Original Article

Journal of Food Safety and Hygiene

Journal homepage: http://jfsh.tums.ac.ir

Effectiveness of clove oil-enriched starch films as packaging materials

Himaja*[1], Dhanya Pulikkottil Rajan[2], Jiya Jain[1]

[1]PG and Research Department of Aquaculture and Fishery Microbiology, M.E.S. Ponnani College, kerala, India.
[2]Department of Aquaculture, M.E.S. Asmabi College, P.Vemballur, kerala, India.

ARTICLE INFO

Article history: Received 20.12.2025
Received in revised form 06.03.2026
Accepted 10.03.2026

Keywords:
Antimicrobial;
Bacillus;
Essential oil;
Fish;
Inhibition;
Slaughterhouse;
Shelf-life

ABSTRACT

Plastics are employed for food packaging; it imparts huge pollution to the environment. Meanwhile, biodegradable materials are employed for the same function, as they not only provide a barrier but also prevent the microbial contamination. The aim of the study is to analyse the film-forming capacity of the starch along with the essential oils and identify its antibacterial property. Starch, due to its abundance and cost-effectiveness, was a choice for packing different food products. For this, 5g of starch was mixed with 100 mL of distilled water along with 1 mL of glycerol as a plasticizer. The antimicrobial agent used was clove oil in a concentration of 0.5, 0.7 and 1 mL; a control sample was made in which no clove oil was added to differentiate the inhibition. The solution was heated and dried into the films. The bacterial strains were isolated from the gills of healthy tuna (*Euthynnus affinis)*, and on conducting total plate counts, pure bacterial strains of *Bacillus cereus* were identified. The FTIR of the derived material was conducted. Resulted starch film was then cut into a 9 mm disc shape and incubated on plates of bacterial colonies at 36°C for 24 h. Significant inhibitory zones were seen, starch films (5 g/100 mL distilled water) with the concentrations of 0.5, 0.7, and 1.0 mL showed inhibition zones of 18.86±1.06, 16±0.81, and 18.66±0.94 mm against the bacteria. The FTIR analysis shows an association of eugenol and its derivative with the starch film. This film suggests an antibacterial property, and further studies have to be done.

Citation: Himaja, Rajan DP, Jain J. **Effectiveness of clove oil-enriched starch films as packaging materials.** J Food Safe & Hyg 2026; 12 (1): 35-44. http://doi.org/10.18502/jfsh.v12i1.22249

1. Introduction

The emerging focus on the sustainable food preservation methodologies has armed into biodegradable antimicrobial packaging, especially for the perishable food commodity like fish (1). This is important for extending shelf life and promoting food safety by blocking microbial populations, thereby resolving food waste and negative impacts of traditional plastics (2, 3). Antimicrobial packaging belongs to active packaging that induces antimicrobial agents into the packaging films, which suppresses the bacterial load and extends the shelf-life (4).

*Corresponding author.
E-mail address: himaja4edu@gmail.com

The most successful process is to use natural antimicrobial substances in the food packaging and thereby reduce the contamination of pathogens. Major natural agents are antimicrobial peptides, organic acids, essential oils, enzymes, and some biopolymers (5). Antimicrobial packaging systems function in different ways; films and biopolymers have been seen as efficient carriers of antimicrobial agents (6). The selection of packaging films is crucial, as it should be able to maintain quality, provide a safe supply, and increase the shelf life of the food products. Generally, petroleum-derived materials dominate the industry since they provide excellent mechanical properties, but they cause huge pollution to the environment, and thereby there is a progressive turn towards biodegradable polymers (7,8).

Natural polymers adhere to antimicrobial properties through the influence of natural agents on polymers. The renewable polymers are proteins, lipids, polysaccharides, and gums, which are derived from either plants or animals (9). Based on the affinity for water, polysaccharides are classified into starch and non-starch polysaccharides (10). Starch is a renewable carbohydrate that sources from wheat, corn, rice, and potatoes and can be considered an emerging polymer that adheres to decomposability, affluence, sustainability, cheap availability, and non-toxicity (8,11). Since it holds high mechanical properties, transparency, tastelessness, and flavorless characteristics, it has been used as food packaging material. Brittleness and poor water vapor barrier have been a disadvantage, which can be modified (12).

Among the natural antimicrobials, plants are considered the foremost and richest source and do possess antimicrobial, antioxidant, and flavoring properties. The plant and its by-products do carry phenolic compounds that reduce the microbial growth, especially essential oils (13). Clove oil extracted from flower buds contains a high quantity of phenols, mainly eugenol, which is responsible for reducing inflammation and boosting the immune system (14). Clove oil is commonly used as a natural or as a medicated food additive to increase the shelf-life against foodborne pathogens like *E. coli*, *S. typhimurium*, *S. aureus*, *B. cereus*, and *L. monocytogenes.* Hence, the films incorporated with clove oil are an emerging food packaging material (15).

Bacillus cereus comes into the genus *Bacillus* as of the genetic similarity. *B. cereus* can be widely seen in a temperature range of 5-50°C, with an optimum of 28°C-35°C. They are seen in a wide range of food items, like raw and baked foods, meat and meat products, seafood products, and vegetables. They form heat-resistant spores, which makes it a threat to the food industry. They are responsible for gastrointestinal and respiratory diseases. Several control measures have to be administered in order to delay the microbial infection (16).

The objective of this study is to analyse the antimicrobial property of biopolymer starch film incorporated with clove oil against *Bacillus cereus*. The film forming capacity of the starch can also be identified. The starch films enriched with different concentrations of clove oil should resist microbial growth and hence would extend the shelf life of fish and fish products. The specific focus is to evaluate the efficacy of natural antimicrobial agents against fish spoilage bacteria.

2. Materials and Methods

http://doi.org/10.18502/jfsh.v12i1.22249

2.1. Materials

Potato starch from Kanton Laboratories, Glycerol from PRS Pharmaceuticals, Clove oil with Refractive index of 1.629- 1.537, specific gravity of 1.043-1.068[a] with characterization of colorless to yellowish color was purchased from Spice Board of India, Nutrient agar from Hi-Media, Sterilized Distilled Water, Normal saline, Gram Staining kit from Hi-Media and Tuna (*Euthynus affinis*) from the munabam harbour were used. All the chemicals were analytical grade. For standardizing the bacterial load McFarland Standard was prepared.

2.2. Instrumentation

Hot plates (Rotek, India), Hot air ovens (Labline, India), FTIR spectra (PerkinElmer Spectrum IR Version 10.6.), Incubators **(**Labline, India**),** Autoclave (Labline, India) were used at different sections of the experiments.

The Fourier Transform InfraRed (FTIR) spectra were collected in total reflectance mode (ATR) with PerkinElmer Spectrum IR Version 10.6. device was administered for the test. Fragments of starch film both control and sample were previously dried, cleaned and powdered with Potassium Bromide. The investigated wavenumber range is 4000- 600 cm^{-1}.

2.3. Isolation of *Bacillus cereus*

10 g of fish from the gills of Tuna (*Euthynnus affinis)* was separated, and macerated with 90 mL of diluent (Normal saline). It gave 10 times the dilution of the sample. ie. 10^{-1} dilution. For further dilution, 1 mL from 10^{-1} dilution is mixed with 9 mL of the diluent (10^{-2} dilution) and the serial dilution is continued to a dilution of 10^{-7}. From the dilution factor of 10^{-7}, 0.5 mL of the solution was plated to nutrient agar and conducted in triplicates to result in the pure strains. The bacterial morphology was conducted (17).

2.4. Preparation of bacterial lawn

Nutrient agar (14 g) was mixed with 500 mL distilled water, which was sterilized in an autoclave at 15 lbs for 15 min. (121^{o}C for 15 min). On solidification of the nutrient plates the bacterial load of 1.5* 10^{8} was prepared with McFarland standards (18).

2.5. Synthesis of biofilm

Starch (5 g) was weighted using weighing balance, and made up to the volume of 100 mL using sterilized distilled water. This solution was mixed and heated for 15 min. 1mL quantity of glycerol was added to the mixture. As soon as the solution thickened, clove oil was added to the solution. Different concentrations of clove oil were used to prepare such starch films and the concentrations (0,5, 0.7, 1.0 %). Similarly control film was also prepared in which no clove oil was added (19). The photographs of starch film formation from sample weighing (a) to disc inoculation to the bacterial lawn (d) is represented. All the photographs were taken by the author. Fig.1 (a) represents the sample weighing, (b) shows the scratch film formation, (c) is the dried film of the solution, Fig.1 (d) is the synthesized film. Fig 1. (e) and (f) is the disc size of 9 mm on inoculation to the bacterial lawn.

The prepared film was cut into a 9 mm disc shape to examine the inhibitory zones when inoculated into the bacterial lawn grown over nutrient agar. Measurement of Starch film disc used for antibacterial testing by vernier caliper.

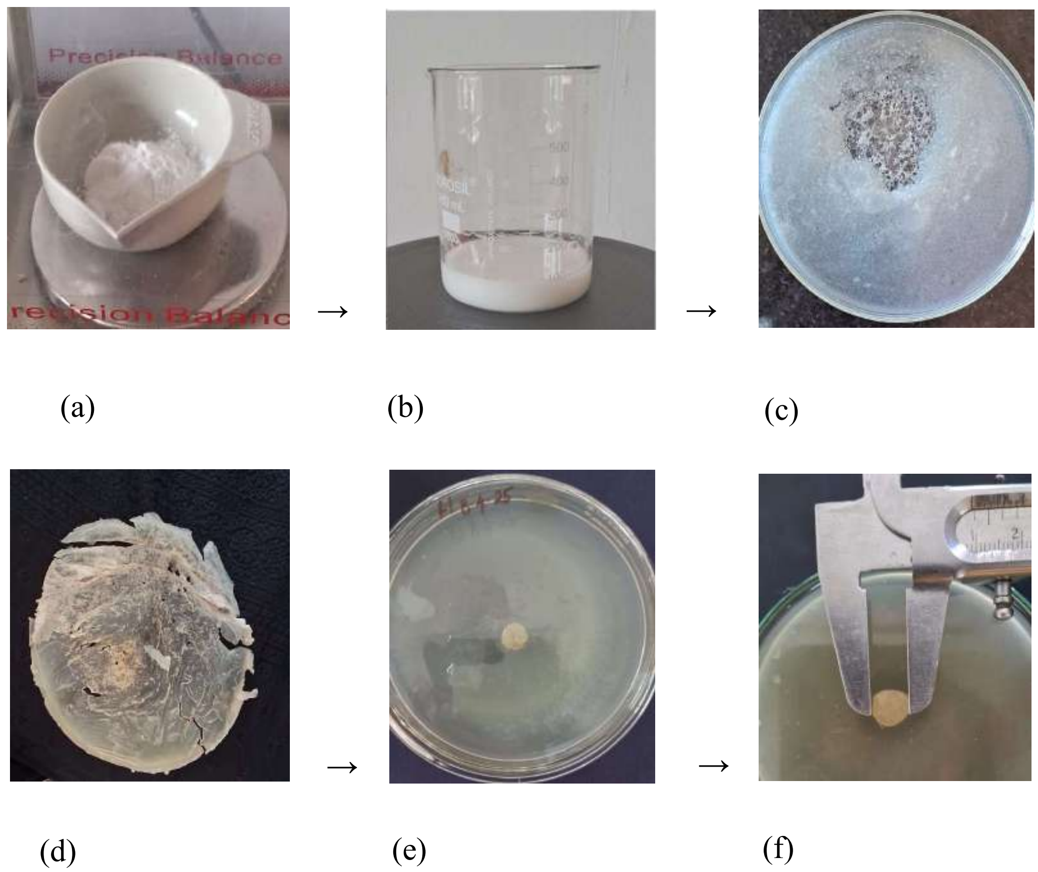

Figure 1. The synthesis of biofilm and insertion of 9mm disc into the bacterial lawn.

2.6. Antibacterial assessment

The antibacterial test was conducted by placing different concentrations of 9mm starch film (0.5,0.7, 1.0 mL) into the nutrient agar plates inoculated with *B. cereus* where a control of zero concentration of clove oil was also placed to define the inhibition. The antimicrobial tests were analyzed in the incubator at 36^{o}C for 24 h. Triplicates of the test were conducted so as to analyze the effectiveness.

2.7. Statistics

The experiments conducted such as film formation, antibacterial analysis, FTIR were performed in triplicates. Results were reported as mean $\pm$ standard deviation. The analysis of variance was evaluated by ANOVA procedure ($p<0.5$) by SPSS Software.

3. Results

3.1. Morphology of the bacterial strains

The pure strains of the bacteria were isolated and identified as irregular, undulated, flat and wrinkled. The strain was gram stained which results in gram-positive.

3.2. FT-IR analysis

The FT-IR spectra is presented in Fig 2. (a, b). Fig 2. (a) represents the spectra of starch film alone and Fig 2. (b) shows the spectra of starch enriched with clove oil. The main difference seen between starch film and starch film with clove was that 1103.69 cm^{-1} to 1637.28 cm^{-1}

stretching the C-O bond and C=C stretching of the aromatic ring in the eugenol group. FTIR spectra of the starch alone have typical peaks at 2,900-3,000 cm^{-1} (C-H stretching), 1,103-1,1150 cm^{-1} (C-O, C-C, and C-O-H

Table 1. FTIR values of starch film

Wavenumber (cm^{-1})	Assignment	Materials	References
925.35	C-O-H	Starch film	(20)
1151.44-1103.69	C-O, C-C, C-O-H	Starch film	(20)
1080.00	C-O	Starch film+ C	(18)
1242.13	C-O	Starch film+ C	(18)
1637.28	C=C	Starch+ clove oil	(21)
2933.60	C-H	Starch + clove oil	(22)
3308.73	O-H	Starch film	(20)
3336.52	O-H	Starch +clove oil	(22)

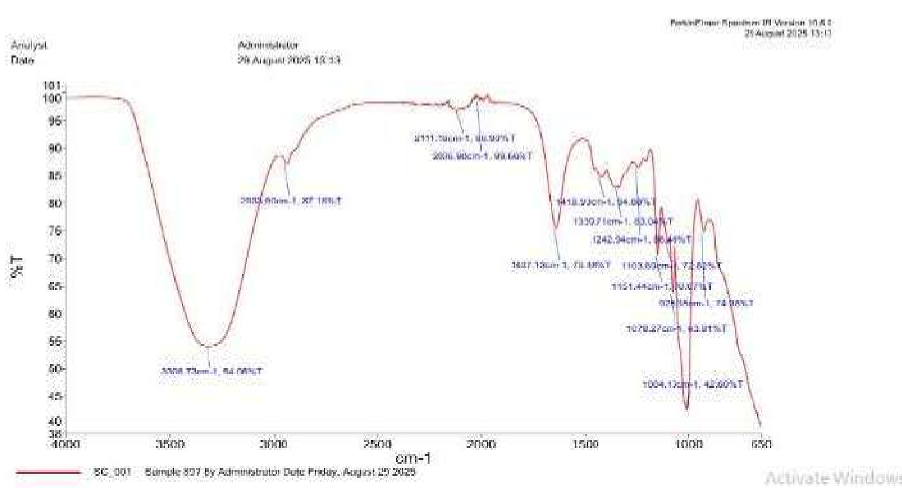

Figure. 2. (a) vibration peaks of starch alone

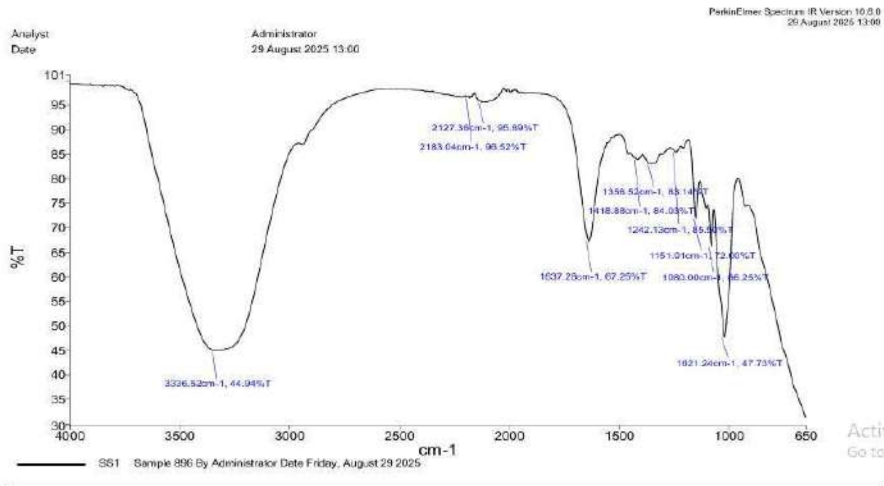

Figure. 2. (b) vibration peaks of starch enriched with clove oil

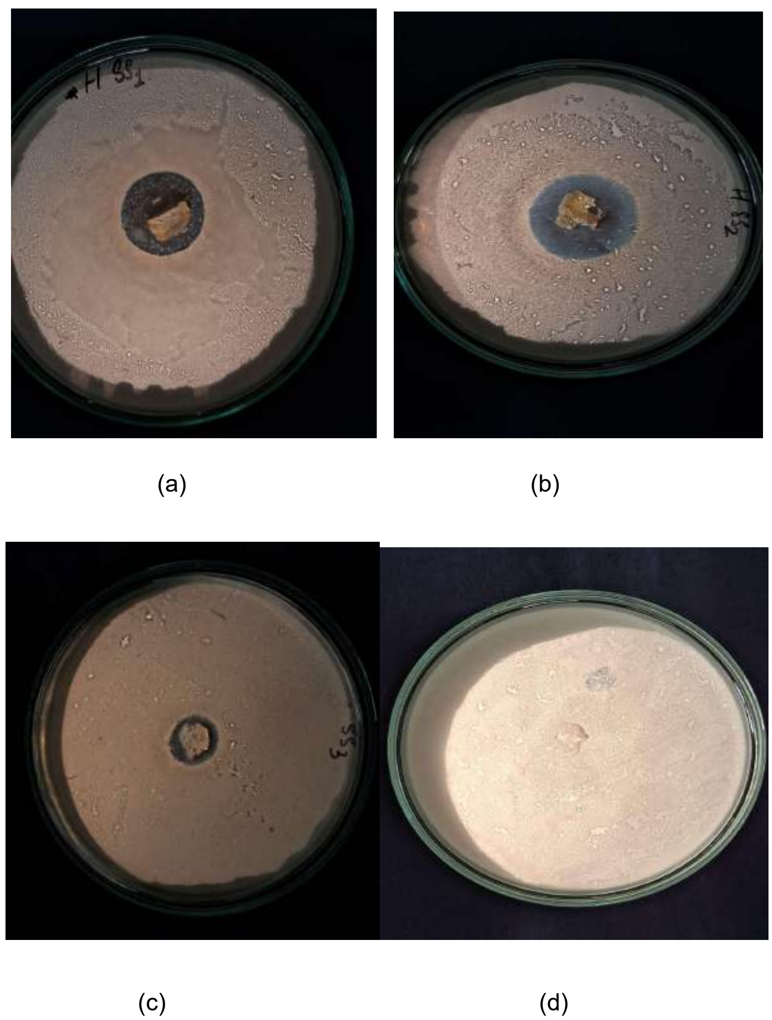

Figure 3. The inhibitory zones of clove enriched starch film of 0.5, 0.7, 1.0 mL and (d) shows no inhibition.

Table 2. The mean value of inhibitory zones of starch enriched film in different concentrations.

Concentration of Clove oil (mL)	Avg. of inhibitory zones
0.5	18.86 ± 1.06
0.7	16 ± 0.81
1	18.66 ± 0.94

stretching), 925 cm^{-1} (C-O-H bending and stretch vibration). From the spectra, there is a peak vibration of glucose backbone at 2,900- 3,000 cm^{-1} C-H stretching, 1,100-1,150 cm^{-1} (C-O, C-C and C-O-H stretching), 900-–1,100 cm^{-1} (C–O–H bending and stretch vibration) (20). Table 1 represents the vibration peaks of the spectra and comparison with previous literature notes. Taraj et al., in the previous literature quoted about the vibration peaks of the eugenol group, in which stretching of C-O bond and C=C stretching in the range of 1103.69 cm^{-1} to 1637.28 cm^{-1} (21). In the literature of Zhuang et al., (19) clearly demonstrates the peak vibration of glucose backbone and when comparing with the resulted peaks of 2,900-3,000 cm^{-1} C-H stretching, 1,100-1,150 cm^{-1} (C-O, C-C and C-O-H stretching), 900-–1,100 cm− 1 (C–O–H bending and stretch vibration) (22). In the previous study conducted by Ruggero et al., (20) note that 1080.00 cm^{-1} shows a C-O stretch, 1242.13 cm^{-1} indicates the peak of C-O vibrations from the given samples. The broad characteristic bands of 1151.91- 1242.13 cm^{-1}, in the studies by Rojas et al., (24) represents asymmetric C-O-C stretching of eugenol and its derivatives like eugenol acetate (23).

3.3. Antimicrobial analysis

After incubating the starch disc in the bacterial lawn for 24 h, significant zones were formed in all concentrations. On computing the mean and standard deviation of the inhibitory zones for 0.5 mL it results 18.866 ±1.06 mm. For the concentration of 0.7 mL, it results 16±0.81mm and for the concentration of clove oil of 1.0 mL it results as 18.66±0.94mm diameter zones. Fig. 3 (a, b, c) shows the inhibitory zones of starch film against *Bacillus cereus* in 0.5, 0.7, and 1.0 mL concentrations, whereas (d) shows no inhibition in all the experiments done.

With the exception of the control biofilm that showed no inhibitory zones were considered as control, all of the different concentrations of biofilm in the present study showed inhibition. In which maximum inhibitory zones were seen in 1.0% clove oil enriched film.

4. Discussion

The ATR-FTIR spectral analysis of the starch film with clove oil shows characteristic absorption bands corresponding to its primary chemical constituents, specifically eugenol and eugenyl acetate (24). This experimental analysis shows the influence of clove essential oil on the starch polymer, which significantly inhibits *Bacillus cereus* growth with different oil concentrations resulting in inhibitory zones (25). This dose-dependent efficacy reveals that increasing the concentration of clove oil elevates the reduction of microbial colonies within the active packaging system (26). Additionally, the incorporation of essential oil into the biofilm would reduce the water vapor transmission and alter the physical barrier performance (27). Even though this integration of bioactive compounds also contributes to the mechanical integrity of the film, it leads to a decrease in tensile strength and an increase in elongation at break as the oil interacts (28).

5. Conclusion

The study clearly demonstrates the application of clove oil to the starch film. Its inhibitory zones show its possibility of making them an ideal material for antimicrobial packaging.

The present study of analyzing the antimicrobial property of starch-enriched biofilms with different concentrations of clove oil shows great inhibition against *Bacillus cereus,* which is a food-spoilage bacteria. The study also demonstrates the chemical bonds of molecules using FTIR, which reveals the presence of eugenol and its derivatives in the prepared film. The volume of 1.0 mL shows a significant inhibition followed by 0.5 mL and 0.7 mL. This study thus contributes to the advancement of antimicrobial packaging. The work is limited to a single bacterial strain, and other physicochemical properties and chemical analyses of the starch film have to be done.

Funding

For the conduct of the particular study no external funding agency is involved.

Authorship contributions

From the conceptualization, and all the authors are involved in the particular study. Major writing and correction were done by the corresponding author and preceded by the second author.

Acknowledgements

For the conduct of the analysis both the organization, PG and Research Department of Aquaculture and Fishery Microbiology, M.E.S. Ponanni College & Department of Aquaculture, M.E.S. Asmabi College, P. Vemballur has supported the conduct of the particular work.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Data availability

All data generated or analyzed during this study are included in this article.

References

1. Melarcode HS, Ertugrul O, Alzate Calderon PC, Kerry JP, Morris MA. Advanced biodegradable antimicrobial coatings by combining biopolymers and natural antimicrobials for the active packaging and extending the shelf-life of fish and other seafood products: a review. Int J Food Sci Technol. 2025;60(2):vvaf168. DOI: https://doi.org/10.1093/ijfood/vvaf168.
2. Bose I, Roy S, Pandey VK, Singh R. A Comprehensive review on significance and advancements of antimicrobial agents in biodegradable food packaging. Antibiotics. 2023;12(6):968. DOI: 10.3390/antibiotics12060968.
3. Nor AHA, Jalil MTM, Seow EK, Idris MHM, Yahya MFZR. Antimicrobial packaging: Mechanisms, materials, incorporation methods, strategies, biodegradability, and smart innovations–a

comprehensive review. Food Mater Res. 2025;5(1). DOI: 10.48130/fmr-0025-0008.

4. Malhotra B, Keshwani A, Kharkwal H. Antimicrobial food packaging: Potential and pitfalls. Front Microbiol. 2015;6:611. DOI: 10.3389/fmicb.2015.00611.

5. Devi LS, Krishnan R, Begum S, Nath D, Mohanty A, Misra M, Kumar S. Curcumin as bioactive agent in active and intelligent food packaging systems: A comprehensive review. Compr Rev Food Sci Food Saf. 2025;24(4):e70228. DOI: 10.1111/1541-4337.70228.

6. Bahrami A, Delshadi R, Assadpour E, Jafari SM, Williams L. Antimicrobial-loaded nanocarriers for food packaging applications. Adv Colloid Interface Sci. 2020;278:102140. DOI: 10.1016/j.cis.2020.102140.

7. Karimi Sani I, Hassani B, Rasul NH, Mansouri E, Eghbaljoo H, Kaveh M, Hassani D, Alizadeh Sani M, Khezerlou A, Gholizadeh H, Mamakani ZS, Jafari SM. Antioxidant packaging films: Application for sustainable food protection. Curr Res Food Sci. 2025;11:101222. DOI: 10.1016/j.crfs.2025.101222.

8. Oluwasina OO, Awonyemi OI. Citrus peel extract starch-based bioplastic: Effect of extract concentration on packed fish and bioplastic properties. J Polym Environ. 2021;29:1-11. DOI: 10.1007/s10924-020-01990-7.

9. Sung SY, Sin LT, Tee TT, Bee ST, Rahmat A, Rahman W, et al. Antimicrobial agents for food packaging applications. Trends Food Sci Technol. 2013;33(2):110-23. DOI: 10.1016/j.tifs.2013.08.001.

10. Anis A, Pal K, Al-Zahrani SM. Essential oil-containing polysaccharide-based edible films and coatings for food security applications. Polymers. 2021;13(4):575. DOI: 10.3390/polym13040575.

11. Onyeaka H, Obileke K, Makaka G, Nwokolo N. Current research and applications of starch-based biodegradable films for food packaging. Polymers (Basel). 2022;14(6):1126. DOI: 10.3390/polym14061126.

12. Gamage A, Thiviya P, Mani S, Ponnusamy PG, Manamperi A, Evon P, Merah O, Madhujith T. Environmental properties and applications of biodegradable starch-based nanocomposites. polymers (Basel). 2022;14(21):4578. DOI: 10.3390/polym14214578.

13. Umaraw P, Munekata PES, Verma AK, Barba FJ, Singh VP, Kumar P, et al. Edible films/coating with tailored properties for active packaging of meat, fish and derived products. Trends Food Sci Technol. 2020;98:10-24. DOI: 10.1016/j.tifs.2020.01.032.

14. Li J, Li C, Shi C, Aliakbarlu J, Cui H, Lin L. Antibacterial mechanisms of clove essential oil against *Staphylococcus aureus* and its application in pork. Int J Food Microbiol. 2022;380:109864. DOI: 10.1016/j.ijfoodmicro.2022.109864.

15. Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li HB. Antibacterial and antifungal activities of spices. Int J Mol Sci. 2017;18(6):1283. DOI: 10.3390/ijms18061283.

16. Kowalska J, Maćkiw E, Korsak D, Postupolski J. Prevalence of *Bacillus cereus* in food products in Poland. Ann Agric Environ Med. 2024;31(1):8-12. DOI: 10.26444/aaem/168580

17. Surendran PK, Thampuran N, Nambiar VN, Lalitha KV, Toms CJ. Laboratory techniques for microbiological examination of seafood. Cochin: ICAR-CIFT; 2013.

18. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493-6.

19. Zhuang Y, Wang Y, Yang H. Characterizing digestibility of potato starch with cations by SEM, X-ray, LF-NMR, FTIR. Food Chem. 2023;424:136396. DOI: 10.1016/j.foodchem.2023.136396

20. Ruggero F, Carretti E, Gori R, Lotti T, Lubello C. Monitoring of degradation of starch-based biopolymer film under different composting conditions, using TGA, FTIR and SEM analysis. Chemosphere.

2020;246:125770. DOI: 10.1016/j.chemosphere.2019.125770

21. Taraj K, Andoni A, Ylli F, Malollari I. Spectroscopic investigation of *Syzygium aromaticum* L. oil by water distillation extraction. J Int Environ Appl Sci. 2019;14(3):122-6.
22. Kuz P, Ates M. Starch-based bioplastic materials for packaging industry. J Sustain Constr Mater Technol. 2020;5(2):399-406. DOI: 10.29187/jscmt.2020.44.
23. Saied M, Ramadan R, Abd-El-Messieh S. A study of the biophysical properties of polystyrene films incorporated with clove oil as bio-based plasticizer. Egypt J Chem. 2021;64(6):3111-20.
24. Rojas J, Cabrera S, Benavides J, Lopera Y, Yarce CJ. Lipidic matrixes containing Clove essential oil: Biological activity, microstructural and textural studies. Molecules. 2021;26(9):2425. DOI: 10.3390/molecules26092425.
25. Rodríguez JDW, Peyron S, Rigou P, Chalier P. Rapid quantification of clove (*Syzygium aromaticum*) and spearmint (*Mentha spicata*) essential oils encapsulated in a complex organic matrix using an ATR-FTIR spectroscopic method. PLoS One. 2018;13(11):e0207401. DOI: 10.1371/journal.pone.0207401.
26. Ahmed M, Saini P, Iqbal U, Sahu K. Edible microbial cellulose-based antimicrobial coatings and films containing clove extract. Food Prod Process Nutr. 2024;6(1):65. DOI: 10.1186/s43014-024-00241-9
27. Pandey VK, Shams R, Singh R, Dar AH, Pandiselvam R, Rusu AV, Trif M. A comprehensive review on clove (*Caryophyllus aromaticus L.*) essential oil and its significance in the formulation of edible coatings for potential food applications. Front Nutr. 2022;9:987674. DOI: 10.3389/fnut.2022.987674.
28. Ulyarti U, Lavlinesia L, Surhaini S, Lisani L, Nazarudin N. Development of yam-starch-based bioplastics with the addition of chitosan and clove oil. Makara J Sci. 2021;25(1):71-9.