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Edible Biodegradable Films Incorporating Essential Oil-based Pickering Emulsions: A Review of Antioxidant and Antimicrobial Properties

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Abstract

This article reviews the antioxidant and antimicrobial properties of biodegradable edible films based on Pickering emulsions containing essential oils. Edible biodegradable films incorporating essential oil-loaded Pickering emulsions are increasingly recognized as a promising option for sustainable food packaging. By incorporating essential oils into the emulsion matrix, the antioxidant and antimicrobial properties of these films significantly improved. Therefore, the key properties discussed in this review include antioxidant activity, antimicrobial effectiveness, and the role of these films in extending the shelf life of food products. The results showed that the incorporation of Pickering emulsions containing essential oils significantly increased the antioxidant capacity of the films, leading to a notable reduction in oxidative degradation of food. Additionally, these films exhibited effective antimicrobial activity against various foodborne pathogens such as *Escherichia coli and Staphylococcus aureus*, which is attributed to the bioactive properties of the incorporated essential oils. The films effectively inhibited microbial growth, directly contributing to enhanced food safety. The findings highlight the great potential of Pickering emulsion-based biodegradable films as a sustainable solution for food packaging with antioxidant and antimicrobial properties, ensuring longer shelf life and higher safety of packaged food products.

Keywords: Antioxidant activity, Antimicrobial, Essential oils, Edible films, Pickering emulsions

Introduction

In recent years, growing concerns over the environmental impact of synthetic plastic packaging have led researchers to explore sustainable alternatives (Bangar, Whiteside, Dunno, Cavender, & Dawson, 2023). Among these, edible biodegradable films have emerged as promising candidates for food packaging applications. These films are typically made from natural biopolymers such as proteins, polysaccharides, and lipids, offering the dual benefits of environmental friendliness and direct edibility without the need for removal before consumption (Majdzadeh, Rajaei, Mirzaee Moghaddam, & Movahednejad, 2018). However, the inherent limitations of pure edible films—such as low mechanical strength, poor barrier properties, and limited bioactivity—have encouraged the incorporation of functional agents to enhance their performance(Muñoz-Tebar, Pérez-Álvarez, Fernández-López, & Viuda-Martos, 2023).

To address these challenges, the concept of active packaging has been introduced. Active packaging not only provides a physical barrier

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but also interacts with the food or its environment to extend shelf life and improve safety. One widely studied approach involves the integration of bioactive compounds, particularly essential oils, known for their potent antioxidant and antimicrobial properties. These natural substances can inhibit the growth of foodborne pathogens and delay lipid oxidation, thereby maintaining food quality (Friedman, Henika, & Mandrell, 2002). However, the direct incorporation of essential oils into film matrices presents several challenges, such as high volatility, light and heat sensitivity, and strong odor, which can adversely affect both stability and consumer acceptance. These issues have driven the search for effective delivery systems that can improve the controlled release and protect the functional properties of essential oils (Shahidi & Hossain, 2022).

One innovative strategy for stabilizing essential oils is their encapsulation within emulsion systems. Among the various techniques, Pickering emulsions have garnered significant attention as a surfactant-free and biocompatible alternative to conventional emulsions (Roy & Rhim, 2021b; Visan, Popescu-Pelin, & Socol, 2021; Wardana, Wigati, Van, Tanaka, & Tanaka, 2023). These emulsions are stabilized by solid particles that irreversibly adsorb at the oil–water interface, preventing coalescence of the dispersed droplets. Solid particles such as cellulose nanocrystals, protein nanoparticles, and biopolymer-based particles have been used to create stable Pickering emulsions (Priyadarshi & Rhim, 2020; Sharkawy, Barreiro, & Rodrigues, 2020; Sun *et al*., 2020; Tavakoli-Rouzbehani *et al*., 2021). These systems offer enhanced physical stability, protection against environmental stressors, and improved retention and the controlled release of encapsulated essential oils, which are crucial for preserving their functional efficacy. (Fan *et al*., 2023).

Incorporating essential oil-loaded Pickering emulsions into edible biodegradable films offers a novel approach to developing high-performance active packaging systems (Pandita *et al*., 2024). These hybrid structures enable the controlled release of essential oils while simultaneously enhancing the mechanical and structural integrity of the film matrix. The presence of stabilizing particles in the emulsions not only improves the dispersion of the oil phase but also facilitates better interactions with the film matrix, leading to enhanced functional and physical properties (Zhang *et al*., 2024). Recent research has explored various parameters affecting these systems, including the type of stabilizing particles, the nature of the essential oil, and the oil-to-water ratio, demonstrating the significant potential of Pickering emulsions in food packaging innovations (Cheng *et al*., 2024).

In recent years, extensive research has explored the synergistic combination of essential oils and Pickering emulsions for incorporation into edible biodegradable films to enhance their antioxidant and antimicrobial properties. For instance, (Zhang *et al*., 2022) developed konjac-based films infused with oregano essential oil encapsulated in zein–pectin nanoparticles, which exhibited significant antioxidant activity. Similarly, (Roy & Rhim, 2021a) developed films using a carrageenan–agar biopolymer matrix reinforced with tea tree essential oil stabilized by nanocellulose fibers, yielding materials with notable antioxidant performance. In another study, (Zhao *et al*., 2023) incorporated clove essential oil into a film composed of potato starch and polyvinyl alcohol, observing strong antibacterial effects, particularly against *Escherichia coli* compared to *Staphylococcus aureus*. A particularly innovative approach by (Bu *et al*., 2022) involved the use of konjac glucomannan and pullulan as a film matrix, combined with tea tree essential oil delivered via cellulose nanofibril-stabilized Pickering emulsions, resulting in a hybrid system with potent antimicrobial activity against both *Escherichia coli* and *Staphylococcus aureus*. Collectively, different studies highlight the efficacy of Pickering emulsions in stabilizing volatile essential oils, facilitating their controlled release, and maintaining their bioactive properties within biopolymer-based films.

This review critically examines the role of Pickering emulsion-based delivery systems in enhancing the functional properties of essential oil-loaded edible films, with a focus on their antioxidant and antimicrobial efficacy. The mechanisms of action of essential oils, the benefits of Pickering emulsions as delivery systems, and their influence on the bioactive performance of edible films were also explored in this review. Furthermore, this review identifies key challenges—such as scalability, sensory compatibility, and long-term stability—while proposing future research directions to advance the development of next-generation active packaging. By integrating fundamental principles with cutting-edge applications, this work aims to bridge critical knowledge gaps and inspire innovative solutions for sustainable, high-performance food preservation systems.

Pickering Emulsions: Fundamentals and Applications

Definition and Mechanism

Pickering emulsions are a type of emulsion stabilized by solid particles, rather than traditional surfactants. These emulsions consist of two immiscible liquids, such as oil and water, with solid particles adsorbed at the interface between the two phases. The solid particles act to stabilize the emulsion by preventing the coalescence of oil droplets, forming a rigid structure at the oil-water interface. In contrast, conventional emulsions, rely on surfactants to reduce the interfacial tension between oil and water, preventing droplet aggregation. In Pickering emulsions, the particles adsorb onto the droplet surface, creating a physical barrier that decreases interfacial tension and provides steric and electrostatic repulsion (Mirzaee Moghaddam, 2019). This prevents the droplets from merging. The solid particles form a more stable and robust emulsion compared to surfactant-based systems, as they are less prone to desorption or leaching under challenging conditions such as temperature fluctuations or pH changes (Yang *et al*., 2017). The stabilization of oil droplets in Pickering emulsions is influenced by factors such as the size, shape, and surface characteristics of the solid particles (Hosseini, Rajaei, Tabatabaei, Mohsenifar, & Jahanbin, 2020). These particles need to be small enough to effectively stabilize the droplets, but also large enough to prevent excessive diffusion or aggregation. The wettability and surface charge of the particles also play a significant role in determining the overall stability and structure of the emulsion. For example, hydrophobic particles typically stabilize oil-in-water emulsions, while hydrophilic particles are more suited for water-in-oil emulsions (Zhao *et al*., 2022). These factors make Pickering emulsions highly effective for encapsulating and stabilizing sensitive compounds, including essential oils.

Benefits in Food Systems

In food systems, the use of Pickering emulsions provides several significant advantages, especially when it comes to encapsulating bioactive compounds such as essential oils. One of the key benefits is the enhanced stability of volatile and sensitive compounds. Essential oils, are volatile and hydrophobic, prone to degradation through factors like heat, light, and oxygen exposure. By encapsulating these oils within Pickering emulsions, their stability is improved significantly. The solid particles at the oil-water interface form a protective barrier, preventing the evaporation or degradation of the essential oils, thereby preserving their activity for longer periods (De Farias *et al*., 2025). In addition to stability, Pickering emulsions offer controlled release of active compounds, which is particularly advantageous in food applications (Nazari, Rajaei, & Moghaddam, 2025). The structure of these emulsions allows for the gradual release of bioactive compounds, such as antioxidants or antimicrobial agents, over time. This controlled release helps extend the shelf life of food products by continuously delivering active compounds that inhibit microbial growth and prevent oxidative rancidity. For instance, essential oils encapsulated within Pickering emulsions can be slowly released, maintaining their antimicrobial activity over an extended period, which is beneficial for active food packaging applications (Monjazeb Marvdashti, Yavarmanesh, & Koocheki, 2016). This approach enables a long-lasting protective effect, making it an ideal solution for food products requiring enhanced preservation. Furthermore, Pickering emulsions can improve the texture and sensory attributes of food products. The formation of stable emulsions with uniform droplet size distribution ensures smooth and consistent textures, which are crucial in products such as sauces, dressings, and beverages, where uniformity is highly valued by consumers. The use of natural stabilizers in Pickering emulsions also supports the growing demand for clean-label products, as consumers increasingly seek food products with minimal synthetic additives (Cheng *et al*., 2024).

Types of Stabilizers

The success of Pickering emulsions largely depends on the choice of stabilizers, which can be categorized into natural biopolymers and inorganic nanoparticles. Both types of stabilizers offer unique advantages and can be selected based on the specific requirements of the food system in question. Natural biopolymers, such as chitosan, cellulose, and starch, are frequently used as stabilizers in Pickering emulsions because they are abundant, biodegradable, and compatible with food systems. Chitosan, derived from the shells of crustaceans, is widely utilized in food applications due to its biocompatibility, biodegradability, and antimicrobial properties. The molecular structure of chitosan facilitates effective adsorption at the oil-water interface, stabilizing emulsions and enhancing their overall stability. Additionally, chitosan can provide functional benefits, such as improving the antioxidant properties of the emulsion, which further contributes to food preservation (Hamed, Özogul, & Regenstein, 2016). Cellulose, a naturally abundant polymer, is another effective stabilizer for Pickering emulsions. Cellulose-based materials, such as cellulose nanocrystals and cellulose nanofibers, improve the stability and rheological properties of emulsions. These materials stabilize the emulsion by forming strong, durable interfacial layers that prevent droplet coalescence and enhance the mechanical strength of the emulsion. The use of cellulose is particularly beneficial in food systems where texture and stability are key considerations (Liu *et al*., 2023). Starch, a versatile biopolymer, is often used in Pickering emulsions for its cost-effectiveness and ease of production. Modified starches and starch nanoparticles have demonstrated excellent emulsifying properties, providing stable emulsions with controlled release characteristics. Starch-based Pickering emulsions can improve both the stability and texture of food products, making them ideal for a wide range of applications (Ramos, Ramírez-López, Pinho, Ditchfield, & Moraes, 2025).

Inorganic nanoparticles are also commonly used as stabilizers in Pickering emulsions due to their excellent mechanical properties, chemical stability, and biocompatibility. Silica nanoparticles are particularly effective in stabilizing oil-in-water emulsions. They have a high surface area and are hydrophilic, which makes them suitable for applications that require high stability, such as encapsulating volatile compounds like essential oils. Silica's ability to form strong interfacial layers enhances the emulsion's stability under varying environmental conditions, such as changes in temperature and pH (Jiang, Sheng, & Ngai, 2020). While titanium dioxide (TiO_2) nanoparticles have been studied for their dual role in stabilizing Pickering emulsions and providing antimicrobial properties, their use in food applications has faced regulatory restrictions in several regions, including a ban in Iran due to safety concerns. As a result, researchers are increasingly exploring alternative inorganic or organic stabilizers—such as starch-based nanoparticles, cellulose nanocrystals, or clay minerals—that offer similar functional benefits without regulatory limitations (Das, Kumar, Singh, & Kayastha, 2024; Omidian, Akhzarmehr, & Chowdhury, 2024). The choice of stabilizer depends on the specific requirements of the food system, including the type of active ingredients to be encapsulated, the desired release profile, and the sensory attributes of the final product. By optimizing Pickering emulsions with approved stabilizers, food scientists can enhance functionality, ensuring improved stability, controlled release, and extended shelf life. These advancements provide a promising pathway for developing safer, more compliant food preservation technologies while maintaining product quality (Nahalkar, Rajaei, & Mirzaee Moghaddam, 2025).

Preparation and Characterization of Essential Oil-Loaded Pickering Emulsions

Formulation Techniques

The formulation of essential oil-loaded Pickering emulsions involves the careful selection of both the essential oils to be encapsulated and the stabilizing agents, which play a pivotal role in ensuring the stability and functionality of the emulsions. Essential oils, due to their lipophilic nature and volatility, require robust encapsulation strategies to maintain their stability and ensure their controlled release. Commonly used essential oils for encapsulation include thyme, oregano, rosemary, and clove oils, which are selected based on their bioactive properties, such as antioxidant, antimicrobial, and antifungal activities. The choice of essential oil is influenced by factors such as the intended application (e.g., food preservation, active packaging), the specific bioactive compound profile, and the compatibility of the oil with the other components of the emulsion (Mirzaee Moghaddam & Rajaei, 2021; Oun, Shin, & Kim, 2022; Priyadarshi & Rhim, 2020; Roy & Rhim, 2021a).

To achieve effective emulsion stabilization, stabilizing particles must exhibit suitable surface characteristics, such as hydrophilicity or hydrophobicity, to ensure their strong adsorption at the oil-water interface. This adsorption forms a protective barrier around dispersed droplets, preventing their coalescence and enhancing the long-term stability of the emulsion. However, recent studies have revealed that particle geometry also plays a crucial role in determining the efficiency of emulsion stabilization. As a result, increasing attention has been given to the design and utilization of non-spherical particles—including rods, fibers, ellipsoids, and cubes—as effective Pickering emulsion stabilizers (Wu & Ma, 2016).

In this context (Madivala, Fransaer, & Vermant, 2009), investigated the effect of particle shape on emulsion stability by mechanically stretching spherical polystyrene particles to create ellipsoidal shapes. These anisotropic particles demonstrated markedly improved stabilizing performance. At higher concentrations, the ellipsoidal particles assembled end-to-end at the oil-water interface, forming triangular mesh-like structures that acted as a physical scaffold, impeding droplet movement and coalescence. At lower concentrations, the particles arranged into striped patterns, still reinforcing interfacial stability. The study found a direct correlation between higher aspect ratios and enhanced emulsion stability, highlighting the influence of geometry on interfacial behavior. In another study (Kalashnikova, Bizot, Cathala, & Capron, 2011), synthesized different nanorods and employed them in the preparation of Pickering emulsions. These nanorods exhibited a strong tendency to interconnect at the interface, forming bridge-like structures that contributed to the formation of super-stable emulsions. Such arrangements substantially increased the energy barrier for droplet coalescence, providing robust resistance against phase separation and other destabilizing factors.

Emulsification methods are the next crucial step in the preparation of essential oil-loaded Pickering emulsions. Two common methods used are ultrasonication and high-pressure homogenization. Ultrasonication utilizes high-frequency sound waves to generate intense shear forces, which break up the oil phase into small droplets and create a fine emulsion. This method is particularly effective for producing emulsions with a narrow droplet size distribution and is widely used for encapsulating essential oils. High-pressure homogenization, on the other hand, involves forcing the oil-water mixture through a small orifice under high pressure, which results in the formation of fine droplets. This method is also efficient for stabilizing emulsions and can be applied at larger scales. Both methods can be optimized to achieve the desired droplet size and emulsion stability, depending on the characteristics of the essential oil and stabilizer used (Barradas & de Holanda e Silva, 2021).

Encapsulation Efficiency

Encapsulation efficiency is an important parameter for evaluating the effectiveness of Pickering emulsions in preserving essential oils and delivering them in a controlled manner. Encapsulation efficiency refers to the proportion of the essential oil that is successfully incorporated into the emulsion, compared to the total amount of oil added during the preparation process. It is typically expressed as a percentage and is influenced by factors such as the type of stabilizer used, emulsification method, and the properties of the essential oil (Cahyana *et al*., 2022). To measure encapsulation efficiency, the amount of free or unencapsulated oil is determined by techniques such as centrifugation, filtration, or solvent extraction (Rajaei, Barzegar, Mobarez, Sahari, & Esfahani, 2010). The amount of oil retained within the emulsion can then be quantified, and the efficiency can be calculated based on the ratio of encapsulated oil to the total amount of oil used in the formulation. High encapsulation efficiency is desirable because it indicates that the majority of the essential oil is effectively incorporated into the emulsion, which maximizes its functional benefits in food packaging applications (Nahalkar, Rajaei, & Mirzaee Moghaddam, (in press)). The controlled release profile of essential oils from Pickering emulsions is another critical aspect of their performance. The release of encapsulated essential oils is influenced by the properties of the stabilizing agents, the droplet size, and the emulsification method. Typically, the release rate can be controlled by adjusting the particle size of the emulsion and the thickness of the interfacial layer formed by the stabilizing agents (Lammari, Louaer, Meniai, & Elaissari, 2020).

The controlled release of essential oils is essential for applications such as active food packaging, where the goal is to provide a gradual and sustained release of bioactive compounds to enhance food preservation without overwhelming the sensory properties of the food (Karimi, Bodaghi, Rajaei, & Mojerlou, 2020). The release behavior can be studied using in vitro methods, where the emulsion is exposed to conditions that simulate real-world environments, such as acidic or alkaline conditions, temperature variations, and exposure to light or oxygen (Visan *et al*., 2021). By monitoring the concentration of released essential oil over time, a release profile can be constructed, showing how the essential oil is gradually released from the emulsion matrix. A slow and sustained release is typically ideal for maximizing the shelf life and effectiveness of essential oils in food applications.

Antioxidant and Antimicrobial Evaluation of Edible Films

In Vitro Antioxidant Activity

The evaluation of antioxidant activity is crucial for assessing the potential of edible films containing bioactive compounds, such as essential oils, to prevent oxidative spoilage and enhance food preservation. Antioxidant assays typically measure the ability of a film to scavenge free radicals or reduce oxidative damage to food components. Several in vitro methods are commonly employed to evaluate the antioxidant properties of edible films, including the DPPH and ABTS radical scavenging assays and the Ferric Reducing Antioxidant Power (FRAP) test (Benbettaïeb, Debeaufort, & Karbowiak, 2019). The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is one of the most widely used methods to assess the free radical-scavenging ability of antioxidants. In this assay, the DPPH radical reacts with an antioxidant present in the edible film, resulting in a color change from purple to yellow (Rajaei *et al*., 2021). The extent of this color change is directly proportional to the antioxidant activity, with a higher reduction in the DPPH radical indicating stronger antioxidant properties. The scavenging ability is quantified by measuring the absorbance at 517 nm and calculating the percentage inhibition of the DPPH radical. This assay provides valuable information about the film's potential to prevent oxidative damage to food during storage (Gulcin & Alwasel, 2023).

Table 1- Some recent studies on the effect of essential oil-loaded Pickering emulsions on the antioxidant properties of edible films

Edible film	Essential oil	Pickering particle	Application	Food Product Analyzed	References
Konjac glucomannan	Oregano	Zein–pectin nanoparticle	DPPH free radical scavenging	–	(Zhang *et al*., 2022)
Carrageenan/agar	tea tree	Nanocellulose fibers	Distinct antioxidant	–	(Roy & Rhim, 2021a)
Gelatin	Cinnamon	Lignocellulose nanocrystals-tannic acid	Improve antioxidant properties	–	(Dai *et al*., 2023)
Chayote tuber starch	Cinnamon	Zein-pectin nanoparticle	Increased antioxidant activity	Ground beef	(Wu *et al*., 2023)
Chitosan	Lemon Myrtle	Alkali lignin	Improve antioxidant properties	–	(Liu, Swift, Tollemache, Perera, & Kilmartin, 2022)
Anthocyanidin/chitosan	Cinnamon-perilla	Collagen	Increase antioxidant activity	Chilled fish fillet	(Zhao, Guan, Zhou, Lao, & Cai, 2022)
Gelatin/agar	Clove	Copper-modified zinc oxide nanoparticles	Improve antioxidant properties	Pork meat	(Roy, Priyadarshi, & Rhim, 2022)
Carboxymethyl cellulose /polyvinyl alcohol	Ginger	Ginger essential oil	High antioxidant activity	Bread	(Fasihi, Noshirvani, & Hashemi, 2023)
Konjac glucomannan	Corn germ oil-oregano essential oil	Zein-pectin nanoparticle	Antioxidant activity	–	(Du *et al*., 2023)
Konjac glucomannan	Thyme	Bacterial cellulose nanofibers/soy protein isolate	Highest total phenol content and antioxidant capacities, as well as the best TEO-release property	–	(Liu, Lin, Li, & Yang, 2022)
Konjac glucomannan	Oregano	Chitin nanocrystal	Improve antioxidant properties	–	(Xu *et al*., 2023)
Chitosan	Grapefruit	Amphiphilic octenyl succinic anhydride konjac glucomannan	High efficiency of DPPH free radical scavenging	–	(Bu *et al*., 2022)
Hydroxypropyl methyl cellulose	Cinnamon	Zein/carboxymethyl tamarind gum	Improve antioxidant activity	Cherry tomatoes	(Yao *et al*., 2023)

The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay is another commonly used test for evaluating antioxidant capacity

(Rajaei, Hadian, Mohsenifar, Rahmani-Cherati, & Tabatabaei, 2017). ABTS is a stable radical that, when mixed with an antioxidant, undergoes reduction, leading to a decrease in absorbance at 734 nm. The degree of inhibition of ABTS radical cation formation is used to quantify the antioxidant activity. Like the DPPH assay, the ABTS assay provides a measure of the antioxidant potential of edible films, reflecting their ability to neutralize reactive oxygen species (ROS) and prevent oxidative damage (Ilyasov, Beloborodov, Selivanova, & Terekhov, 2020).

Together, these in vitro antioxidant assays provide a comprehensive evaluation of the antioxidant capacity of edible films and can be used to compare different formulations, such as films containing essential oils or other bioactive agents. Table 1 shows some recent studies on the effect of Pickering emulsions containing essential oils on the antioxidant properties of edible films. Films with strong antioxidant properties are particularly valuable for extending the shelf life of perishable foods by reducing lipid oxidation, preserving flavor, and maintaining nutritional quality. The incorporation of Pickering emulsions containing essential oils into edible films can significantly enhance their antioxidant properties. For example in a study, a bio-based film was formulated using konjac glucomannan, thyme essential oil, and a composite of bacterial cellulose nanofibers and soy protein isolate. The resulting material exhibited the highest total phenol content and antioxidant capacity among the tested formulations. Additionally, it demonstrated the most efficient release of thyme essential oil, making it a promising candidate for active packaging applications aimed at enhancing food preservation and extending shelf life (Liu *et al*., 2022). In another study, a bioactive film composed of chitosan, lemon myrtle essential oil, and alkali lignin was developed to enhance the antioxidant properties of the material. The incorporation of lemon myrtle and alkali lignin significantly improved the film's ability to scavenge free radicals, indicating strong antioxidant activity (Liu *et al*., 2022).

Essential oils are rich in phenolic and bioactive compounds, but their high volatility and instability under environmental conditions limit their effectiveness. Pickering emulsions, stabilized by solid nanoparticles or microparticles, help reduce oxidation, control the gradual release of antioxidant compounds, and prolong their bioactivity within the edible film. This leads to increased resistance of the film against lipid oxidation and damage caused by free radicals, ultimately improving the quality and safety of packaged food products (Mirzaee Moghaddam & Rajaei, 2021; Roy & Rhim, 2021a; Roy & Rhim, 2021b; Xu *et al*., 2023).

Antimicrobial Assays

In addition to antioxidant activity, antimicrobial evaluation is an essential step in determining the efficacy of edible films for active food packaging applications. The antimicrobial properties of edible films help protect food from spoilage and contamination by inhibiting the growth of harmful microorganisms. Various in vitro methods are used to assess the antimicrobial activity of edible films, including disc diffusion, well-diffusion methods, and the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (Benbettaïeb *et al*., 2019). The disc diffusion method is a commonly used technique for assessing the antimicrobial activity of edible films against a wide range of microorganisms. In this assay, the edible film is applied to a sterile disc, which is then placed on an agar plate inoculated with the target microorganism. The film gradually releases its antimicrobial agents, creating a zone of inhibition around the disc where bacterial growth is prevented. The size of the zone of inhibition is measured, and the larger the zone, the stronger the antimicrobial effect of the edible film. This method provides a qualitative measure of antimicrobial efficacy and is useful for screening the antimicrobial potential of films containing essential oils or other bioactive compounds (Benbettaïeb *et al*., 2019).

The well-diffusion method is another technique used to evaluate antimicrobial activity, similar to the disc diffusion method but with a slight variation. In this method, wells are punched into an agar plate, and the edible film or its extract is placed into the well. The antimicrobial agents diffuse radially outward from the well, creating a zone of inhibition. This method is often employed when higher concentrations of antimicrobial agents need to be tested, and it allows for the determination of the minimum concentration of the active compound required to inhibit microbial growth (Balouiri, Sadiki, & Ibnsouda, 2016). To further quantify the antimicrobial effectiveness of edible films, the MIC and MBC are determined. The MIC is the lowest concentration of an antimicrobial agent that prevents visible growth of the microorganism in the presence of the edible film or its extract. The MIC can be determined by preparing serial dilutions of the film or its active components and inoculating them with the target microorganism. The results are interpreted by observing the absence of microbial growth, which indicates the MIC. The MBC is the lowest concentration of the antimicrobial agent that results in the complete eradication of the microorganism, measured by the absence of growth on a subculture plate. These tests provide quantitative data on the effectiveness of edible films in inhibiting or killing microorganisms at different concentrations (Rao, Chen, & McClements, 2019).

In terms of antimicrobial activity, edible films can be tested against both gram-positive and gram-negative bacteria, as well as fungi and viruses. Gram-positive bacteria like *Staphylococcus aureus* and *Listeria monocytogenes* are often used in studies of antimicrobial edible films due to their association with foodborne illnesses. Gram-negative bacteria, such as *Escherichia coli* and *Salmonella spp.*, are also critical targets in antimicrobial food packaging, as they are responsible for a significant proportion of foodborne infections. The differences in cell wall structure between gram-positive and gram-negative bacteria may influence the effectiveness of antimicrobial agents, with gram-negative bacteria often being more resistant to certain compounds due to the presence of an outer membrane that can act as a barrier (Valencia-Chamorro, Palou, Del Río, & Pérez-Gago, 2011).

Antimicrobial tests are essential for determining the suitability of essential oil-loaded Pickering emulsions in edible films. Films containing essential oils such as thyme, oregano, or clove oil are known for their potent antimicrobial properties and have shown significant efficacy against common foodborne pathogens. For example in a recent study, a combination of polyvinyl alcohol, oregano essential oil, cinnamon essential oil, and cellulose nanocrystals (CNCs) was used to enhance the antibacterial properties. The results demonstrated significant antimicrobial activity, with *E. coli* showing greater sensitivity to cinnamon essential oil, while *Staphylococcus aureus* was more sensitive to oregano essential oil. These findings highlight the strong potential of this formulation for use in bio-based packaging materials with effective antibacterial properties (Oun *et al*., 2022). In another study, a composite film based on starch, ginger extract, and TEMPO-oxidized cellulose nanocrystals was developed to enhance antibacterial activity, particularly for food packaging applications. The formulation showed improved antimicrobial effectiveness, making it suitable for preserving perishable items such as tomatoes (Chen *et al*., 2023). Table 2 shows some recent studies on the effect of Pickering emulsions containing essential oils on the antimicrobial properties of edible films. The results from these antimicrobial assays guide the optimization of edible films for food packaging applications, ensuring that they offer both preservation and safety benefits by reducing microbial contamination and extending the shelf life of food products.

Table 2- Some recent studies on the effect of essential oil-loaded Pickering emulsions on the antimicrobial properties of edible films

Edible film	Essential oil	Pickering particle	Application	Food Product Analyzed	References
Chitosan / gelatin	Cinnamon	Zein nanoparticles	High antimicrobial against *Pseudomonad paralactis* MN10 and *Lactobacillus sakei* VMR17	–	(Fan *et al.*, 2023)
Hydroxypropyl methyl cellulose	Cinnamon	Zein/carboxymethyl tamarind gum	Improved antibacterial activity	Cherry tomatoes	(Yao *et al.*, 2023)
Chitosan	Cinnamon	Cellulose nanocrystal	Improve antibacterial properties	Pork meat	(Liu *et al.*, 2022)
Konjac glucomannan	Oregano	Zein–pectin nanoparticle	Antibacterial effect	–	(Zhang *et al.*, 2022)
Carrageenan/agar	Tea tree	Nanocellulose fibers	Antibacterial activity	–	(Roy & Rhim, 2021a)
Gelatin	Cinnamon	Lignocellulose nanocrystals-tannic acid	Improve antibacterial properties	–	(Dai *et al.*, 2023)
Chayote tuber starch	Cinnamon	Zein-pectin nanoparticle	Increased antimicrobial activity	Ground beef	(Wu *et al.*, 2023)
Chitosan	Lemon Myrtle	Alkali lignin	Improve antibacterial properties	–	(Liu *et al.*, 2022)
Potato starch and polyvinyl alcohol	Clove	Clove essential oil	Antibacterial property (showed more potent inhibition of *Escherichia coli* than *Staphylococcus aureus*).	Pork meat	(Zhao *et al.*, 2023)
Konjac glucomannan and Pullulan	Tea tree	Cellulose nanofibrils	Exhibited antimicrobial activity against *E. coli* and *Staphylococcus aureus*	–	(Bu *et al.*, 2022)
Gelatin/agar	Clove	Copper-modified zinc oxide nanoparticles	100% eradication of *L. monocytogenes* and 50% decrease in the E. coli	Pork meat	(Roy *et al.*, 2022)
Carboxymethyl cellulose /polyvinyl alcohol	Ginger	Ginger essential oil	High antimicrobial activity	Bread	(Fasihi *et al.*, 2023)
Konjac glucomannan	Corn germ oil-oregano essential oil	Zein-pectin nanoparticle	Antibacterial activity	–	(Du *et al.*, 2023)
Sodium alginate	Lemongrass	Cellulose nanofibers	Antifungal properties (*Penicillium digitatum* and *P. italicum)*	–	(Wardana *et al.*, 2023)
Chitosan	Clove	Zein and sodium caseinate	Improved antibacterial *(Escherichia coli and Staphylococcus aureus)*	–	(Hua *et al.*, 2021)
Konjac glucomannan	Oregano	Chitin nanocrystal	Improve antibacterial properties	–	(Xu *et al.*, 2023)
Polyvinyl alcohol	Oregano and cinnamon	Cellulose nanocrystals	Improved antibacterial (*E. coli* was more sensitive to CEO, while *S. aureus* was sensitive to OEO)	–	(Oun *et al.*, 2022)
Chitosan	Grapefruit	Amphiphilic octenyl succinic anhydride konjac glucomannan	Antibacterial activity	–	(Bu *et al.*, 2022)
Tapioca strach/polyvinyl alcohol	*Thymus vulgaris*	Cellulose nanocrystals	Prevent the growth of microorganisms	Fish fillets	(Guo *et al.*, 2024)
Starch	Ginger	tempo-oxidized cellulose nanocrystals	Improved antibacterial activity	tomato	(Chen *et al.*, 2023)

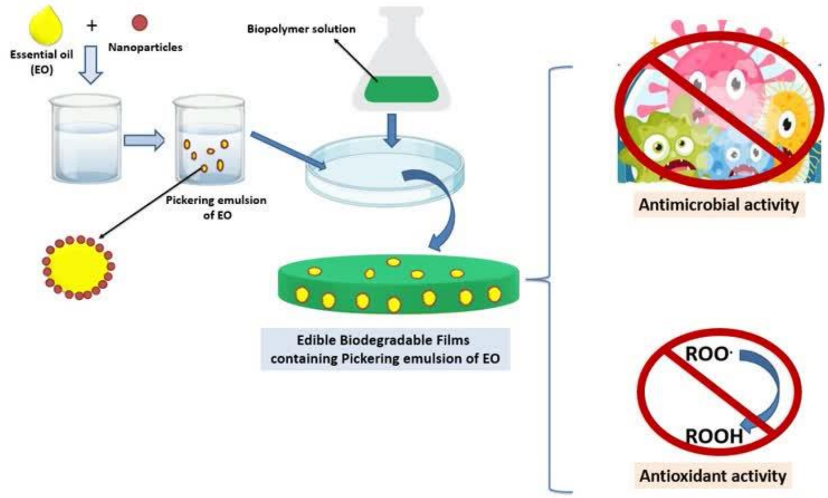

Fig. 1. Effect of Pickering emulsion containing essential oil on antioxidant and antimicrobial properties of edible film

The incorporation of Pickering emulsions containing essential oils into edible films has a significant impact on their antimicrobial properties. Essential oils contain active compounds with antimicrobial effects, but their high volatility and instability reduce their effectiveness in edible films. Pickering emulsions, by stabilizing essential oils through solid nanoparticles or microparticles, enable controlled release of antimicrobial compounds and enhance their stability. This feature helps inhibit the growth of pathogenic and spoilage microorganisms, increases food safety, and improves the shelf life of products packaged with these edible films. Fig. 1 shows the effect of Pickering emulsion containing essential oil on the antioxidant and antimicrobial properties of edible films.

Food Applications and Practical Implications

Potential Use in Food Packaging

The potential application of essential oil-loaded Pickering emulsions in food packaging represents a significant advancement in the development of active packaging systems. These systems are designed not only to provide physical protection but also to actively interact with the contents of the package to improve food preservation. Essential oils, encapsulated within Pickering emulsions, offer dual benefits in food packaging by providing antioxidant and antimicrobial properties. This dual functionality can be applied to a wide range of food products, such as meat, dairy, and fresh produce, to enhance their shelf life and ensure food safety. In the case of meat products, which are highly susceptible to spoilage due to microbial growth and oxidative rancidity, the use of edible films containing essential oils can significantly extend shelf life. Essential oils, such as oregano, thyme, or rosemary oil, have been shown to inhibit the growth of spoilage microorganisms, including Lactic acid bacteria and Enterobacteriaceae, which are commonly found in meat. Additionally, the antioxidant properties of essential oils can prevent lipid oxidation, thereby preserving the flavor and nutritional quality of the meat. By using these active films, the need for synthetic preservatives and additives can be reduced, aligning with consumer demand for cleaner, more natural products (Sánchez-Ortega *et al*., 2014). For example in a study, the use of chayote tuber starch, cinnamon, and zein-pectin nanoparticles has shown promising results in enhancing the functional properties of ground meat. Specifically, these ingredients have been found to increase antimicrobial activity against pathogens such as *Staphylococcus aureus* in ground beef. This combination of natural ingredients effectively reduced microbial growth, improved the safety and shelf life of the meat. Additionally, the incorporation of chayote tuber starch, cinnamon, and zein-pectin nanoparticles in pork meat showed a significant increase in antioxidant activity (Wu *et al.*, 2023).

Dairy products, such as cheese, milk, and yogurt, are also highly prone to microbial contamination and lipid oxidation. Essential oil-loaded Pickering emulsions offer a solution by providing antimicrobial protection against pathogens like *Listeria monocytogenes* and *Salmonella* spp., which are of particular concern in dairy processing and storage. Furthermore, the incorporation of antioxidant essential oils can help prevent the rancidity of fats in dairy products, thereby preserving their sensory properties, such as flavor and texture. The use of such packaging could also potentially extend the shelf life of dairy products, reducing food waste while maintaining product quality over time (El-Sayed, Ibrahim, & Farag, 2022).

For fresh produce, such as fruits and vegetables, the primary concerns include microbial contamination and moisture loss. Essential oil-infused edible films can prevent microbial growth, particularly mold and bacteria, which often cause spoilage in fresh produce. The antioxidant effects can also help to slow down the degradation of vitamins and other bioactive compounds in fruits and vegetables, preserving their nutritional value. Moreover, the use of such films can reduce the need for refrigeration, as they help maintain the desired humidity levels within the packaging, and further extending the freshness of the produce. By offering a biodegradable alternative to conventional plastic packaging, essential oil-loaded Pickering emulsions could also contribute to reducing the environmental impact of food packaging (Qadri, Yousuf, & Srivastava, 2015). In all these applications, the use of essential oil-based films provides an environmentally friendly solution, as they are biodegradable and derived from renewable sources, making them more sustainable than traditional petroleum-based plastics. The ability to encapsulate essential oils within Pickering emulsions further enhances the stability and controlled release of these active compounds, ensuring a prolonged protective effect throughout the shelf life of the food.

Consumer Acceptability and Regulatory Aspects

The adoption of essential oil-loaded Pickering emulsions in food packaging not only requires technical efficacy but also the acceptance of consumers and compliance with regulatory frameworks. The sensory attributes of the packaging, such as taste, aroma, and texture, play a critical role in consumer perception, especially when essential oils are involved. Sensory evaluation is, therefore, an essential part of determining the consumer acceptability of edible films used in food packaging. Sensory evaluation typically involves tests where consumers or trained panels assess the sensory characteristics of the packaged food, including any potential impact on flavor, odor, or appearance. Essential oils, while offering antimicrobial and antioxidant benefits, can impart strong aromas or flavors to the food, which might not always be desirable depending on the type of food product. For instance, essential oils like oregano or thyme may impart a noticeable flavor to meats or dairy products, which could either enhance or detract from the product's sensory appeal. As such, it is crucial to carefully select and balance the type and concentration of essential oils in the film formulations to ensure that their sensory impact is minimal or complementary to the food product (Sipos, Nyitrai, Hitka, Friedrich, & Kókai, 2021). In addition to sensory factors, consumer perception plays a significant role in determining whether these active packaging systems will be accepted in the marketplace. The growing consumer preference for natural and clean-label products has driven the demand for safer, more sustainable packaging materials. However, it is important to address concerns about the safety and potential toxicity of the essential oils used, especially in food applications. Consumers may have concerns regarding allergies to specific essential oils or the possibility of chemical residues from packaging components leaching into the food. Therefore, clear communication about the safety and benefits of these materials is necessary to foster consumer trust (Krishna, 2012).

Safety considerations regarding the use of essential oils in food packaging are also governed by regulatory agencies, such as the FDA (Food and Drug Administration) in the United States and the EFSA (European Food Safety Authority) in the European Union. These agencies establish strict guidelines and regulations to ensure the safety of food contact materials. For instance, the FDA regulates substances that come into contact with food under the Food, Drug, and Cosmetic Act, which requires that any food-contact material be proven safe for its intended use. Essential oils used in food packaging must meet specific safety standards, including toxicological assessments to determine acceptable exposure levels. Furthermore, any essential oils or other ingredients used in edible films must be approved for use in food packaging through the FDA's Food Contact Notification (FCN) process or by being listed as Generally Recognized as Safe (GRAS). In the EU, EFSA evaluates the safety of food contact materials through risk assessments, ensuring that substances do not migrate into food at levels that could pose a risk to human health. Both the FDA and EFSA require comprehensive data on the migration behavior of essential oils from the packaging into the food product, ensuring that the concentrations remain within safe limits. Additionally, any claims made about the antimicrobial or antioxidant properties of essential oil-loaded films must be substantiated with scientific evidence to comply with food labeling regulations (Muncke *et al*., 2017). The regulatory approval process for essential oil-loaded Pickering emulsions in food packaging involves rigorous testing to confirm that the films are non-toxic, effective, and meet all safety standards. By meeting these regulatory requirements, manufacturers can ensure that essential oil-based edible films are not only safe for consumers but also meet the quality standards expected by the food industry.

Conclusion and Future Perspectives

Summary of Key Findings

The incorporation of essential oil-loaded Pickering emulsions into edible films has emerged as a promising strategy for enhancing food packaging. The fundamental principles of Pickering emulsions, where solid particles replace traditional surfactants to stabilize oil droplets, have enabled the creation of highly stable, functionalized films with significant antioxidant and antimicrobial properties. These films offer multiple benefits, including extending shelf life, improving food safety, and reducing the need for synthetic preservatives. The ability of essential oils, such as thyme, oregano, and rosemary, to act as natural preservatives due to their potent bioactive components—carvacrol, thymol, and eugenol—has been demonstrated to effectively inhibit the growth of common foodborne pathogens like *E. coli*, *Salmonella* spp., and *Listeria monocytogenes*. Additionally, their antioxidant capabilities prevent the oxidation of fats and oils, preserving the quality and nutritional integrity of food products. The encapsulation of essential oils in Pickering emulsions offers a controlled and sustained release mechanism, enhancing the stability of these volatile compounds, which is critical for their efficacy in food packaging. The use of biopolymers such as chitosan, cellulose, and starch, as well as inorganic nanoparticles like silica, titanium dioxide, and calcium carbonate, as stabilizers for these emulsions, has further improved their applicability in food systems. These biopolymers provide not only structural support but also contribute to the overall biocompatibility and sustainability of the films, making them more suitable for food contact applications. Overall, the integration of essential oil-loaded Pickering emulsions into edible films presents an innovative and environmentally friendly approach to developing active food packaging materials. These films have the potential to reduce food waste, enhance food safety, and meet the growing consumer demand for clean-label products, ultimately advancing the field of food packaging technology.

Challenges and Limitations

While the use of essential oil-loaded Pickering emulsions in edible films shows great promise, several challenges and limitations remain. One of the primary concerns is the sensory impact of essential oils, particularly their strong aroma and flavor, which may not be suitable for all food products. The strong scents of certain essential oils can alter the taste and aroma profile of the food, which may not be desirable for certain types of food products, such as fruits, dairy, or mild-flavored meats. Therefore, selecting appropriate essential oils and optimizing their concentration in the film formulations is crucial to minimize any negative sensory effects. Another challenge is the stability of the emulsions over time, especially during storage and distribution. Although Pickering emulsions are generally more stable than traditional emulsions, issues such as phase separation and changes in droplet size can still occur over time. The stability of these emulsions can be influenced by factors such as temperature, humidity, and the specific nature of the stabilizing agents used. Ensuring the long-term stability of essential oil-loaded emulsions is essential for their effectiveness in real-world applications. The scalability and cost-effectiveness of producing these films on an industrial scale remains another limitation. While laboratory-scale studies have demonstrated the potential of these films, large-scale production requires efficient and cost-effective manufacturing processes. The production of Pickering emulsions involving natural biopolymers and inorganic nanoparticles may incur higher costs compared to traditional plastic-based packaging materials. As a result, further research is needed to optimize production techniques and reduce the cost of raw materials to make these films more economically viable for widespread commercial use. Moreover, regulatory approval for the use of essential oil-loaded films in food packaging remains a critical hurdle. Regulatory agencies such as the FDA and EFSA have strict guidelines regarding the safety and migration of substances from food packaging materials into the food itself. Comprehensive toxicological studies are required to ensure that essential oils and their encapsulating materials do not pose any health risks to consumers. In addition, standardized testing protocols for the performance of active packaging films in different food systems need to be developed to facilitate the approval process.

Future Research Directions

Future research in the field of essential oil-loaded Pickering emulsions for food packaging should focus on several key areas to overcome the existing challenges and expand their applications. One promising avenue for future research is the development of nanoemulsion approaches. Nanoemulsions are similar to Pickering emulsions but involve the use of smaller droplet sizes, typically in the nanometer range, which could offer enhanced stability, increased surface area, and more efficient release of active ingredients. This could improve the performance of essential oil-loaded films in terms of antimicrobial efficacy and antioxidant activity. The use of advanced nanotechnology to tailor the size, distribution, and surface properties of oil droplets could lead to the creation of even more effective and versatile packaging materials. Additionally, smart nanoemulsions, where the release of active compounds is triggered by external stimuli such as temperature, pH, or humidity, could offer enhanced functionality for food preservation. Another exciting direction is the development of smart packaging systems integrated with real-time monitoring sensors. These sensors could provide consumers and food producers with real-time information about the quality and safety of the packaged food. For instance, sensors that detect changes in the pH, gas composition, or temperature within the packaging could signal when the food is nearing the end of its shelf life or when contamination occurs. These sensors could be coupled with the essential oil-loaded Pickering emulsions to create an integrated, multifunctional packaging system that not only preserves the food but also actively monitors and communicates its status. The integration of such sensors into edible films would provide a more proactive approach to food safety, helping to reduce food waste and ensuring that products are consumed at their optimal quality. Furthermore, research should also explore the optimization of essential oil selection and dosage. While essential oils provide significant antimicrobial and antioxidant benefits, their effectiveness can vary depending on the type of food and the particular pathogen or spoilage organism involved. Investigating the synergistic effects of different essential oils and their combinations could lead to more effective formulations for specific food products. Additionally, research in the area of interactions between essential oils and food components such as proteins, lipids, and carbohydrates will be critical for ensuring that the films do not interfere with the sensory qualities or nutritional value of the food.

Author Contributions

H. Mirzaee Moghaddam: Conceptualization, Project administration, Data curation, Visualization, Writing–review & editing. **A. Nahalkar:** Methodology, Data Curation, Writing–Original Draft. **A. Rajaei:** Conceptualization, Supervision, Data curation, Visualization, Writing–review & editing.

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فیلم‌های خوراکی زیست‌تخریب‌پذیر حاوی امولسیون‌های پیکرینگ دارای اسانس: مروری بر خواص آنتی‌اکسیدانی و ضدمیکروبی

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چکیده

این مقاله به بررسی خواص آنتی‌اکسیدانی و ضدمیکروبی فیلم‌های خوراکی زیست‌تخریب‌پذیر دارای امولسیون‌های پیکرینگ حاوی اسانس می‌پردازد. امروزه، فیلم‌های خوراکی زیست‌تخریب‌پذیر که امولسیون‌های پیکرینگ حاوی اسانس را در خود جای داده‌اند، به‌طور فزاینده‌ای به‌عنوان گزینه‌ای امیدبخش برای بسته‌بندی پایدار مواد غذایی شناخته می‌شوند. افزودن اسانس به ماتریس امولسیون، عملکرد آنتی‌اکسیدانی و ضدمیکروبی این فیلم‌ها را به‌طور قابل‌توجهی افزایش می‌دهد. بر این اساس، ویژگی‌های کلیدی مورد بحث در این مرور شامل فعالیت آنتی‌اکسیدانی، اثربخشی ضدمیکروبی و نقش این فیلم‌ها در افزایش ماندگاری محصولات غذایی است. نتایج نشان داده‌اند که افزودن امولسیون‌های پیکرینگ حاوی اسانس، ظرفیت آنتی‌اکسیدانی فیلم‌ها را به‌طور قابل توجه‌ای افزایش داده و موجب کاهش محسوس در تخریب اکسیداتیو مواد غذایی شده است. علاوه بر این، این فیلم‌ها فعالیت ضدمیکروبی مؤثری در برابر عوامل بیماری‌زای مختلف غذایی مانند اشریشیا کلی و استافیلوکوکوس اورئوس از خود نشان داده‌اند که این عملکرد به خواص زیست‌فعال اسانس‌های افزوده‌شده نسبت داده می‌شود. این فیلم‌ها با مهار مؤثر رشد میکروبی، به بهبود ایمنی مواد غذایی کمک مستقیم می‌کنند. این یافته‌ها، پتانسیل بالای فیلم‌های زیست‌تخریب‌پذیر حاوی امولسیون‌های پیکرینگ را به‌عنوان راهکاری پایدار برای بسته‌بندی مواد غذایی با خواص آنتی‌اکسیدانی و ضدمیکروبی تأکید می‌کنند که به افزایش ماندگاری و ایمنی بیشتر محصولات غذایی بسته‌بندی‌شده منجر می‌شود.

واژه‌های کلیدی: اسانس‌ها، فیلم‌های خوراکی، امولسیون‌های پیکرینگ، فعالیت آنتی‌اکسیدانی، ضدمیکروبی

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