves to maintain the microbiological quality of meat while soaking.
2. Materials and Methods

2.1. Beef Preparation

The sample for this study was 1 kg of Aceh beef thigh purchased from a local market. The beef was cut into 18 pieces weighing 50 grams each. In addition, the meat was washed and drained aseptically in preparation for use as research samples.

2.2. Preparation of Basil Leaf Extract

*Ocimum basilicum L.*

The basil leaves used in this study came from homegrown garden plants. The basil leaves were young, fresh, and whole, with no flaws or holes. Furthermore, the basil leaves were cleaned, washed, and rinsed with distilled water. A total of 500 grams of basil leaves were mashed in a blender with 200 ml of sterile water in the ratio of basil leaves: distilled water = 500 grams:200 ml, then filtered with filter paper to obtain basil leaf extract. The meat was then soaked in basil leaf extract for 0, 4, and 8 hours at 28°C.

2.3. Total Plate Count (TPC)

Beef samples soaked in basil leaf extract were weighed up to 10 grams, placed in plastic (polyethylene), and crushed with a stomacher. A 10 ml sample was vortexed in a bottle containing 90 ml of distilled water (10⁻¹). In addition, 1 ml of the suspension from the 10⁻¹ dilution was mixed with 9 ml of the 10⁻² dilution, and the dilution was repeated to 10⁻³ to 10⁻⁶ dilutions. Then, in duplicate, 1 ml of suspension from the 10⁻³ and 10⁻⁶ dilutions was pipetted into a petri dish, followed by 20 ml of nutrient agar media, and homogenized. The petri dish was allowed to solidify before incubating at 37°C for 48 hours [7]. The number of colonies in each dish is calculated using the same procedure as the TPC enumeration with different diluent factors and media. A total of 1 ml of the dilution (10⁻¹) suspension was pipetted into the center of the petri dish in duplicate. The VRBA media was poured on top of it again. The sample cooled and solidified in a petri dish before incubating at 37°C in an inverted state for 48 hours [7]. The number of colonies in each dish is calculated using the formula (2).

\[
TPC \left( \text{CFU/ml} \right) = \frac{\text{number of growing colonies} \times \text{dilution factor} \times 10^{-2}}{10}
\]  

2.4. *Staphylococcus aureus* Colony Count

The total pathogenic bacteria enumeration followed the same procedure as the TPC enumeration with different diluent factors and media. A total of 1 ml of the dilution (10⁻¹) suspension is added to the dilution solutions 10⁻² to 10⁻³. Then, in duplicate, 1 ml of the suspension from the 10⁻³ dilution was placed in a petri dish, followed by 20 ml of nutrient agar media, and homogenized. The petri dish was allowed to solidify before incubating at 37°C for 48 hours [7]. Based on the dilutions, the number of colonies in each plate was calculated using the same formula.

2.5. *Escherichia coli* Colony Count

An overlay system was used to count the total *E. coli* bacteria. The first stage involved pouring up to 20 ml of Violet Red Bile Agar (VRBA) media into each petri dish and allowing it to solidify. Furthermore, after the VRBA media had solidified, 1 ml of the dilution (10⁻²) suspension was pipetted into the center of the petri dish in duplicate. The VRBA media was poured on top of it again. The sample cooled and solidified in a petri dish before incubating at 37°C in an inverted state for 48 hours [7]. The number of colonies in each dish is calculated using the formula (2).

\[
TPC \left( \text{CFU/ml} \right) = \frac{\text{number of growing colonies} \times \text{dilution factor} \times 10^{-2}}{10}
\]  

2.6. Meat pH Measurement

The beef sample was weighed up to 10 grams, placed in a plastic bag, filled with 100 ml of distilled water, mashed with a stomacher, and poured into a glass beaker. The calibrated electrode (pH meter) was immersed in the sample-containing glass beaker. The pH reading is taken when the pH meter scale is stable [9].

3. Result

Statistical analysis revealed that soaking beef in basil leaf extract affected TPC, *E. coli*, and pH but did not affect total *S. aureus* in beef. Table 1 shows the findings. The results of Duncan's multiple-distance further test (Table 1) show that the TPC of treatment T0 (6.19 CFU/ml) was not different from T1 but significantly different from T2. At 4 and 8 hours of immersion, the duration of soaking beef with basil leaves significantly reduced the amount of TPC in beef. The total microbes growing on beef were 6.19 CFU/ml without soaking for 0 hours (T0), 6.01 CFU/ml after 4 hours of soaking (T1), and 5.96 CFU/ml after 8 hours of immersion (T2).

Table 1. The average TPC, *S. aureus*, *E. coli*, and beef pH levels after soaking in basil (*Ocimum basilicum L.*) leaf extract

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Immersion time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₀</td>
</tr>
<tr>
<td>Total plate count (CFU/mL)</td>
<td>6.19 ± 0.20</td>
</tr>
<tr>
<td><em>S. aureus</em> (log CFU/mL)</td>
<td>3.04 ± 0.11</td>
</tr>
<tr>
<td><em>E. coli</em> (log CFU/mL)</td>
<td>2.21 ± 0.18</td>
</tr>
<tr>
<td>pH</td>
<td>6.12 ± 0.22</td>
</tr>
</tbody>
</table>

Information: *a b* Different superscripts revealed significant (P<0.05) (TPC) and very significant (P<0.01) differences. Total *E. coli* and pH T₀ = no soaking (the parameters were measured as soon as the treatment without soaking), T₁ = 4 hours of soaking, T₂ = 8 hours of soaking.

4. Discussion

The reduction in total microbes was caused by basil leaf extract, which positively inhibits microbial growth. This is supported by Budiman and Aprinda (2014), who discovered that basil leaves contain active antibacterial substances such as eugenol, linalool, flavonoids, saponins,
and tannins [10]. Eugenol disrupts the stability of microbial cell membranes, causing potassium ion leakage and cell death; additionally, the activity of the ATPase enzyme in microbes is inhibited, and thus the energy required for cell growth is not formed. Furthermore, linalool's antibacterial activity is mediated by damaging the microbial cell membrane, suppressing the translation of a specific gene product, and working to inhibit enzymes in microbes, thereby inhibiting microbial growth.

Because basil leaves contain bioactive substances, they are more effective at inhibiting microbial growth in beef. According to the findings of phytochemical tests in the research of Kumalasari and Andiarna (2020), the ethanolic extract of basil leaves contains bioactive compounds such as flavonoids, alkaloids, saponins, and tannins that can act as antibacterial agents [11]. According to Maryati al. (2007), one of the bioactive substances in basil leaves is an essential oil containing eugenol compounds that have antiseptic effects that can damage cell membranes in microbes [12]. Furthermore, binding phenolic compounds to microbial cells would interfere with membrane permeability and microbial cell transport processes, resulting in the loss of macromolecules and cations in cells and disrupting microbial cell growth.

According to the statistical analysis findings (Table 1), soaking beef in basil leaf extract did not affect the total number of *S. aureus*. This is possible because *S. aureus* is a gram-positive bacterium with a thick layer of the peptidoglycan cell wall and a compact cell wall structure. It is difficult for bioactive substances in basil leaves to penetrate and lyse the cell membrane of bacteria *S. aureus* [12]. This statement is consistent with the findings of Tortora and Derrickson (2012), who discovered that the cell walls of gram-positive bacteria have many layers of peptidoglycan, which causes the structure of the cell walls to thicken and stiffen [13]. Furthermore, the content of teichoic acid and teichuronic acid in the cell wall can regulate the function of elasticity, porosity, tensile strength, and electrostatic properties of the cell wall.

The bacterial cell wall protects bacteria from osmotic stress, maintains cell shape, regulates cell division processes, and determines the properties of bacterial antigens [14]. Basil leaf essential oil is more effective against gram-negative bacteria than gram-positive bacteria. This is related to bacterial cell wall permeability, which is influenced by the thickness of the peptidoglycan layer in the bacterial cell. However, when observed during immersion, *S. aureus* decreased the longer the total immersion time.

Statistical analysis revealed that the length of soaking beef in basil leaf extract had a highly significant (P<0.01) effect on total *E. coli* bacteria. Table 1 shows the results of observations on *E. coli* bacteria. Duncan's multiple-distance further test revealed that the pH in treatment T4 differed significantly from treatments T1 and T5, but not from treatments T1 and T2.

The pH drop is thought to be caused by microorganisms that degrade carbohydrates in the form of glycogen into lactic acid. This is consistent with Santosos and Ranti (2004). They stated that the pH decreased after soaking due to the formation of lactic acid due to glycogen breakdown by the activity of microbial enzymes found in meat [19]. According to Lawrie (2003), the pH of meat during soaking includes the pH of fresh meat, which generally ranges from 5.4 to 5.8, where the meat has an open structure, is bright red, has a more favorable flavor, and is more resistant to microbial damage [20]. Furthermore, high storage temperatures can increase the rate of pH decrease [21].

5. Conclusions

Soaking beef in basil leaf juice for an extended period can suppress microbial growth, reduce total *E. coli*, and...
keep the meat's pH at fresh meat levels. However, the beef sample used in this study came from a single cow. As a result, additional research using different meat samples is required to strengthen the findings of this study.

Acknowledgements

Acknowledgments to the Meat Processing Technology laboratory team for their assistance with the implementation of this research.

References


