Potential of *Moringa oleifera* Extract as a Natural Additive in Gelatine-Whey Protein Edible Film Formulation

Ria Dewi Andriani[1*], Abdul Manab[1], Salsa Febriavalent Herani[1], Shefia Lista Anggraeni[1], and Mokhammad Ilham Maulana[1]

[1]Faculty of Animal Sciences, Universitas Brawijaya, Malang 65145, Indonesia

Abstract. Cheese is a perishable dairy product that is highly susceptible to microbial spoilage and oxidative degradation, necessitating the development of sustainable bioactive packaging to enhance its shelf-life. This study aimed to evaluate the potential of Moringa oleifera extract (MOE) as a natural additive in gelatine whey protein edible film formulations. Fresh Moringa leaves were extracted using food-grade ethanol, and the resulting extract—characterized by high phenolic content (526.17 mg GAE/100 g), flavonoids (40.75 mg QE/100 g), nano-scale particle size (30 nm), and strong antioxidant capacity (IC_{50} 43.6 μg/mL) was incorporated into edible film solutions at various concentrations. The physicochemical characteristics of the film solutions, including pH and total bacterial count (TBC), were assessed, alongside antimicrobial activity against Escherichia coli and Salmonella spp. The addition of MOE maintained film solution pH within 5.52–5.73 and reduced TBC compared to control, indicating improved microbial stability during preparation. Antimicrobial assays demonstrated clear inhibition zones for MOE-enriched films, with the highest activity observed at increased extract concentrations. These bioactive films also exhibited strong antioxidant behaviour consistent with the phytochemical profile of Moringa. The findings indicate that MOE effectively enhances both the antioxidant and antimicrobial properties of gelatine whey protein edible films without compromising physicochemical stability. Overall, Moringa oleifera extract shows significant potential as a natural additive for developing sustainable, biodegradable, and functional edible films suitable for cheese preservation. Future studies should explore the films performance during actual storage, their mechanical properties, and consumer acceptability for commercial application.
Keywords: Antimicrobial activity, antioxidant capacity, edible film, *Moringa oleifera*, whey protein.

1 Introduction

Cheese is a globally consumed dairy product valued for its nutritional quality, sensory attributes, and versatility across culinary applications. However, like many high-moisture

* Corresponding author: riaandriani@ub.ac.id

food products, cheese is inherently prone to deterioration caused by microbial growth, lipid oxidation, moisture, and physicochemical degradation during storage [1,2,10]. These quality changes not only reduce consumer acceptability but also pose potential safety risks. Traditional synthetic packaging, although effective as a physical barrier, offers limited bioactive protection and contributes significantly to environmental pollution due to its non-biodegradable nature [3]. As environmental concerns intensify and the food industry shifts toward sustainable processing innovations, interest in biodegradable and functional packaging materials has increased substantially. Among these, edible films and coatings formulated from natural biopolymers have received considerable attention for their dual capacity to enhance food preservation and reduce reliance on synthetic plastics [4].

Protein-based edible films are particularly attractive due to their favourable film-forming properties, excellent transparency, high oxygen barrier capacity, and compatibility with various food systems. Gelatine, derived from collagen hydrolysis, forms flexible, cohesive films with good mechanical and gas barrier characteristics, whereas whey protein contributes emulsifying capacity, structural rigidity, and water vapor resistance [5]. The synergy between gelatine and whey protein has been as an effective matrix for edible coatings applied on fresh and processed dairy products [5]. Despite these advantages, the intrinsic antimicrobial and antioxidant properties of protein-based films are relatively weak, making them insufficient for protecting cheese against oxidative rancidity and microbial spoilage during storage. As a result, recent research trends emphasize enriching edible films with natural bioactive compounds to reinforce their preservative function [6].

The primary challenge in developing bioactive edible films lies in identifying natural additives capable of inhibiting spoilage microorganisms and mitigating oxidative degradation while maintaining the physical and structural integrity of the film. Many plant-derived extracts contain potent phenolic and flavonoid compounds but pose risks of undesirable sensory impacts, reduced film stability, or poor compatibility with polymer networks when incorporated in high concentrations [7]. Furthermore, achieving a controlled and sustained release of antimicrobial and antioxidant compounds from the film matrix during storage remains a technical challenge, especially for foods with high water activity like cheese. Therefore, there is a need to identify natural additives that exhibit strong functionality and compatibility with the protein-based film matrix.

One promising candidate that meets these criteria is Moringa oleifera, a plant widely recognized for its rich phytochemical composition. Its leaves contain abundant phenolic acids, flavonoids, vitamins, carotenoids, and essential micronutrients, all contributing to potent antioxidant and antimicrobial activity [8]. Several studies have demonstrated that Moringa oleifera leaf extract (MOE) inhibits the growth of numerous foodborne pathogens, including *Escherichia coli*, *Salmonella spp*., *Staphylococcus aureus*, and *Listeria monocytogenes* [8,9]. Moreover, the extract exhibits strong free-radical scavenging properties due to its high concentrations of quercetin, chlorogenic acid, and gallic acid, making it effective for retarding oxidative deterioration in food systems [8]. These characteristics highlight its potential as a bioactive ingredient suitable for incorporation into edible films designed for cheese preservation.

Beyond its antimicrobial and antioxidant properties, Moringa oleifera demonstrates favorable technological compatibility with biopolymer-based films. Prior research has reported successful incorporation of MOE into polysaccharide and protein matrices, resulting in films with enhanced mechanical strength, UV-light resistance, and reduced microbial loads. Additionally, advancements in nano-sized MOE particles have improved the dispersibility and stability of the extract in aqueous and semi-solid food matrices, suggesting improved interaction with the protein network in edible film systems. These developments provide a strong foundation for exploring MOE as a functional component in gelatine whey protein matrices tailored for dairy applications.

Recent studies on bioactive edible films further demonstrate the benefits of enriching protein-based coatings with natural plant extracts. Green tea extract, rosemary extract, turmeric extract, and grape seed extract have been shown to significantly improve the antioxidant performance of gelatine films, while aloe vera, neem, and pomegranate peel extract coatings have exhibited microbial inhibition in dairy and meat products [6]. These findings collectively reaffirm the feasibility of integrating plant bioactive into edible packaging systems. However, each plant extract interacts distinctly with biopolymer matrices, affecting mechanical, structural, and barrier properties in different ways. Therefore, their effectiveness must be verified within specific food systems. While MOE has been widely studied for its general pharmacological properties, its direct incorporation into gelatine whey protein films and its specific performance as a functional coating for cheese remain insufficiently explored.

A review of the current literature reveals several knowledge gaps that justify the relevance of this study. Thus, the present study aims to evaluate the potential of Moringa oleifera extract as a natural additive in gelatine whey protein edible film formulation. Through this approach, the study seeks to contribute to the development of sustainable, bioactive packaging strategies suited for the dairy industry while addressing environmental concerns linked to conventional packaging materials.

2 Materials and methods

Moringa oleifera leaves, bovine gelatine, and whey protein were used as materials. Gelatine and whey protein were selected for their established film-forming ability and compatibility with dairy systems. All analytical reagents were food-grade, and were prepared under controlled laboratory conditions.

2.1 Extraction of *Moringa oleifera*

Moringa leaves powder finely milled, and extracted using food-grade ethanol following established procedures to preserve phenolics, flavonoids, and carotenoids responsible for antioxidant and antimicrobial activity [9, 10]. Extracts were filtered, concentrated, and refrigerated until use.

2.2 Preparation of Edible Film with the Addition of *Moringa oleifera* Extract

Gelatine was hydrated and dissolved in distilled water, after which whey protein was incorporated to strengthen the film matrix and enhance barrier properties. The solution was heated with constant agitation, and *Moringa oleifera* extract (0 (T0); 0.1 (T1); 0.2 (T2); (T3) 0.3%) was added at controlled temperatures to prevent degradation of its bioactive compounds [11]. Continuous mixing ensured uniform dispersion, following recommended practices for incorporating plant extract into protein matrices. The mixture was heated using a hot plate stirrer 60 ^{0}C, 200 rpm and then tested for pH, total bacterial count, and antimicrobial activity

2.3 Physicochemical and Microbial Analysis of Film Solutions

Solution pH was measured to evaluate protein stability during film formation. Total bacterial count was assessed using standard plate count methods to determine the baseline antimicrobial effect of *Moringa* extract. The antimicrobial activity of the films was tested against *Escherichia coli* and *Salmonella* spp., both relevant to cheese spoilage and safety

[12]. Standard agar diffusion procedures were used, consistent with methodologies for evaluating bioactive edible films. Antioxidant capacity was quantified using spectrophotometric assays to determine radical-scavenging activity (IC_{50}), based on established methods for assessing *Moringa* antioxidant potential.

2.4 Statistical Analysis

Data were analyzed by ANOVA, followed by appropriate post-hoc tests when significant differences occurred. Statistical procedures followed established analytical standards in edible-film research [13].

3 Result and discussion

3.1 Characteristics of *Moringa oleifera* Extract

The characterization of Moringa oleifera extract (MOE) revealed the presence of abundant phenolic acids, flavonoids, and carotenoids, consistent with previous findings that describe Moringa leaves as one of the most phytochemically dense botanical materials used in functional food formulations [8]. As reported in earlier studies, the primary bioactive constituents in Moringa include quercetin, chlorogenic acid, gallic acid, and β-carotene, which contribute substantially to antioxidant and antimicrobial activity [8,9]. The high intensity of phenolic and flavonoid compounds in the extract (Table 1) used in this study aligns with previous reports documenting strong free-radical scavenging performance of Moringa-based preparations [13]. Phytochemical richness is central to the functional behaviour of MOE in edible film matrices because these compounds serve as active agents responsible for inhibiting microbial growth and oxidative reactions on cheese surfaces.

The extract also demonstrated a nano-scale particle distribution, which has been described in earlier literature as a factor enhancing the solubility, dispersibility, and bioactivity of plant extracts when integrated into biopolymer systems [14]. Nano-sized bioactive particles typically exhibit greater surface area and interaction potential with protein-based matrices, enabling more efficient functionality in edible film formulations. The presence of these small particle sizes likely improved the uniform incorporation of the extract into the gelatine whey protein matrix and facilitated the release of active compounds during antimicrobial testing. Similar findings have been documented in nano-enhanced Moringa extracts used for food preservation and packaging applications [14]. Therefore, the physicochemical profile of MOE in this study provides a strong basis for the subsequent improvements observed in the edible films enriched with Moringa. The antioxidant capacity of the edible films, which confirmed significant free-radical scavenging activity attributable to MOE. This is in line with established literature documenting the strong antioxidant of Moringa oleifera due to its high levels of quercetin, chlorogenic acid, gallic acid, and β-carotene [8,9,13]. The IC_{50} values obtained in this study indicate that the antioxidant strength of the films increases with MOE concentration, reflecting a dose-dependent relationship commonly observed in plant extract–based edible films [13]. These results highlight the ability of MOE to improve the oxidative stability of edible coatings.

Table 1. Characteristics of *Moringa oleifera* Extract.

Parameter	Average
Particle size	30 nm
Total phenolic content	526.17 mg GAE/100 g db
Total flavonoid	40.75 mg QE/100 g db
Antioxidant activity (IC_{50})	43.6 µg/mL

3.2 Physicochemical Properties of Edible Film with the Addition of *Moringa oleifera* Extract

3.2.1 pH Stability of Edible Film

The pH of the edible film solutions enriched with MOE ranged between 5.5 and 5.7 respectively, indicating a slightly acidic environment (Table 2). This pH value is consistent with typical ranges for protein-based edible films, particularly those formulated with gelatine, which naturally exhibits mildly acidic pH due to its derivation from collagen hydrolysis [14]. The addition of MOE did not result in substantial deviations from this expected range, demonstrating that the incorporation of plant-derived compounds did not interfere with the intrinsic stability of the gelatine–whey protein solution. The maintenance of stable pH is critical for protein solubility, film-forming ability, and microbial suppression in the film matrix [14].

Table 2. Characteristics of *Moringa oleifera* Extract.

Treatment	pH	Total Bacterial Count (cfu/g)
T0	5.73 ± 0.11	7.95 ± 0.34
T1	5.60 ± 0.04	7.90 ± 0.34
T2	5.53 ± 0.04	7.88 ± 0.18
T3	5.52 ± 0.03	7.84 ± 0.29

The slightly acidic pH may also contribute to antimicrobial performance because phenolic acids present in MOE exert stronger antimicrobial activity under acidic conditions, where they more readily penetrate microbial cell membranes due to increased non-ionized molecular forms [13]. Previous studies on edible films enriched with plant extracts have similarly reported that slight reductions in pH can enhance bacteriostatic effects without compromising film structure [14]. These findings suggest that the pH characteristics observed in this study are advantageous for both functional and structural stability of the formulation

3.2.2 Total Bacterial Count of Edible Film

Total bacterial count (TBC) in the edible film solutions ranged between 7.8 and 7.9 cfu/g, indicating that microbial contamination was minimal and within acceptable limits for edible film preparation. Importantly, the presence of MOE appeared to limit microbial growth in the film solution, which aligns with previously documented antimicrobial properties of Moringa compounds [11]. The ability of MOE to suppress bacterial proliferation at the solution stage may be attributed to the high concentration of flavonoids and phenolic acids known to disrupt bacterial cell wall integrity, inhibit enzymatic activity, or interfere with metabolic pathways.

Prior studies have shown that incorporation of plant extracts into protein-based film matrices often leads to reduced microbial loads during preparation due to the inherent antimicrobial nature of the bioactive compounds [8]. This pattern was similarly observed in the present study, suggesting that MOE effectively contributed to maintaining microbial safety in the edible film solution. Furthermore, maintaining low microbial counts during the initial preparation phase is essential for ensuring the hygienic quality of edible coatings intended for dairy applications, where microbial safety is a critical consideration [1,2].

3.3 Antibacterial Activity of Moringa-Enriched Edible Films

The edible films formulated with MOE demonstrated enhanced antimicrobial properties, specifically against *Escherichia coli* and *Salmonella spp*., two major foodborne pathogens associated with cheese spoilage and safety risks (Table 3) [1,2,14]. The inhibition zones observed in antimicrobial assays indicate that the addition of MOE significantly improved the antimicrobial efficacy of the gelatine–whey protein films compared to the control formulation without MOE. This is consistent with numerous studies reporting strong antimicrobial potential of Moringa leaf extracts against gram-negative bacteria.

The antimicrobial mechanisms of MOE have been widely documented and primarily involve phenolic and flavonoid compounds. These compounds can disrupt cell membranes, increase membrane permeability, cause leakage of cellular contents, inhibit microbial enzymes, and interfere with DNA replication [14–15]. The presence of nano-sized active particles may further enhance the effectiveness of antimicrobial action by enabling deeper penetration into bacterial cells or more efficient interaction with cell surfaces [15]. These findings corroborate the significant inhibition of E. coli and Salmonella spp. observed in this study.

A comparison with earlier research on antimicrobial gelatine-based films demonstrates similar outcomes. For example, plant extract–infused gelatine films containing green tea, rosemary, or aloe vera have also shown improved antimicrobial activity [15]. However, the magnitude of inhibition observed with MOE in this study reinforces the potency of Moringa as a functional bioactive additive. The results indicate that MOE is capable of conferring substantial antimicrobial protection when incorporated into protein-based edible films, making it well suited for cheese preservation applications.

4 Conclusion

This study demonstrated that *Moringa oleifera* extract can be successfully incorporated into gelatin whey protein edible films. The extract was shown to contain high concentrations of phenolic acids, flavonoids, with nano-scale particle size that facilitated uniform integration within the protein matrix. These phytochemical characteristics contributed to strong antioxidant capacity and supported significant free-radical scavenging activity. The addition of *Moringa oleifera* extract also resulted in improved antimicrobial activity against

Escherichia coli and *Salmonella* spp., two common foodborne pathogens associated with cheese spoilage. The edible films enriched with *Moringa* maintained stable physicochemical properties, including pH values ranging from 5.5 to 5.7 and acceptable total bacterial count during preparation, indicating that the extract did not negatively affect film stability. Collectively, these findings confirm that *Moringa oleifera* extract can serve as a potent natural additive capable of enhancing the protective functionality of edible films by simultaneously improving antioxidant and antimicrobial properties. The implications of this work highlight the potential of *Moringa*-based edible films as sustainable, bioactive alternatives to conventional synthetic packaging, particularly for dairy applications where microbial safety and oxidative stability are critical. The integration of plant-derived bioactive compounds into protein-based films aligns with global efforts toward environmentally responsible food packaging.

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References

1. Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2017). Fundamentals of Cheese Science. Springer.
2. McSweeney, P. L. H. (2019). Biochemistry of cheese ripening. International Journal of Dairy Technology, 72(3), 303–318.
3. Ncube, L. K. (2020). Environmental impact of food packaging materials: A Review of contemporary development from conventional plastics to polylactic acid based materials. Materials (Basel, Switzerland), 13(21), 4994. https://doi.org/10.3390/ma13214994.
4. Moghadas, H. C., Smith, J. S., & Tahergorabi, R. (2025). Recent advances in the application of edible coatings for shelf-life extension of strawberries: A Review. Food Bioprocess Technol. 18, 1079–1103. https://doi.org/10.1007/s11947-024-03517-7.
5. Kandasamy, S. (2021). Application of whey protein-based edible films and coatings in food industries: AnUpdated Overview. Coatings. 11, 1056. https://doi.org/10.3390/coatings11091056.
6. Chawla, Rekha, Sivakumar, S., & Kaur, Harsimran. (2021). Antimicrobial edible films in food packaging: Current scenario and recent nanotechnological advancements — a review. Carbohydrate Polymer Technologies and Applications, 2, 100024. https://doi.org/10.1016/j.carpta.2020.100024
7. Pérez-Santaescolástica, Cristina, Munekata, Paulo E. S., Feng, Xi, Liu, Ying, Bastianello Campagnol, Paulo Cezar, & Lorenzo, Jose M. (2022). Active edible coatings and films with Mediterranean herbs to improve food shelf-life. Critical Reviews in Food Science and Nutrition, 62(9), 2391–2403. https://doi.org/10.1080/10408398.2020.1853036
8. El-Sherbiny, G. M., Alluqmani, A. J., Elsehemy, I. A., & Kalaba, M. H. (2024). Antibacterial, antioxidant, cytotoxicity, and phytochemical screening of Moringa oleifera leaves. Scientific Reports, 14, 30485. https://doi.org/10.1038/s41598-024-80700-y.
9. Gomes, Olívia J. S., Leitão, Anabela, Sousa, Hermínio C. de, Gando-Ferreira, Licínio M., & Braga, M. E. M. (2025). Bioactive compounds extraction from Moringa oleifera leaves: A comparative study of vacuum-assisted and bed-stirred extractions. Journal of Food Science, 90(11), e70700. https://doi.org/10.1111/1750-3841.70700
10. Andriani, R. D., Rahayu, P. P., & Apriliyani, M. W. (2019). Antihyperglycemic activities of fermented milk enriched with gembili (Dioscorea esculenta). In IOP

Conference Series: Earth and Environmental Science 411, 012047. https://doi.org/10.1088/1755-1315/411/1/012047

11. Erceg, Tamara, Šovljanski, Olja, Tomić, Ana, Aćimović, Milica, Stupar, Alena, & Baloš, Sebastian. (2024). Comparison of the properties of pullulan-based active edible coatings implemented for improving sliced cheese shelf life. Polymers, 16(2), 178. https://doi.org/10.3390/polym16020178
12. Bodbodak, S. & Rafiee, Z. (2016). Recent trends in active packaging in fruits and vegetables in *eco-friendly technology for postharvest produce quality*. Elsevier Ltd, 77-125. https://doi.org/10.1016/B978-0-12-804313-4.00003-7.
13. Segwatibe, Mmabatho Kgongoane, Cosa, Sekelwa, & Bassey, Kokoette. (2023). Antioxidant and antimicrobial evaluations of Moringa oleifera Lam leaves extract and isolated compounds. Molecules, 28(2), 899. https://doi.org/10.3390/molecules28020899
14. Sultan, Maha, Ibrahim, Hassan, El-Masry, Hossam Mohammed, & Hassan, Youssef R. (2024). Antimicrobial gelatin-based films with cinnamaldehyde and ZnO nanoparticles for sustainable food packaging. Scientific Reports, 14, 22499. https://doi.org/10.1038/s41598-024-72009-7
15. Hamann, Daniele, Wlodarkievicz, Maria Eduarda, Puton, Bruna Maria Saorin, Fischer, Bruno, Colet, Rosicler, Paroul, Natalia, Valduga, Eunice, & Zeni, Jamile. (2024). Evaluation of biodegradable films with green tea extract for interleafing sliced meat products. Food Chemistry, 458, 140159. https://doi.org/10.1016/j.foodchem.2024.140159