

Basil (*Ocimum basilicum* L.) leaves as a natural pathogenic bacterial inhibitor in beef

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Abstract

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%. Meat is a protein-rich diet vulnerable to physical, chemical, and microbiological alterations. Basil (*Ocimum basilicum* L.) is a consumable herb that contains active chemicals, such as flavonoids and eugenol. This research aimed to investigate the potential of basil leaves as 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 *Escherichia coli* bacteria growth and meat pH, but no effect ($P > 0.05$) on total *Staphylococcus aureus* bacteria growth. The results indicate that soaking beef in basil leaf extract for 8 hrs 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.

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% (Prasetyo *et al.*, 2013). According to Suardana and Swacita (2009), meat is easily contaminated by pathogenic bacteria due to its high nutrient and water content. The pH also supports bacterial growth. Bacterial infection will reduce meat quality by generating physical, chemical, and microbiological changes.

Staphylococcus aureus, a gram-positive bacterium, is one indicator of microbiological alterations in meat (Radji, 2010). This bacterium is very harmful since it can infect beef and lead to enterotoxin production, which can induce poisoning symptoms in humans. This bacterial infection can happen when butchering meat or slaughtering livestock. *Staphylococcus aureus* can also spread through livestock slaughter equipment and by slaughterhouse staff. In addition, *Escherichia coli*, a gram-negative bacterium, is also frequently found in meats. *Escherichia 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 and the quality of the meat is the pH value. Beef has a relatively low pH of 5.5 to 5.8 (Abustam, 2012). The rate of glycolysis has a significant influence on the pH of meat after slaughter. The longer the beef remains at room temperature, the more bacterial activity will increase, and a spoilage process will occur, 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 (Atikah, 2013). Basil leaves contain bioactive compounds that act as antibacterial and antioxidants. They also contain 11.8% alkaloids, 11.5% flavonoids, 3.55% tannins, and 0.28% saponins (Nadeem *et al.*, 2020). Basil leaves contain antifungal, antioxidant, and antibacterial compounds that can inhibit the growth

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of fungi, *Mycobacterium tuberculosis*, *S. aureus*, and other pathogenic bacteria. However, there has been no research on the benefits of basil leaves in overcoming beef contamination. Thus, 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 g each. Then, the meat was washed and drained aseptically for samples.

2.2 Preparation of basil leaf extract

The basil leaves used in this study were obtained from home-grown garden plants. The basil leaves were young, fresh, and whole, with no flaws or holes. The basil leaves were cleaned, washed, and rinsed with distilled water. A total of 500 g of basil leaves was mashed in a blender with 200 mL of sterile water in the ratio of basil leaves: distilled water = 500 g:200 mL, then filtered with filter paper to obtain basil leaf extract. The meat was then soaked in basil leaf extract for 0 (T_0), 4 (T_1), and 8 (T_2) hrs at 28°C.

2.3 Total plate count

Beef samples soaked in basil leaf extract were weighed up to 10 g, placed in plastic (polyethylene), and crushed with a stomacher. A 10 mL sample was vortexed in a bottle containing 90 mL of distilled water (1:9). 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^{-5} to 10^{-6} dilutions. Then, in duplicate, 1 mL of suspension from the 10^{-5} 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 hrs (Hanum et al., 2017). The number of colonies in each dish is calculated using the formula (1).

$$\text{Total Plate Count (CFU/mL)} = \text{number of growing colonies} \times 1/(\text{diluent factor}) \times 10 \quad (1)$$

2.4 *Staphylococcus aureus* colony count

The total pathogenic bacteria enumeration followed the same procedure as the TPC enumeration with different diluent factors and Vogel Johnson Agar (VJA) media. A total of 1 mL of the diluted sample (10^{-1}) was re-diluted 10 to 100 folds, obtained 10^{-2} and 10^{-3} dilutions. Then, in duplicate, 1 mL of the suspension from the 10^{-3} dilution was placed in a petri dish, followed by 20 mL of Vogel Johnson Agar and homogenized. The sample was allowed to solidify in a

petri dish before being incubated inverted at 37°C for 48 hrs (Hanum and Wanniatie, 2015). 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 hrs (Hanum et al., 2017). The number of colonies in each dish is calculated using the formula (2).

$$\text{Escherichia coli count (CFU/mL)} = \text{number of growing colonies} \times 1/\text{Dilution factor} \times 10^{-2} \quad (2)$$

2.6 Meat pH measurement

The beef sample was weighed up to 10 g, 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 (National Standardization Agency, 1992).

3. Results

Statistical analysis revealed that soaking beef in basil leaf extract affected the total plate count (TPC), *E. coli* count, and pH but did not affect total *S. aureus* count in beef. Table 1 shows the findings.

The results of Duncan's multiple-distance further test (Table 1) show that the TPC of treatment T_0 (6.19 CFU/mL) was not different from T_1 but significantly different from T_2 . At 4 and 8 hrs 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 hrs (T_0), 6.01 CFU/mL after 4 hrs of soaking (T_1), and 5.96 CFU/mL after 8 hrs of immersion (T_2).

4. Discussion

The TPC test resulted in a reduction in total microbes that showed the ability of basil leaf extract to inhibit 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. 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, thus, the energy required for cell growth is not formed. 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.

Table 1. The average total plate count, *Staphylococcus aureus* count, *Escherichia coli* count, and beef pH after soaking in basil leaf extract.

Parameter	Immersion time (hrs)		
	T ₀	T ₁	T ₂
Total plate count (CFU/mL)	6.19±0.20 ^a	6.01±0.12 ^a	5.96±0.06 ^b
<i>S. aureus</i> count (CFU/mL)	3.04±0.11	2.97±0.11	2.89±0.07
<i>E. coli</i> count (CFU/mL)	2.21±0.18 ^a	1.98±0.11 ^b	1.85±0.00 ^b
pH	6.12±0.22 ^a	5.41±0.22 ^b	5.59±0.39 ^b

Values are presented as mean±SD. Values with different superscripts in the same column are highly significantly different (P<0.01). T₀: no soaking, T₁: 4 hrs of soaking, T₂: 8 hrs of soaking.

In a previous study, Kumalasari and Andiarna (2020) conducted a phytochemical test on the ethanolic extract of basil leaves, reporting the bioactive compounds of the extract, such as flavonoids, alkaloids, saponins, and tannins. Those compounds can act as antibacterial agents. According to Maryati *et al.* (2007), one of the bioactive substances in basil leaves is eugenol, which has antiseptic effects. It can damage cell membranes in microbes. The binding of 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 (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 *S. aureus* (Maryati *et al.*, 2007). 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 (Tortora and Derrickson, 2012). 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 (Jawetz and Adelberg, 2005). Basil leaf essential oil is more effective against gram-negative bacteria than gram-positive bacteria (Semeniuc *et al.*, 2017). This is related to bacterial cell wall permeability, which is influenced by the thickness of the peptidoglycan layer in the bacterial cell. However, the longer the immersion time, the total of *S. aureus* decreased.

Statistical analysis revealed that the soaking time of 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 results show that the T₀ treatment significantly differed from the T₁ and T₂ treatments. Meanwhile, T₁ and T₂ had no significant difference. The total *E. coli* growing on beef was higher in samples without soaking (T₀), then decreased at 4 hrs (T₁) and 8 hrs (T₂). The reduction in the total *E. coli* bacteria in this study was caused by basil leaves' antibacterial activity, which could inhibit the growth of gram-negative bacteria like *E. coli*.

This present study demonstrates that gram-negative bacteria are susceptible to antibacterial bioactive compounds found in basil leaves, such as eugenol, flavonoids, linalool, and citral. These findings are consistent with Angelina *et al.* (2015), who discovered that the essential oil in basil extract has strong antibacterial properties and can effectively inhibit bacterial growth. Basil leaves contain flavonoid compounds, which can act as antibacterial agents by damaging the bacterial cell membrane, inhibiting nucleic acid synthesis and bacterial energy metabolism, and impairing the function of the bacterial cell cytoplasmic membrane. The hydrogen ions of flavonoid compounds cause cell membrane damage by attacking the cell membrane's phosphate group (polar), causing phospholipids to degrade into glycerol, phosphoric acid, and carboxylic acid. According to Nurmashita *et al.* (2015), basil leaves flavonoid compounds act as antibacterial by damaging the bacterial cell membrane on the phospholipid portion of the cell wall, impairing permeability and damaging bacterial cells.

Flavonoid compounds act as antibacterial agents by inhibiting energy metabolism, nucleic acid synthesis, and the function of bacterial cell membranes. One of the flavonoid compounds found in basil leaves is anthocyanin. When anthocyanin attacks *E. coli* bacteria, they can cause irregularities in their cells' outer membrane and cytoplasmic leakage. The ability of basil

leaves to inhibit the growth of *E. coli* is caused by its cell wall structure, which includes gram-negative bacteria, which have a cell wall with a layer of lipopolysaccharide, lipid, and lipoprotein (Pratiwi, 2008). Gram-negative bacteria have a peptidoglycan cell wall that accounts for only about 10% of the cell wall's dry mass, resulting in a thinner bacterial cell wall that is more easily penetrated by antibacterial compounds (Putri et al., 2018).

The duration of soaking beef in basil leaf extract had a highly significant ($P < 0.01$) effect on the final pH of the meat. Table 1 shows the average pH value obtained in each treatment. Duncan's multiple-distance further test revealed that the pH in treatment T_0 differed significantly from treatments T_1 and T_2 , but T_1 and T_2 showed no significant difference.

The pH drop could be due to the microorganisms that degrade carbohydrates from glycogen into lactic acid. This is consistent with Santoso and Ranti (2004). They reported that the pH decreased after soaking due to the formation of lactic acid resulting from glycogen breakdown by the activity of microbial enzymes. According to Lawrie (2003), fresh meat and soaking meat usually have pH ranges from 5.4 to 5.8, where the meat has an open structure, bright red color, more favorable flavor, and is more resistant to microbial damage. Furthermore, high storage temperatures can increase the rate of pH decrease (Soeparno, 2009).

5. Conclusion

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.

Conflict of interest

The authors declare no conflict of interest.

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