



Active edible films and coatings with enhanced properties using nanoemulsion and nanocrystals

Fatemeh Kalateh Seifari¹, Hamed Ahari^{1*}

¹ Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran

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ABSTRACT

Environmental concerns related to using plastics for food packaging and consumers' demand for the extended shelf life of foods conducted the researches to develop new strategies for food packaging. The shelf life of food systems is related to the existence and growth of food pathogenic and spoilage microorganisms during food storage. Also, the environmentally friendly aspects of edible films and coatings make them appropriate substitutes for plastics in food packaging systems. Therefore, edible films and coatings which contain a food preservative introduced as hopeful novel systems for extending shelf life and preserving the quality of foods. The antimicrobial agents could be used in edible films and coating for restriction or stopping the microbial growth for prolonging the shelf life of foods. Due to the weak barrier and mechanical characteristics of most edible films and coatings, natural nanocrystals could be employed to improve the properties of them. In this review, the nanoemulsion encapsulation introduced as a technique for improving antimicrobial properties, while minimizing the antimicrobial agent impacts on the foods' organoleptic properties. Also, using natural nanocrystals proposed as a reinforcing agent for edible packaging material. The shelf life of food systems is related to the existence and growth of food pathogenic and spoilage microorganisms during food storage.

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1. Introduction

In recent years, due to the environmental concern associated with the un-renewability of food packaging materials, biodegradable and edible films and coatings have become the issues of interest for researchers (1-3). Extending the shelf life, protection against physical, chemical, and microbial hazards with retention of the safety and quality of food products during storage and transportation are the expected characteristics of food packaging (4). The duration in which a product is safe to eat from the microbial, sensorial aspects is defined as the product shelf life (5). Polysaccharides, proteins, and lipids are among the candidates used in the preparation of edible and biodegradable films and coatings in the food industry (6). Low cost, high processability, appropriate mechanical and thermal properties, biodegradability, chemical resistance, antimicrobial, and gas permeability are the main expected characteristics of food packaging materials (7). One of the strategies to prevent food spoilage due to the presence of

pathogenic microorganisms in them is the use of compounds with antimicrobial properties in the preparation of edible films and coatings. Therefore, active antimicrobial films and coatings can be introduced as an efficient method to reduce microbial growth in food products (8). Many attempts have been made for employing new materials and improving the performance of edible antimicrobial film and coatings. In this regard, the use of antimicrobial compounds with natural origin like essential oils is preferred over synthetic compounds (9, 10). For the preservation of the antimicrobial properties of these compounds, encapsulation using various strategies have been suggested to protect these volatile antimicrobial agents against the degradation and evaporation (11, 12). Different encapsulation techniques have been employed to entrap an antimicrobial agent into other substances, which resulted in the production of a millimeter, micrometer, and nanometer-scale capsules (13). In addition to the antimicrobial effect of the essential oils, some edible films and coatings could inhibit the growth of selective microorganisms by disrupting the oxygen

* Corresponding author: Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
E-mail address: dr.h.ahari@gmail.com (Hamed Ahari).

access or reacting with the cell wall of microorganisms (14). This review focused on edible coatings and films containing natural components and using nanotechnology for the enhancement of antimicrobial and mechanical properties of these packaging materials.

2. Edible packaging materials

Usually, edible films and coatings could be made using a conventional film preparation method. Generally, spraying, dipping, and brushing methods are used for coating, while casting, and extrusion methods are used for film preparation (15, 16). It should be mentioned that the edible films are formed distinctly and then applied to the surface of the food products, while coatings are prepared directly onto the food surfaces (17). Film and coating characteristics such as mechanical, thermal, barrier, and optical properties depend on the used raw materials and preparation methods. The edible film and coating properties are generally weaker than synthetic ones. Therefore, this will limit their application to particular purposes (18). Polysaccharides, proteins, and lipids are dominant raw materials for edible film and coating preparation (6).

2.1. Polysaccharides

Polysaccharides are long chains of carbohydrate molecules in which monosaccharides bind to each other through glycosidic linkages. These molecules are the most abundant biopolymers in nature that act primarily as a source of energy for humans, animals, and plants. The structure of polysaccharides derived from natural materials is remarkably complex and diverse. There are differences at the molecular level of polysaccharides that strongly influence their properties (19). In comparison with other biopolymers, these macromolecules have great potential for altering the molecular structure, because monosaccharide units in polysaccharides can be interconnected at several points and form a wide variety of structures (20). Polysaccharides with plant or marine origins such as starches, cellulose, gums, seaweed derivatives, and chitosan, commonly were used as edible films and coatings in the food industry (21). However, microbial polysaccharides with unique properties have been used as novel biopolymers in the preparation of edible films and coatings (22). The low cost, high availability, and some specific characteristics of polysaccharides such as selective permeability to some gases like oxygen, and dioxide carbon, and resistance to lipid migration, made them good materials for using in edible films and coatings preparation in the food industry. Nevertheless, the poor moisture barrier property of polysaccharides due to the high hydrophilic nature of them caused dehydration of fresh products (17). This disadvantage could be overcome by making edible film and coating composites by organic or inorganic fillers incorporating into the polysaccharide's matrix (23).

2.2. Proteins

Protein has been made from linked amino acids to each other by peptide bonds. The difference among proteins is due to their various sequence of amino acids, which caused the different three-dimensional structure (24). Edible proteins due to their high nutritional value and high intermolecular binding potential as a result of their unique structure are an important class of materials employed for the development of edible films and coatings. Proteins utilized in the production of edible films and coatings can be obtained from sources and by-products of animal and plant origin (25). Collagen, gelatin, surimi, casein, whey protein, creatine, and egg white are among the animal-based proteins used in edible film and coating in the food industry. Also, soy protein, wheat gluten, zein, proteins extracted from beans, sesame, pumpkin, sunflower have good film-making capacity for packaging films, which are the plant-based proteins. However, low mechanical strength, high sensitivity to moisture, and weak water vapor barrier properties because of their hydrophilic nature, limit employing the protein films and coatings compared to other polymers used in food packaging. Various methods have been manipulated to improve the most important properties of protein films, including mechanical strength and moisture barrier properties (26). Physical modifications including the heat curing, shearing, hydrostatic pressure, ultrasonication, ultraviolet, and Gama irradiation, thermoplastic processing such as compression molding and extrusion, chemical modifications including the reactions with chemical agents (alkylation, acylation, acetylation, succinylation, and grafting), and modification by pH alteration (hydrolysis, change in protein structure), biochemical modifications include transformation by enzymes, composite films and addition of bioactive compounds were used for improving the properties of protein films (24).

2.3. Lipids

Lipids are small hydrophobic or amphiphilic small molecules. Unlike polymers, they do not consist of repeating units attached via covalent bonds to make a large molecular structure. These molecules can form structures such as vesicles and multilayer liposomes in an aqueous solution. Neutral lipids, waxes, fatty acids, and resins are among the most important lipids usually utilized for the production of edible lipid-based films and coatings. Proper compatibility of lipids with other film ingredients and their good barrier properties against water vapor and gases has made them a candidate for use as edible films and coatings. As lipids are small molecules, they don't have the ability to form cohesive, independent films. Therefore, lipid-based films and coatings are composited with hydrocolloids for better characterization (27, 28). The chemical structure of different lipids (including the chemical group distribution, aliphatic chain length, and lipid polarity) could significantly influence the final properties of lipid-based films and coatings (17). It is reported that the lipid-based films and coatings had a good barrier to moisture, while the waxy taste and texture, potential rancidity, and greasy surface formation are the drawbacks of using them (29).

3. Antimicrobial agents

Antimicrobial agents as an additive to edible films and coatings increase shelf life and reduce the risk of pathogen growth on food surfaces. Bacteriocins (e.g., nisin), natural preservatives (e.g., essential oils, lysozyme), and other safe preservatives (e.g., benzoates, propionates, sorbates, parabens) as antimicrobial components in edible films and coatings are utilized in the food industry (30, 31). However, essential oils of plants as natural antimicrobial agents have attracted much attention. Essential oils are a complex mixture of non-volatile and volatile compounds with hydrophobic nature and wide biological activity. Studies have shown that many essential oils have strong antibacterial, antiviral, and antifungal activities, which caused the increment use of them in food products as natural antimicrobials (32). Antimicrobial activity of different essential oils against various bacterial, viral pathogens, fungal, and drug-resistance microorganisms was reported in numerous studies. The hydrophobic nature of essential oils allows them to interact with fatty acids of the dense microbial cell membrane. Whereas, the antimicrobial mechanism of essential oils depends on the type of essential oils and the microorganism strain (33-35). Using encapsulated antimicrobial compounds in edible films and coatings could minimize their impacts on the organoleptic properties of foods and preserve their biological activity. The encapsulation of antimicrobial compounds using nanoemulsion preparation is reported for the enhancement of their performance in the food industry (36).

4. Nanoemulsions

An emulsion system consists of two immiscible liquids, which one of them is dispersed in drops. Emulsions with a droplet size of about nanometer and typically in the range of 20 to 200 nanometers are called nanoemulsions. The unique structure and properties of nanoemulsions in comparison to conventional emulsions have created advantages for their application in many industries, including the food industry. The encapsulation of antimicrobial compounds in nanoemulsions and its application in edible film and coating minimizes the effect of antimicrobial agents on the organoleptic properties of food products, as well as enhancing their biological activity, due to their sub-micron size and better diffusion (37).

4.1. Nanoemulsions preparation

Typically, the processes of preparing nanoemulsions can be classified into two classes of high energy emulsion and low energy emulsion methods. In high-energy emulsion methods, the break-up of large droplets into smaller pieces occurred following by the adsorption of emulsifiers onto newly formed surfaces. The concentration and type of emulsifiers and the method of mechanical energy application for the decomposition of droplets and the process conditions affect the final properties of the prepared nanoemulsions. The most

widely used nanoemulsion production systems, which require large amounts of energy to transfer into the process fluid are colloid milling, high-pressure homogenizers, and ultrasonication. Nanoemulsions could be produced by physicochemical processes, which are known as low energy emulsion methods. Membrane emulsification, solvent substitution, and phase inversion are common low-energy methods for the production of nanoemulsions. Despite their many advantages, these methods need large amounts of surfactants and careful material selection (38, 39).

4.2. Application of nanoemulsions in edible films and coatings

Due to the transparency or slight turbidity of nanoemulsions, they could be used in edible films and coatings in the food industry. Also, food-grade surfactants such as natural gums, proteins, lecithins, sugar esters, and polysorbates must be used as emulsifying agents in nanoemulsion preparation for use in edible films and coatings (36). Despite the strong antimicrobial properties of some essential oils, the direct use of them in edible films and coatings is limited due to their strong flavor and low stability, which affects the organoleptic characteristics properties of food products. Therefore, the direct application of essential oils in edible film and coating is a challenging task that could be achieved using the encapsulation of essential oils into nanoemulsions (40). For years, edible films and coatings have been employed for shelf life extension of food by controlling the mass transport by creating a physical barrier among the environment and food. Earlier researches have revealed that the incorporation of essential oils in edible films and coatings remarkably affect their mechanical properties. However, encapsulated essential oils into nanoemulsions could be improved properties of films and coatings such as homogeneity, antimicrobial activity, and stability of the compounds under stress conditions (40, 41). The incorporation of nanoemulsions of *Zataria multiflora* essential oil into basil seed gum-based films resulted in increasing the food product shelf life by retarding the release of volatile constituents of the essential oil. Whereas, employing the *Zataria multiflora* nanoemulsions in the basil seed gum-based film matrix improved the mechanical properties of the film by significant changes in the microstructure. Also, the microbial investigation revealed that the resulted films were effective against potential foodborne pathogens (42). The prepared nanoemulsion films using hazelnut meal protein enriched with clove essential oil due to homogenization with ultrasound showed good mechanical properties, poor moisture permeability, and antibacterial effects upon *P. aeruginosa*, *L. monocytogenes*, *S. aureus*, *P. aeruginosa*, *E. coli*, and *B. subtilis* (43). Gelatin-based active films contained ginger essential oil nanoemulsion and reinforced with montmorillonite prepared with enhancing physical properties for food packaging. The results showed that the utilization of ginger essential oil nanoemulsions increased the thickness and reduced the solubility in water, moisture content, and hydrophobicity of films (44). The

improvement in the film integrity and mechanical properties and reduction of water vapor. The improvement in the film integrity and mechanical properties and reduction of water vapor permeability of the whey protein isolate-based films were reported due to the incorporation of *Grammosciadium ptrocarpum* Boiss essential oil nanoemulsions (GEO). The results demonstrated the potential of activated whey protein isolate-based films by nanoemulsions of GEO for food preservation (45). The preparation of hydroxyl propyl methylcellulose (HPMC) films contain *Thymus daenensis* essential oil nanoemulsions revealed the plasticizing effect of the nanoemulsions on the films. Also, activated HPMC edible films using *Thymus daenensis* essential oil nanoemulsions illustrated remarkable antimicrobial activity towards selected microorganisms (46).

4.2.1. Meat products

The active nanoemulsion loaded edible coatings and films significantly prolonged the shelf life and decreased the total viable bacterial, yeast, and mold counts, pH changes, and TBARS values, during refrigeration storage of meat products (47, 48). The feasibility study of using *Ferulago angulata* essential oil nanoemulsions for improving the efficiency of chitosan coating on extending the shelf life of Rainbow trout fillets during refrigerator storage showed that the nanoemulsion of the essential oil had a significantly better inhibitory effect on bacterial growth of refrigeration stored fillets. Moreover, using the essential oil nanoemulsions decreased the TVB-N and lipid peroxidation in fish fillets (49). Comparison of *Zataria multiflora* Boiss essential oil nanoemulsion and coarse emulsion effect on the microbial properties of fish fillets during cold storage revealed that nanoemulsion coating was more effective than coarse emulsion to extend the shelf life of fish fillets. Also, the nanoemulsion coating showed more inhibition of microbial flora, compared with coarse emulsions during the storage time (50). The investigation of the effect of pectin edible coating loaded oregano essential oil nanoemulsion on fresh pork loin showed the great potential of the active coating on fresh meat preservation by inhibiting lipid and protein oxidation, decreasing the pH and color change, keeping meat tenderness, and retarding microbial growth (48). Incorporation of nanoemulsified lemongrass essential oil into the glycerol-plasticized cassava starch films demonstrates improving the dispersion of essential oils in water, imparting increased functionality, and reduced degradation (51). Evaluation of the effect of gelatin films loaded food-grade nanoemulsions (curcumin, gallic acid, and quercetin) as chicken meat packaging material showed nanoemulsions loaded films increased the fresh broiler meat shelf life compared with the control (52). Loading nanoemulsions of oregano essential oil into the hydroxypropyl methylcellulose films reduced the UV and water vapor transmittance. The composite films showed an antibacterial effect against *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and

Vibrio parahaemolyticus (53). Comparison of the effects of corn starch films containing conventional and nanoemulsion form of *Zataria multiflora* essential oil and cinnamaldehyde on ground beef patties during refrigerator storage showed the corn starch film incorporated *Zataria multiflora* nanoemulsions resulted in the better oxidative stability and sensory properties (54). The increasing the shelf life of chicken breast fillets were reported by employing sodium caseinate -based edible coating containing ginger essential oil nanoemulsion onto the fillets. The antimicrobial potential of coatings contains nanoemulsion was significantly increased when nanoemulsion was fabricated. The aerobic psychrophilic bacteria of chicken fillets significantly decrease during refrigerator storage for 12 days (55).

4.2.2. Fruit and vegetables

Numerous preservation methods could be employed to decrease the spoilage of fresh fruits and vegetables, which have the problems of short shelf-life and reduce nutritional value during storage. Cold storage, using edible coatings contain chemical additives or natural plant extracts, and various new methods such as the development of nanocapsules or multi-layered systems could be applied to minimize the spoilage of fresh fruits and vegetables (56). The active edible films and coatings supplemented with essential oil nanoemulsion remarkably reduced the loss mass, firmness, total soluble solids, and titratable acidity of vegetables and fruits (57). Pullulan coating containing the cinnamon essential oil nanoemulsion was used for evaluation of the effect of cinnamon nanoemulsion incorporation on the fresh strawberries shelf life during room storage. Besides, the coating presented effective antimicrobial activity against bacteria and molds (57). The comparison of the performance of alginate-based coating supplemented with lemongrass essential oil and citral nanoemulsions on Rocha pears revealed that alginate coating enriched with the lemongrass essential oil nanoemulsion had better firmness. Alginate coating enriched with the lemongrass essential oil did not demonstrate the scald symptoms, while alginate coating incorporated citral nanoemulsions showed the strong internal browning and scald symptoms. The microbial population was under the safety limits in all treatments. Coatings did not affect soluble solids content and titratable acidity (58). Evaluation of the effect of *Myristica fragrans* essential oil chitosan nanoemulsions as the coating on the microbial characteristics and safety of stored rice seeds for controlling the postharvest losses of food commodities revealed effective protection against fungal infestation (15 foodborne molds), aflatoxin B1 contamination, and lipid peroxidation (59). Incorporation of bergamot oil nanoemulsions stabilized with nanocellulose into the whey protein isolate films provided a noticeable antimicrobial and antioxidant activity for the prepared edible films. The mechanical strength of composite films was higher than the net film. The water vapor barrier property of whey protein isolate film increased due to the incorporation of bergamot oil nanoemulsions (60).

5. Properties enhancement

As mentioned before, using active edible films and coatings in the food industry has been confined due to the weak mechanical properties and high-water vapor permeability of them. The properties enhancement of films and coatings could be considerably enhanced using some fillers to create composite films and coatings. Because of the nanometric size, which leads to large specific surface areas and unique properties, nanofillers are currently considered as high potential materials for the improvement of films and coatings properties. The nanofillers can be either organic (natural biopolymers (e.g. starch, cellulose, chitin, and chitosan)) or inorganic (e.g. clay, metals, and metal oxides) (61). Among nanofillers, natural nanocrystals with various shapes based on sources and preparation methods attracted much interest for properties enhancement of active edible films and coatings.

5.1. Natural nanocrystals

Recently, natural nanocrystals such as starch, cellulose, and chitin nanocrystals with high crystallinity, biocompatibility, nontoxicity, and large specific surface area have received considerable attention. Starch nanocrystals could be obtained due to the preservation of crystalline structures after the destruction of the starch granules amorphous regions (62). Moreover, cellulose nanocrystals as renewable and biodegradable nanomaterials with prominent mechanical properties could be produced from different natural sources (47). Chitin nanocrystals are another possible natural nanofiller that can be employed for reinforcing edible films and coatings (63). Several methods including acidic hydrolysis (64), ultrasonic treatment (65) ultrasonic pretreatment and acidic hydrolysis (66), enzymatic pretreatment and acidic hydrolysis (67), ball milling with acid hydrolysis (68), heat-moisture treatment with acid hydrolysis (69) could be used for starch nanocrystals production. Also, various methods such as acidic hydrolysis (70), enzymatic hydrolysis (71), ionic liquids treatment (72), subcritical water hydrolysis (73), and combined mechanical treatments (ball mill pretreatment, a high-pressure homogenizer, etc.) with acid hydrolysis have been employed for the preparation of cellulosic nanocrystals (74, 75). Acid hydrolysis (76), ammonium persulfate (77), and TEMPO-oxidation (78) methods could be employed for the preparation of chitin nanocrystals. However, starch, cellulose, and chitin nanocrystals are normally prepared using acidic hydrolysis due to treatment with a strong mineral acid, such as sulfuric acid and hydrochloric acid. It could be mentioned that the physicochemical properties of the nanocrystals depend on their preparation methods (79-81).

5.2. Application of nanocrystals in edible films and coatings

Cross-linked cassava starch nanocomposite edible film reinforced by starch nanocrystals was successfully prepared. Huangguan pears were coated with the prepared coating effectively extended the pears shelf life. However, starch

nanocrystals did not change the color of the coating and were appropriate for reinforcing and improving the barrier property of the coating (82). Starch nanocrystals and sour lemon peel extract were used for the preparation of bioactive coating solutions to preserve the quality of chicken fillets during the cold-storage. The optimized conditions achieved using the response surface method. The results showed the coating of chicken fillets with the optimal coating solution significantly improved the physicochemical, textural, and sensory characteristics in comparison with the control during twelve-day refrigerator storage (64). Mango seed starch films were produced with different levels of starch nanocrystals from mango seed kernels, and mango seed shells cellulose nanocrystals. The effect of cellulose nanocrystals on the enhancement of the water vapor barrier property and mechanical strength was higher than starch nanocrystals (83). Comparison of the tensile strength and moisture barrier property of amaranth protein films supplemented with amaranth starch granules and nanocrystals revealed that nanocrystals successfully enhanced the amaranth film properties. It could be due to the nanosize, uniform distribution, and robust interactions of starch nanocrystals with amaranth proteins (84). Incorporation of 5% mango starch nanocrystals into edible mango kernel starch films resulted in an increase in modulus and tensile strength in about 120% and 90%, respectively. Also, water vapor permeability reduces about 15%. However, the elongation of the film has been decreased, and the opacity increased (85). Reinforcement of the cassava starch films with cassava starch nanocrystals influenced the properties of the edible film, including water-vapor transmission rate, tensile strength, and elastic modulus. The results showed the film contains 10% starch nanocrystals showed a 43% decrease in water vapor permeability, 200% and 616% increases in traction resistance, and elasticity modulus in comparison with the control (86). UV barrier property and the oxygen permeability of alginate-based films loaded cellulose nanocrystals were evaluated at different relative humidity. The results showed the alginate films supplemented with cellulose nanocrystals had a UV barrier effect and decreased oxygen permeability. The edible coating on chicken breasts also demonstrated a decrease in lipid peroxide value and thiobarbituric acid reagent substances (87). Cationic starch films enriched with montmorillonite and nanocrystalline cellulose were produced by the solution casting method. The comparison of the net cationic starch film with the enriched film with 5 wt% montmorillonite and 5 wt% nanocrystalline cellulose demonstrated the tensile strength and modulus of the film increased up to 61% and 73%, respectively. The enriched film showed a high disintegrability rate and a significant reduction in moisture and oxygen permeability obtained with the addition of nanocrystalline cellulose (88). Edible apple pectin coating reinforced with cellulose nanocrystals and supplemented with lemongrass essential oil as an antimicrobial agent was employed to increase the shelf life of strawberries due to direct film forming on the surface of the strawberries. The prepared coatings effectively minimized the weight loss, without weakening the

other properties (89). Incorporation of the cellulose nanocrystals into the poly (lactic acid) film contained *Tanacetum balsamita* essential oil and propolis ethanolic extract, significantly increased the mechanical properties of poly (lactic acid) composite. The enhanced mechanical strength films considerably increased the vacuum-packed cooked sausages shelf life (90). Active carboxymethyl cellulose-based films contained chitin nanocrystal and supplemented with grapefruit seed extract were prepared using isolated chitin nanocrystals from shrimp shells powder. The results revealed that the addition of chitin nanocrystals enhanced the mechanical and water vapor barrier properties of the resulted films. Also, the films contained grapefruit seed extract showed strong antibacterial activity against foodborne pathogenic bacteria, *Escherichia coli*, and *L. monocytogenes* (63). The chitin nanocrystals were used to enhance the mechanical properties of poly (lactic acid) for food packaging applications. For enhancing the compatibility of chitin nanocrystals with the poly (lactic acid) matrix, acylation with dodecanoyl chloride acid and anhydride acetic was done for modification of chitin nanocrystals. The functionalized chitin nanocrystals enhanced the hydrophobicity of the poly (lactic acid) nanocomposite films and enhanced their antifungal activity (91). The mechanical properties of the plasticized poly (lactic acid) improved with nanocomposite preparation of poly (lactic acid) with cellulose nanocrystals and chitin nanocrystals. The results showed that nanocomposite films contain chitin nanocrystals were more transparent than the nanocomposite films containing cellulose nanocrystals (92). The incorporation of chitin nanocrystals and chitin nanofibers into thermoplastic starch-based matrices revealed that the chitin nanoparticles enhanced the mechanical, thermal, barrier properties of the nanocomposite films in comparison to pure thermoplastic starch film. The results showed that the shape and size of chitin nanoparticles have an important role in the final characteristics of the starch nanocomposite films (93).

6. Conclusions

The advent of active, biodegradable, renewable, and edible materials creates a novel path for the production of an ecofriendly way for storage, transportation, and extending the shelf life in the food industry. The edible films and coatings are biomaterial incorporated with various natural antimicrobial agents. Essential oils of plants as the natural antibacterial, antiviral, and antifungal components are an appropriate source of bioactive material for producing active edible films and coatings. The nanoemulsion of antimicrobial compounds could be used for enhancing antimicrobial compounds' performance in active edible films and coatings. The intrinsic properties of edible films and coatings such as low mechanical properties and high-water vapor permeability could be enhanced with natural nanocrystals as a new class of edible nanofillers.

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