Comparative Evaluation of Antimicrobial Effects and Sensory Impact of Lemongrass Essential Oil in Tofu Preservation

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ABSTRACT

The growing demand for natural and minimally processed food products has increased interest in the use of plant-based preservatives for improving food safety and shelf life. Tofu is a protein-rich and high-moisture food product. It is highly vulnerable to microbial spoilage during storage, leading to deterioration in quality and reduced shelf life. The present study was conducted to evaluate the antimicrobial effectiveness of lemongrass essential oil in tofu preservation as well as to assess its impact on the sensory quality of the product. Microbiological analysis was performed against spoilage-associated microorganisms isolated from tofu samples, including *total viable bacteria*, *coliforms*, *Enterobacteriaceae*, *yeast and molds*. The antimicrobial activity of lemongrass essential oil was evaluated using an agar well diffusion assay. The results demonstrated clear inhibitory activity of lemongrass oil against the tested microorganisms, with higher concentrations exhibiting greater antimicrobial effectiveness. The observed inhibitory action may be associated with the presence of bioactive compounds such as citral and related terpenoid constituents naturally present in lemongrass essential oil. Sensory evaluation of treated tofu samples indicated that the optimised concentration of lemongrass oil maintained acceptable sensory characteristics without causing major undesirable changes in flavor, texture, or appearance. The treated samples showed improved microbiological quality compared to untreated controls, suggesting the potential of lemongrass essential oil as a natural preservative for tofu preservation systems. Overall, the findings of the study support the application of lemongrass essential oil as an alternative to synthetic preservatives for improving the microbial safety and shelf life of tofu. The study also provides a basis for future research involving edible coating systems, encapsulation techniques, and advanced natural preservation strategies for sustainable food preservation applications.

Keywords: Tofu Preservation, Antimicrobial activity, Plant-based preservatives, Microbial Inhibition.

INTRODUCTION

Tofu is a highly nutritious soybean-based food product that contains high moisture and protein, making it particularly susceptible to microbial spoilage during storage. The increasing consumer demand for natural food preservatives has enthused researchers to make the use of essential oil from plants as an alternative to synthetic antimicrobial agents (Arya et al., 2019, p. 2174). Consumers are becoming more interested in the antimicrobial properties of essential oils. Furthermore, consumers who are "clean labels" have developed the market of essential oils (Hyldgaard et al., 2012, p. 18). Essential oils, extracted from different parts of plants, appear to be effective against an important group of foodborne pathogens (including *E. coli*, *Listeria monocytogenes*, and *Salmonella spp*.) and have the potential to extend the shelf-life and safety of food products (Salanță & Cropotova, 2022). More research is specifically needed on the application of essential oils to proteinaceous food systems like tofu, because of the effects of essential oils, antimicrobial activity and potential interactions with food components (Gutiérrez et al, 2008, p. 111). This study addresses a critical gap by comparatively evaluating the antimicrobial efficacy and sensory effects of lemongrass essential oil as a natural preservative in tofu, a widely consumed plant-based protein. The investigation quantifies the reduction in microbial load attributable to each essential oil and examines their influence on the organoleptic properties of tofu, thereby providing essential data for their potential application in sustainable food preservation strategies (Hanková et al., 2023). This research is particularly important because many food products are highly perishable and vulnerable to bacterial and fungal contamination, which necessitates effective preservation methods to maintain quality and safety throughout preparation, storage, and distribution (Lucera et al., 2012, p. 1). The growing consumer demand for natural, plant-based products has intensified interest in essential oils as bio-preservatives in functional foods, highlighting the need to understand their effects on both microbial load and sensory characteristics (Adekunbi et al., 2025). The incorporation of essential oils into food systems requires balancing effective antimicrobial activity with the preservation of desirable organoleptic qualities. High concentrations of essential oil may introduce undesirable flavors and aromas (Hassoun & Çoban, 2017, p. 50). The search for natural alternatives to synthetic preservatives is driven by increasing concerns regarding the toxicity and environmental impact of conventional additives, as well as the rise of microbial resistance (Maurya et al., 2021, p. 2).

METHODOLOGY

Materials and Methods

The fresh Tofu samples were aseptically collected from local retailer(s) and immediately transported to the microbiology laboratory in hygiene condition for analysis. The samples were promptly processed to avoid contamination and to protect their original microbial quality during the experiment.

Selected essential oil and experimental conditions

Essential oil such as Lemongrass (*Cymbopogon citratus*) was chosen on the basis of previous literature evidence demonstrating its potential antimicrobial effect on food preservation systems. The concentrations were prepared using different doses of the same essential oil in a laboratory sterile condition. The concentration of the essential oil applied on Tofu was 1.53 mg/ml, 3.06 mg/ml, and 6.125 mg/ml.

Design of experiment

The experiment was carried out in two phases:

Phase I: Microbiological screening of Tofu Samples without essential oil treatment

The untreated tofu samples were microbiologically examined for the presence of spoilage microorganisms, hygiene indicators, and selected food-borne pathogens to compare the bacterial load in tofu before treatment with essential oil.

Phase II: Tofu samples were treated with essential oils

The tofu samples were treated with Lemongrass essential oil at varying concentrations and then subjected to microbiological examination.

Preparation of Sample Suspension and Serial Dilution

Ten g of the tofu sample was aseptically homogenized in sterile diluent (Maximum Recovery Diluent) under laboratory conditions to prepare the suspension, which will be serially diluted. Serial dilutions were prepared with sterile dilution blanks using standard microbiological procedures following an ISO-based analytical method.

Microbiological Analysis

All microbiological analyses were done according to the ISO standard under aseptic laboratory conditions.

Total Plate Count (TPC)

The total viable bacterial load of tofu samples was evaluated following ISO 4833-1:2013 Amendment 1:2022. For preparation of the test sample, 10 g of tofu was aseptically blended with 90 mL of Maximum Recovery Diluent (MRD) to obtain a uniform suspension. Serial dilutions were prepared under sterile laboratory conditions. From suitable dilutions, 1 mL aliquots were transferred into sterile Petri dishes, after which molten Plate Count Agar (PCA) was added and mixed carefully using the pour plate method. Once solidified, the plates were incubated at 30°C for 72 hours. After incubation, plates showing countable colonies were selected, and the colonies were enumerated. The microbial load was expressed as colony-forming units per gram (CFU/g) using the standard ISO calculation formula. Media blanks and dilution controls were maintained simultaneously to ensure the validity and sterility of the procedure.

Yeast and Mold Count

Yeast and mold enumeration were carried out following the guidelines of ISO 21527-2:2008. Initially, 10 g of the tofu sample was aseptically transferred into 90 mL of sterile diluent and homogenized thoroughly to prepare the primary suspension. Further serial dilutions were prepared under sterile conditions. Depending upon the water activity of the sample, either Dichloran Glycerol 18 Agar (DG18) or Dichloran Rose Bengal Chloramphenicol Agar (DRBC) was selected for fungal isolation and counting. The inoculated plates were incubated at 25°C for five days. Along with the test samples, dilution blanks and uninoculated media controls were also maintained to monitor sterility and procedural accuracy. After incubation, plates showing suitable fungal growth were chosen for counting, and the colonies were assessed based on their appearance, texture, pigmentation, and other characteristic morphological features of yeasts and molds.

Coliform Count

Coliform analysis was performed according to ISO 4832:2006 using Violet Red Bile Agar (VRBA). For sample preparation, 10 g of tofu was blended with 90 mL sterile diluent to obtain the initial homogenate, followed by serial dilution preparation using aseptic techniques. Appropriate dilutions were pour-plated on VRBA and incubated at 37°C for 24 hours. After incubation, characteristic coliform colonies appeared as dark reddish-purple colonies, generally larger than 0.5 mm in diameter, sometimes accompanied by a surrounding precipitation halo. Presumptive colonies were selected and inoculated into Brilliant Green Lactose Bile (BGLB) broth for confirmation. The inoculated broth tubes were further incubated at 37°C for 24 ± 2 hours, and gas formation was considered evidence of positive coliform activity. Final counts were calculated according to standard ISO procedures using confirmed colonies.

Enterobacteriaceae Count

The presence of *Enterobacteriaceae* was determined according to ISO 21528-2:2017 using Violet Red Bile Glucose Agar (VRBGA). A 10 g tofu sample was homogenized in 90 mL of sterile diluent, after which serial dilutions were prepared aseptically. Suitable dilutions were inoculated onto VRBGA plates and incubated at 37°C for 24 hours. Colonies suspected to belong to the Enterobacteriaceae family typically developed as pink to reddish-purple colonies with surrounding precipitated zones. Selected colonies were purified on Nutrient Agar plates and incubated again at 37°C for 24 hours for further verification. Confirmation of isolates was carried out through oxidase testing and glucose fermentation reactions. Isolates that were oxidase negative and capable of fermenting glucose were considered positive for *Enterobacteriaceae.*

Detection of Staphylococcus aureus

Isolation and enumeration of *Staphylococcus aureus* were performed in accordance with ISO 6888-1:2021 using Baird-Parker Agar (BPA). For analysis, 10 g of tofu sample was mixed with 90 mL sterile diluent and homogenized properly before serial dilutions were prepared. Appropriate dilutions were spread onto BPA plates and incubated at 35–37°C for 24–48 hours. Typical colonies of *S. aureus* appeared as smooth, shiny black colonies surrounded by clear halos and thin whitish margins. Presumptive colonies were sub-cultured into Brain Heart Infusion Broth (BHIB) and incubated at 35–37°C for 24 hours. Confirmation was achieved using the coagulase test, where 0.1 mL of the actively grown culture was mixed with 0.3 mL rabbit plasma and incubated for 6–24 hours at 35–37°C. Development of visible clotting in the plasma was interpreted as a positive reaction confirming the presence of *Staphylococcus aureus.*

Detection and Enumeration of *Escherichia coli*

Analysis of *Escherichia coli* was conducted according to ISO 16649-2:2001 using Tryptone Bile X-glucuronide (TBX) agar. Initially, 10 g of the tofu sample was homogenized with 90 mL of sterile diluent to prepare the primary suspension. Serial dilutions were subsequently prepared under aseptic conditions. Appropriate dilutions were inoculated onto TBX agar plates and incubated at 44°C for 24 hours. Following incubation, characteristic blue-green colonies were considered presumptive *E. coli* due to β-glucuronidase activity. Plates containing typical colonies were selected for enumeration, and the results were calculated and expressed as CFU/g of sample.

Detection of Salmonella spp.

The presence of *Salmonella spp*. was investigated according to ISO 6579-1:2017 Amendment 1:2020. For pre-enrichment, 25 g of the tofu sample was mixed with 225 mL Buffered Peptone Water (BPW) and incubated at 37°C for approximately 18–24 hours. After the pre-enrichment stage, selective enrichment was performed by transferring 0.1 mL of culture into Rappaport–Vassiliadis Soya (RVS) broth and 1 mL into Muller–Kauffmann Tetrathionate-Novobiocin (MKTTn) broth. The RVS broth was incubated at 41.5°C, whereas the MKTTn broth was incubated at 37°C for 24 hours.

Following selective enrichment, cultures were streaked onto Xylose Lysine Deoxycholate Agar (XLDA) and Brilliant Green Agar (BGA), followed by incubation at 37°C for 24 hours. Typical presumptive colonies on XLDA appeared red with black centers, while colonies on BGA appeared pink to light-colored. Representative colonies were purified on Nutrient Agar plates and incubated further at 37°C for 24 hours. Purified isolates producing off-white colonies were subjected to biochemical characterization using Triple Sugar Iron (TSI) agar, urease test, lysine decarboxylase broth, indole test, and β-galactosidase test. Confirmation of *Salmonella* isolates was based on the collective interpretation of morphological and biochemical characteristics.

Detection of Listeria monocytogenes

Detection of *Listeria monocytogenes* was performed according to ISO 11290-1:2017. Briefly, 25 g of tofu sample was aseptically transferred into 225 mL of Half Fraser Broth supplemented with selective additives and incubated at 30°C for 24 hours for primary enrichment. Following enrichment, 0.1 mL of the culture was transferred into Full Fraser Broth and incubated again at 37°C for another 24 hours.

After enrichment, a loopful of culture was streaked onto ALOA agar and Oxford agar plates, which were incubated at 37°C for 24–48 hours. On ALOA agar, presumptive *Listeria monocytogenes* colonies appeared blue green with surrounding opaque halos, whereas on Oxford agar, the colonies appeared small, dark brown to black with black halos. Suspected colonies were purified on Tryptone Soya Yeast Extract Agar (TSYEA) and incubated at 37°C for 24 hours. Further confirmation was carried out through observation of colony morphology and standard biochemical identification methods recommended in ISO protocols.

Agar Well Diffusion Assay

Lemongrass essential oil showed strong antimicrobial activity against spoilage microorganisms isolated from tofu using the agar well diffusion method. Fresh microbial cultures were inoculated onto sterile agar plates, and different concentrations of the oil were added to sterile wells. Antimicrobial activity was assessed by measuring the Zone of Inhibition (ZOI) after incubation.

Lemongrass oil exhibited concentration-dependent antimicrobial efficacy, producing ZOI values ranging from 15.8 ± 1.6 mm to 18.9 ± 2.1 mm. Its antimicrobial action is mainly attributed to bioactive compounds such as cital, gerani, and limonene, which disrupt microbial cell membranes and inhibit essential metabolic functions, leading to cell death.

Treatment of tofu with Lemongrass essential oil significantly reduced microbial load during storage compared to untreated samples, confirming its preservative potential in perishable protein-rich foods. Sensory evaluation indicated that treated tofu maintained acceptable color, texture, flavor, aroma, and overall acceptability, with scores ranging from 7.2 ± 0.4 to 7.7 ± 0.5. Lower and moderate concentrations were most preferred, while higher concentrations produced a stronger aroma that slightly reduced acceptability.

Conclusion

The findings of the present study indicate that lemongrass essential oil can serve as an effective natural antimicrobial agent against spoilage-causing microorganisms in tofu. Due to its high moisture and protein content, tofu is highly susceptible to microbial deterioration during storage; therefore, natural preservation strategies are essential to improve its microbial safety and shelf stability. The incorporation of lemongrass essential oil significantly reduced microbial growth while maintaining acceptable sensory characteristics, highlighting its potential application as a bio-preservative in tofu and other plant-based food products. Furthermore, optimized delivery systems such as nanoemulsions, edible coatings, and controlled-release packaging may further enhance the antimicrobial efficacy and stability of lemongrass essential oil in food preservation systems.

Sensory Evaluation

Sensory evaluation of untreated and treated tofu samples was carried out to assess the influence of essential oil treatment on sensory attributes including aroma, flavor, texture, and overall acceptability. Comparative observations were recorded to identify treatments showing acceptable sensory quality while maintaining antimicrobial effectiveness.

Observation and Interpretation of Results

Microbiological observations recorded before and after treatment with lemongrass essential oil demonstrated a significant reduction in microbial load in tofu samples during storage. The antimicrobial efficacy of lemongrass oil was evaluated through microbial enumeration, pathogen detection, and Zone of Inhibition (ZOI) analysis using ISO-based microbiological procedures followed throughout the study. Untreated tofu samples showed substantial microbial growth, indicating rapid spoilage under normal storage conditions. The total plate count (TPC) ranged from approximately 1.0×10^6 to 1.8×10^6 CFU/mL, while *Yeast and molds*, *Coliforms*, and *E. coli* were also detected in untreated samples, confirming the high susceptibility of tofu to microbial deterioration.

In contrast, tofu samples treated with lemongrass essential oil exhibited complete inhibition of microbial growth throughout the storage period. No detectable colonies were observed for total plate count (TPC), *Yeast and Mold count (YMC)*, *Coliforms*, *E. coli*, or *Enterobacteriaceae* in the treated samples. The absence of microbial growth indicated the strong antimicrobial potential of lemongrass essential oil against spoilage and indicator microorganisms associated with tofu deterioration. The findings suggest that lemongrass essential oil effectively improved the microbiological quality and storage stability of tofu by suppressing the growth of spoilage-causing microorganisms. Therefore, lemongrass essential oil may serve as a promising natural bio-preservative for enhancing the safety and shelf life of tofu products.

Results for Untreated Sample:

1. Screening of Microbial Load:

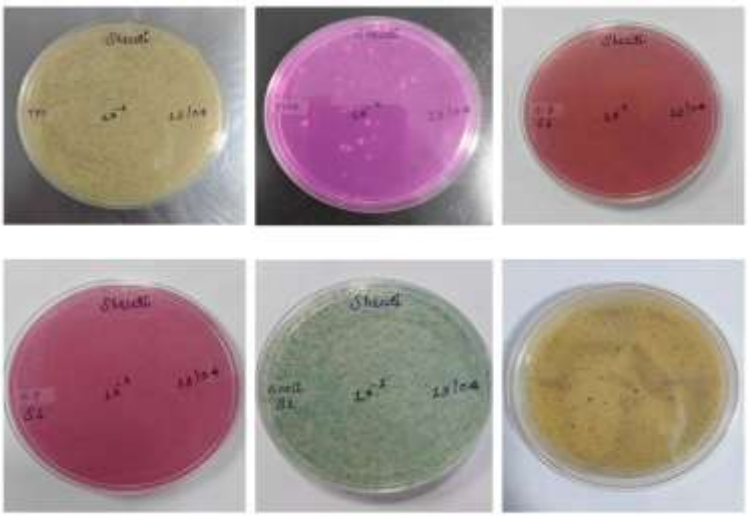
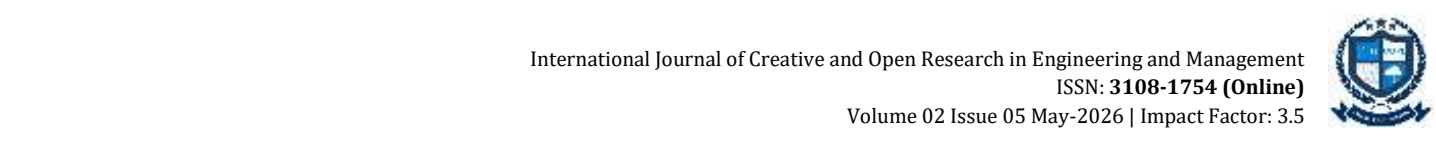


Figure 1: Growth of microorganisms in an untreated tofu sample on different selective media for *total plate count (TPC)*, *yeast and mold(y/m)*, *Enterobacteriaceae*, *Coliforms*, *E. coli*, *and Staphylococcus aureus* at 10^{-1} dilution.



2. Confirmation and Biochemical Characterization:

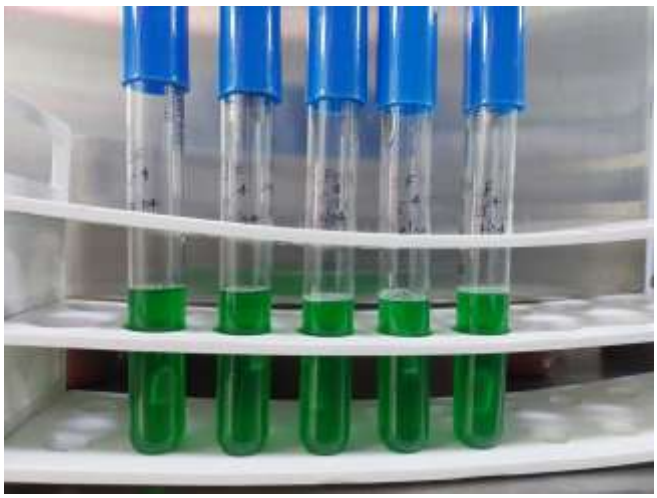


Figure 2: Showing gas formation for coliforms which confirms the presence of coliforms in Durham tubes when the colonies were inoculated in brilliant green lactose bile broth.

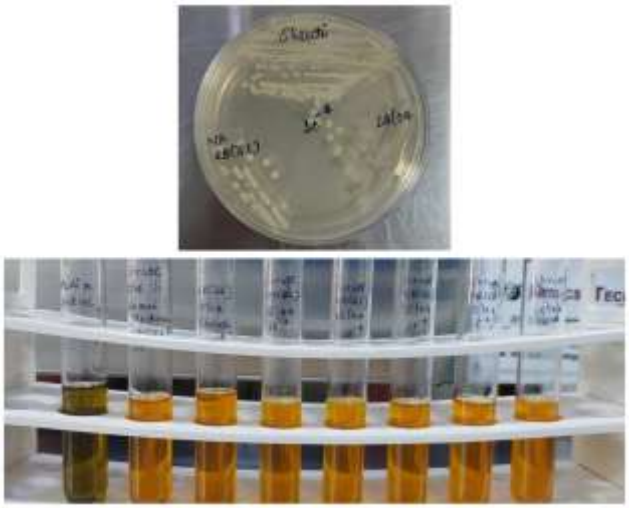


Figure 3: Demonstration of streaking onto nutrient agar for confirmation and further biochemical characterization by the glucose fermentation test in the glucose of medium.

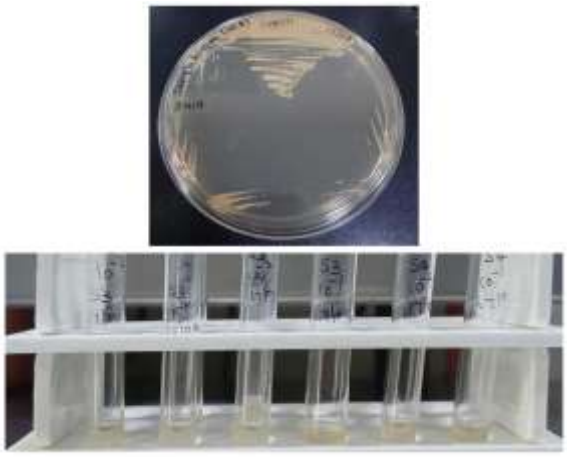


Figure 4: Inoculation for *Staphylococcus aureus*: atypical colonies were onto brain heart and infusion agar (BHIA), as well as confirmation was done by the catalase test, which showed negative results.

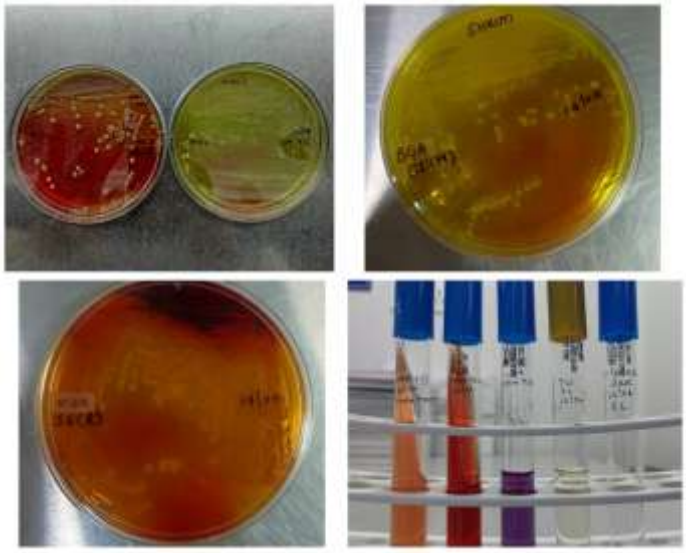


Figure 5: Plates of XLD and BG Agar for *Salmonella spp*. were streaked from MKTTN and RVS for further streaking for confirmation and biochemical characterization which also showed negative results.

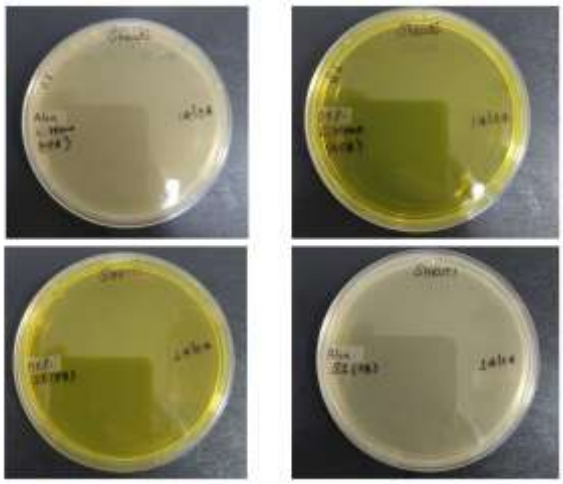


Figure 6: Streaking onto ALOA and oxford agar from Half Fraser Broth, for *Listeria monocytogenes:* which resulted in the growth of "blue and off-white color colonies"; further images show no growth and resulted in negative.

Results for the Treated Sample with Lemongrass essential oil

1. Screening of Microbial Load

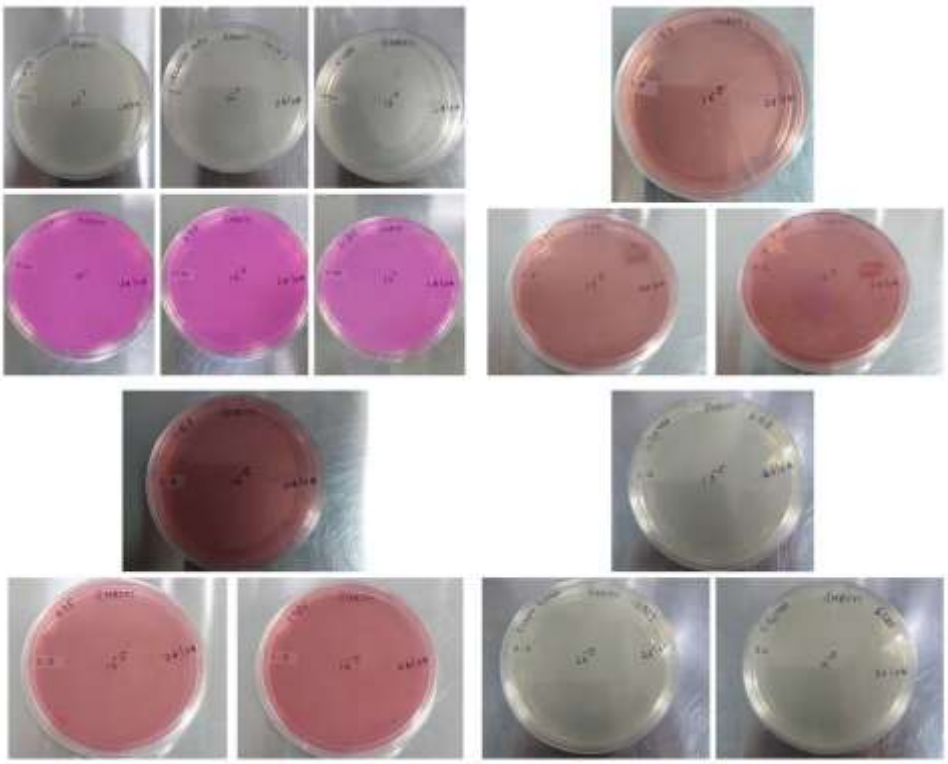


Figure 7: **M**icrobiological assessment of untreated tofu samples treated with different concentrations (1.53, 3.06, and 6.125 mg/ml) of essential oils. plates represent *Total Plate Count (TPC), Yeast and Mold (Y/M), Coliforms (C.F.), Enterobacteriaceae (E.B.), and Escherichia coli (E.C.)* analysis after incubation, showing very little microbial growth patterns and comparative antimicrobial activity of the treatments.

2. Zone of Inhibition of Lemongrass Essential Oil:

Figure 8: Demonstration of the zone of inhibition of lemongrass essential oil with different zones as shown in the figure.

Discussion

The present study demonstrated the potential application of plant essential oil as a natural antimicrobial agent for tofu preservation. Due to its high moisture content and nutrient-rich composition, tofu is highly susceptible to microbial contamination and rapid spoilage during storage. The microbiological analysis of untreated tofu samples revealed the presence of elevated microbial load including total viable bacteria, coliforms, *Enterobacteriaceae*, yeast and mold, indicating the highly perishable nature of tofu and the necessity for effective preservation strategies. Previous studies have similarly reported that tofu provides favorable conditions for microbial growth because of its high-water activity and protein content (Burt, 2004; Hyldgaard et al., 2012).

Treatment of tofu samples with lemongrass essential oil resulted in a noticeable reduction in microbial growth compared with untreated samples. Lemongrass essential oil also demonstrated considerable antimicrobial effect. Lemongrass oil is mainly associated with citral and related terpenoid compounds capable of damaging microbial cell structures and inhibiting enzymatic activity. Silva et al. (2013) reported that lemongrass essential oil exhibited significant antimicrobial activity against several foodborne microorganisms, supporting the findings obtained in the present study. Apart from microbial inhibition, sensory observations indicated that the treated tofu samples retained acceptable quality characteristics during storage. The mild citrus-like aroma of lemongrass oil contributed positively to the overall sensory profile without causing undesirable changes in texture or appearance at optimized concentrations. This suggests that lemongrass essential oil may provide both preservative and sensory benefits when incorporated into tofu preservation systems.

The antimicrobial activity observed during agar well diffusion assay confirmed the effectiveness of selected essential oil against microorganisms isolated from tofu. Increasing concentrations of essential oil resulted in improved inhibition, indicating concentration-dependent antimicrobial activity. These findings are consistent with previous reports describing the relationship between essential oil concentration and microbial inhibition efficiency in food preservation systems (Gutierrez et al., 2008).

The microbiological analyses performed according to ISO-based standard methods further strengthened the reliability and reproducibility of the study. Enumeration of hygiene indicators such as coliforms and *Enterobacteriaceae*, along with detection of foodborne pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.*, *and Listeria monocytogenes*, provided important information regarding the microbial quality and safety of untreated and treated tofu samples.

Overall, the study highlights the promising potential of lemongrass essential oils as natural preservatives for tofu preservation systems. The results support the growing demand for clean-label food products and natural alternatives to synthetic preservatives. Furthermore, the findings provide a basis for future studies involving nano-emulsion systems, edible antimicrobial coatings, and synergistic combinations of essential oils for enhanced shelf-life extension and food safety applications.

Conclusion

The present study demonstrated that lemongrass essential oil (LEO) possesses significant antimicrobial activity against spoilage and pathogenic microorganisms associated with tofu, highlighting its potential as a natural alternative to synthetic preservatives. Due to its high moisture and protein content, tofu is highly prone to microbial spoilage during storage; therefore, effective natural preservation strategies are essential to enhance its microbial safety and shelf life.

Treatment with Lemongrass essential oil significantly reduced microbial growth and improved microbiological quality compared to untreated control samples, confirming its preservative potential in tofu and other plant-based food systems. The antimicrobial effect increased with higher concentrations of lemongrass essential oil;

however, sensory quality remained an important consideration. Optimized concentrations provided an effective balance between microbial inhibition and acceptable sensory properties, including aroma, texture, appearance, and overall acceptability.

Overall, the findings support the application of lemongrass essential oil as a promising bio-preservative for clean-label food preservation. Furthermore, advanced delivery approaches such as nano emulsions, edible coatings, and controlled-release packaging may further enhance its stability and antimicrobial efficacy. Future studies focusing on extended storage evaluation, combined preservation techniques, and large-scale industrial applications are recommended to fully establish its commercial potential in tofu preservation systems.

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