Polysaccharide-Based Edible Films Incorporated with Essential Oil Nanoemulsions

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Edible films with essential oils (EOs) are becoming increasingly popular as an alternative to synthetic packaging due to their environmentally friendly properties and ability as carriers of active compounds. However, the required amounts of EOs to impart effective antimicrobial properties generally exceed the organoleptic acceptance levels. However, by nanoemulsifying EOs, it is possible to increase their antimicrobial activity while reducing the amount required. By incorporating EOs nanoemulsions into the packaging matrix, these edible films can help to extend the shelf-life of food products while also improving the quality and safety of the food product during storage. It can be concluded that these edible films have the potential to be used in the food industry as a green, sustainable, and biodegradable method for perishable foods preservation.

food preservation | essential oil | nanoemulsion | edible films | polysaccharide-based film

1. Introduction

Foodborne illnesses caused by pathogens that threaten food safety are a major concern all over the world. According to the US Department of Agriculture, global food-borne illnesses cost more than US$15.6 billion each year. According to a World Health Organization (WHO) report issued in 2015, nearly 600 million individuals were affected by foodborne illnesses in 2015, with more than 420,000 deaths recorded worldwide [1]. Besides that, microbial contamination of food products has an impact on the economic growth of countries that rely on agricultural product exports, which contributes to the economic burden [2]. As a result, much recent research has focused on producing and utilizing bio-based polymers made from a variety of industrial by-products or food waste products, in order to work toward the development of environmentally friendly strategies for preventing microbial contamination of food products [3]. This increased interest is also due to the rising consumer demand for natural food preservation methods, which has led to the development of alternative protection methods, such as the use of edible biopolymer-based films or coatings derived from renewable sources or industrial by-products [4]. In this regard, in the last few decades, the development of innovative edible films and coatings has emerged as a new research area in food science.

Edible biopolymers and food-grade additives or ingredients are used to develop edible films and coatings. Polysaccharides (e.g., carbohydrates and gums) and proteins (e.g., gluten, casein, gelatin) are all examples of film-forming biopolymers [5]. Plasticizers such as glycerol and other additives, crosslinking agents, emulsifiers, and reinforcements or lipids (namely waxes) can be combined with the film-forming biopolymers to modify the physico-chemical, mechanical properties or the functionality of films. Edible films are thin layers of edible material that are formed as a protective coating or layer on foods and can be consumed alongside the food products [6]. They serve as a barrier between oxygen, moisture, and the surrounding environment. They can also be used to separate different compartments of the same food [7]. By limiting the exchanges between foods and their surrounding atmosphere, they can reduce water loss, oxidation reaction rates, and respiration rates, ultimately extending the shelf life of the food product [8]. Since most film-forming biopolymers are hydrophilic, their mechanical and physico-chemical properties can be altered by water. Therefore, decreasing the sensitivity of biopolymer-based films to water, in order to render possible their use in direct contact with rapidly perishable high moisture foods is a subject of intensive research. The crosslinking of biopolymers or the addition of hydrophobic compounds such as lipids in the formulation of films are common strategies which were successfully employed by several authors to decrease their sensitivity to water [9]. Furthermore, edible films may act as a carrier of active compounds such as antimicrobials, which can significantly improve the functionality of edible films in controlling or preventing microbial spoilage of food products by preventing microbial growth during the storage period, thereby not only extending the shelf-life of the food products but also maintaining their quality and safety [2][10].

Antimicrobials, antioxidants, colouring agents, flavours and nutraceuticals are among the active ingredients incorporated into film-forming suspensions to enhance the stability, quality and safety of packaged foods [11]. Interestingly, some of these ingredients may exhibit excellent antibacterial, antifungal, or antioxidant activity when incorporated into edible films and extend the shelf life and/or improve the safety of foods. Essential oils (EOs), aromatic oily liquids extracted from plant materials, are among the most common antimicrobial compounds incorporated in edible films [12]. Clove oil, lemon oil, cinnamon oil, tea tree oil, lavender oil, oregano oil, and peppermint oil are the most commonly used EOs in the production of active edible films or coatings. The key benefit of EOs is their antimicrobial activity against a wide range of microorganisms. Moreover, most of them have been approved by the United States Food and Drug Administration (US-FDA) as safe additives for food applications [2]. However, the amount of EOs used to impart effective antimicrobial activity may exceed organoleptic acceptance levels [13]. As a result, studies have proposed nanoemulsions as a new delivery system for encapsulating and releasing EOs from edible films into food products.

2. Characterization of Essential Oil Nanoemulsion Loaded Edible Films

Recent research has shown that EOs-loaded nanoemulsions have improved the physical and mechanical properties of films when compared to the effect of incorporation of conventional emulsions. Furthermore, higher antibacterial activity has also been observed in nanoemulsified EOs [14]. For instance, pullulan-cinammon EO nanoemulsion coated strawberries had the highest inhibitory activity against bacteria and moulds at the end of the storage period, with log CFU/g values of 2.544 and 1.958, respectively, whereas the pure pullulan coated samples all had values that exceeded 10^3 (bacteria) and 10^4 (moulds) CFU/g [14]. In this regard, polysaccharide-based edible films such as pectin and alginate could be incorporated with EO nanoemulsions as antimicrobial agents during the manufacturing process, resulting in a new generation of active edible coatings/packagings.

It is necessary to evaluate the wettability, the physical, chemical as well as the mechanical (tensile strength, elongation at break), thermal, optical (brightness, opacity), and morphological properties of edible films incorporated with nanoemulsified EOs. This is necessary since the EO type and its interaction with the matrix determine the effectiveness of the edible films as food packaging materials [15]. Moreover, edible films also influence the movement of gases and act as a barrier to aromatic compounds transfer; they can thus also be used to create a modified atmosphere [16]. In this context, it is necessary to evaluate whether the gas barrier properties of edible films are affected by the nanoemulsified EOs addition in their formulation. Furthermore, when edible films are applied to food products for storage, their properties may influence consumer acceptability and their industrial applicability [15]. As a result, it is essential to consider the physico-chemical and mechanical properties of edible films when they are combined with EO nanoemulsions.

2.1. Physical Properties

2.1.1. Colour

Colour is one of the optical properties of edible films that can improve or degrade the overall appearance of food products, thereby influencing consumer acceptance. Colour can be measured by using a *L*, a*, b** colorimeter [10][17]. Acevedo-Fani et al. (2015) [10] reported that alginate-based films containing thyme EO nanoemulsions had the highest positive value (6.9 ± 0.6) expressed by coordinate *b**, indicating that these films had a light greenish-yellowish tone. This might be due to the presence of phenolic compounds in thyme EO, which may have low wavelength light absorption [18]. Similarly, the addition of cinnamon EO nanoemulsions into edible films increased the yellowness (*b**), indicating that increasing the concentration of cinnamon EO nanoemulsions caused the colour of the alginate bio-composite films to become slightly yellowish in colour [19].

Lightness (*L**), redness (*a**), and whiteness were significantly reduced by increasing the concentration of cinnamon EO nanoemulsions in soluble soybean polysaccharide-based edible films, according to Ghani et al. (2018) [20]. Adding cinnamon EO nanoemulsions increased yellowness (*b**), as in alginate films. The results revealed that increasing the concentration of cinnamon EO nanoemulsions changed the colour of soluble soybean polysaccharide-based edible films from light yellow to red. Unlike with cinnamon EO, Restrepo et al. (2018) [17] reported that increasing the concentration of rosemary and lemongrass EO nanoemulsions did not affect the *L*, a**, and *b** values of banana starch edible films.

2.1.2. Film Thickness

Thickness is an important parameter when studying the mechanical and water vapour barrier properties of films [21]. The film thickness was found to have a significant relationship with the droplet size of EO nanoemulsions [10]. Aisyah et al. (2018) [22] reported that corn starch-based edible films with 3% (*v*/*v*) nutmeg EO nanoemulsion had the highest thickness, while the incorporation of a 1% (*v*/*v*) nutmeg EO nanoemulsion resulted in the lowest thickness. The results show that the higher the concentration of nutmeg EO, the thicker the edible films produced. Consistently, Moghimi et al. (2017) [23] observed that hydroxyl propyl methyl cellulose (HPMC) films incorporating the *Thymus daenensis* EO nanoemulsion were significantly thicker than the control films that did not contain nanoemulsions.

The components of EO nanoemulsion, which consist of EO, water, and surfactant, may be responsible for the increase in the thickness of edible film. When the volume of solution poured on each plate is the same, the difference in thickness is caused by differences in the concentration of total solids in the film-forming suspensions [22]. The increase of the viscosity of the film-forming suspensions following the addition of EOs nanoemulsions could also result in thicker edible films. Indeed, Bertuzzi et al. (2007) [24] also demonstrated that increasing the glycerol concentration increased the viscosity of the solution, thereby increasing the film thickness.

The thickness of basil seed gum-based edible films incorporated with *Zataria multiflora* EO nanoemulsion ranged from 114 to 130 µm [13]. There was thus no significant difference between the thickness of basil seed gum control films and of those incorporated with *Zataria multiflora* EO nanoemulsion. Acevedo-Fani et al. (2015) [10] even reported that alginate-based films containing thyme EO and sage EO nanoemulsions presented a significantly smaller thickness compared to control films. Furthermore, incorporation of 2.5 and 5% (*v*/*v*) marjoram EO nanoemulsion significantly decreased the thickness of pectin-based edible films [21]. Previous research suggested that thickness reduction in edible films could be caused by emulsions with small droplet sizes. This effect was attributed to possible EO phase losses during the formation of films, which could reduce the total amount of solids concentration in the film matrix [10].

2.1.3. Film Solubility in Water

Film solubility in water is one of the most important criteria for selecting a film for a specific application. As can be seen in Ghani et al. (2018) [20], the solubility of soluble soybean polysaccharide-based edible films decreased with the increasing content of cinnamon EO nanoemulsions. Furthermore, when banana starch edible films were combined with nanoemulsions of lemongrass and rosemary EOs, the film solubility at 25 °C and 95 °C of the films decreased [17]. The findings obtained in both studies could be explained by the inclusion of hydrophobic compounds that do not solubilize in water, thus maintaining the edible film’s structure in water [20].

Hashemi et al. (2017) [13] observed that the solubility of basil seed gum-based edible films in water was reduced when the edible films were incorporated with *Zataria multiflora* EO nanoemulsion. It could be attributed to the increased hydrophobicity of *Zataria multiflora* EO-loaded basil seed gum films, as well as to network strengthening as also discussed for mechanical properties [20]. Network strengthening can be caused by extensive hydrogen bondings (dipole-dipole interaction) between polar groups, particularly hydroxyl groups, present along the polymer backbone and polar head groups of surfactant molecules present at the interface of dispersed nanodroplets [13].

2.2. Physico-Chemical Properties

2.2.1. Water Vapour Permeability

Water vapour permeability (WVP) is one of the most important factors to consider when selecting a packaging for food storage [20]. This parameter can be used to estimate the barrier property of a film by measuring the diffusion of water molecules through its cross-section [10]. Films used as packaging or coatings must control moisture transport from the product to the environment to prevent or reduce food dehydration, therefore, the WVP of edible films should be as low as possible [25].

EOs are known to decrease the WVP of polysaccharide-based films due to their hydrophobic properties [10]. Incorporation of 0.2% (*v/v*) of cinnamon EO nanoemulsion into soluble soybean polysaccharides-based edible films had no significant influence on WVP when compared to the control films. However, when the cinnamon EO nanoemulsion concentration was increased from 0.4 to 0.8% (*v/v*), the WVP of soluble soybean polysaccharides-based edible films was significantly lowered when compared to the control films [20]. Increasing the concentration of EOs nanoemulsion up to a certain threshold concentration reduced the WVP of films, most likely because the inclusion of nanoemulsion increased the hydrophobic character of the film solutions [10]. Above this threshold concentration of EO nanoemulsion, the high contact between soluble soybean polysaccharides and EO nanoemulsion droplets lowers the soluble soybean polysaccharides chain aggregation forces, thereby reducing the conjunction forces of the polymer network [20].

Similarly, Acevedo-Fani et al. (2015) [10] also observed a significant reduction of WVP when sage EO nanoemulsion was incorporated into alginate-based edible films. This could be explained by the corresponding nanoemulsions' small droplet size, which leads to a more even dispersion of the oily phase in the film structure. Nanocomposites containing well-dispersed nanoemulsions up to an "optimum" proportion have been observed to have improved barrier characteristics [26]. Furthermore, when methyl cellulose (MC)/cellulose nanocrystal (CNC)-based nanocomposite films containing a blend of thyme and oregano EOs nanoemulsion were compared to MC-based control film in Hossain et al. (2018) [27], the WVP values reduced by 9%. This significant decrease in WVP was attributable to CNC dispersion in the matrix, which resulted in a longer path length for water vapour diffusing molecules due to the tortuosity effect.

2.2.2. Moisture Absorption

Most food products contain a significant amount of water. Variations in moisture content can cause major changes in food stability and quality [28]. As a result, moisture absorption is another aspect to consider when looking into the water barrier qualities of film samples. The weight loss observed by the bio-composite films after 24 h of oven drying until a consistent dry weight was reached can be used to measure their moisture content [19].

The percentage of moisture absorption of pectin-based edible film was significantly increased after the addition of 2.5 and 7.5% (*v/v*) marjoram EO nanoemulsion in pectin-based edible films [21]. The control and alginate-based edible films with high cinnamon EO nanoemulsion content demonstrated higher moisture contents than those with a moderate quantity of incorporated cinnamon EO nanoemulsion, according to Frank et al. (2018) [19]. However, the variations were negligible, with the moisture content of the films remaining between 10% and 12%. According to Jouki et al. (2014) [18], the rise in moisture content was attributed to the glycerol concentration in the film, while

Ghasemlou et al. (2013) [29] proposed that this is due to the hydrophobic character of EOs, which has a direct effect on the water retention of the films.

2.3. Mechanical Properties

Tensile strength (TS) and elongation at break (EAB), which are closely related to the chemical structure of edible films, are the most commonly used characteristics to evaluate their mechanical properties [30]. The mechanical attribute of an edible film's tensile strength reflects the maximum stress that the film can withstand before breaking [22]. Elongation at break, on the other hand, refers to a film's ability to withstand changes in shape without breaking [23]. A high elongation is desirable for edible films since it improves the film's capacity to wrap and package food. **Table 1** shows the tensile strength and elongation at break of different polysaccharide-based edible films incorporating various EO nanoemulsions. Since water content of polysaccharides-based films varies with the relative humidity of their surrounding atmosphere, it is of utmost importance to pre-equilibrate films in an atmosphere with a relative humidity and a temperature similar to the environmental conditions for their application and to determine its mechanical properties under these conditions. Indeed, the water content of films varies with relative humidity, and water acts as a plastifier of polysaccharide-based films, thereby affecting its mechanical properties.

Table 1. Tensile strength and elongation at break values of different polysaccharide-based edible films incorporating various EO nanoemulsions.

Edible Film	Essential Oil Nanoemulsion	Tensile Strength (MPa)	Elongation at Break (%)	References
hydroxypropyl methyl cellulose	*Thymus daenensis*	22.6	14.2	[23]
corn starch	*Myristica fragrans*	15.8	32.9	[22]
pectin	*Origanum majorana*	1.3	21.1	[21]
basil seed gum	*Zataria multiflora*	34.6	39.5	[13]
methyl cellulose	*Origanum vulgare* *Thymus vulgaris*	71.6	20.0	[25]
banana starch	*Cymbopogon citratus* *Salvia rosmarinus*	3.2 3.4	23.5 20.5	[17]

According to Moghimi et al. (2017) [23], there are significant differences in the tensile strength (TS) between control and hydroxypropyl methyl cellulose-based films containing nanoemulsions of *Thymus daenensis* EO. The TS of methyl cellulose edible films when incorporated with *Thymus daenensis* EO nanoemulsions (22.6 ± 0.7 MPa) is lower than the control (36.2 ± 0.7 MPa). When nutmeg EO nanoemulsion was added to a corn starch-based edible film at various concentrations, a similar trend was reported [22]. The observed tensile strengths ranged from 15.1 to 18.9 kgf/mm^2 and the higher the concentration of nutmeg EO nanoemulsion, the lower the TS of the film.

The TS value was reduced following incorporation of 5 and 7.5% (*v*/*v*) of marjoram EO nanoemulsion in pectin-based edible films [21], which is consistent with the results obtained in previous studies [23][22]. The TS values of pectin-based edible films incorporated with 5 and 7.5% (*v*/*v*) of marjoram EO nanoemulsion obtained from the study were 1.9 MPa and 1.3 MPa, respectively. The decrease in TS could be attributed to the use of EOs in the production of edible films, which makes the films more flexible but reduces their resistance. Because the interaction between polymer and EOs is weaker than the interaction between polymer and polymer, a barrier between hydrocolloid polymer chains and EOs has formed. As a result, in the presence of EOs, the mechanical properties of edible films are compromised [23].

On the other hand, it was reported that incorporation of 3% (*v/v*) of *Zataria multiflora* EO nanoemulsion in basil seed gum-based edible films increased the TS value from 19.7 MPa (control) to 34.6 MPa [13]. Furthermore, the TS of methylcellulose-based films increased significantly from 54.2 to 71.7 MPa when incorporated with a plant EO blend (oregano and thyme) nanoemulsion (7.5% *w/w*), which is approximately a 30% increase [25]. This phenomenon can be explained by network strengthening caused by extensive hydrogen bondings (dipole-dipole interactions) between polar groups, particularly hydroxyl groups, present along the polymer backbone and polar head groups of surfactant molecules present at the interface of dispersed nanodroplets [13].

Alginate-based edible films incorporated with thyme EO, sage EO, and lemongrass EO nanoemulsions were as resistant as alginate films with no significant differences in TS values [10]. Similarly, the nanoemulsion concentration had no significant effect on TS values, ranging from 3.09 MPa in the edible films without nanoemulsions to 3.43 and 3.23 MPa for edible films with 0.5% (*v/v*) of rosemary and lemongrass nanoemulsions, respectively [17]. The reason for this limited variation of TS is most likely due to the low EO nanoemulsion content in the edible films [10].

2.3.2. Elongation at Break

Acevedo-Fani et al. (2015) [10] reported that alginate-based edible films with sage EO nanoemulsions were the most stretchable (78%) when compared to control and other edible films. However, films containing lemongrass EO (32%) and thyme EO (41%) nanoemulsions did not show significant differences compared to the EAB of control films (38%). The influence of the electrical charge of nanoemulsions in the film structure could explain some of the variations in film flexibility. In the case of a charged polymeric film structure, repulsive forces between molecules of the same charge can increase the distance between polymers, resulting in a plasticizing effect [31].

The EAB of basil seed gum composite films increased when incorporated with *Zataria multiflora* EO nanoemulsion (39.5% compared to 21.6% for the control film without *Zataria multiflora* EO nanoemulsion) [13]. Furthermore, there was an increase in EAB in edible films when rosemary and lemongrass EO nanoemulsions (0.5% *w/w*) were added, with values of 20.45 ± 1.5% and 23.54 ± 0.8%, respectively, when compared to 10.98 ± 1.2% for the control films [17]. This phenomenon could be explained by incorporating EOs, which weaken the intermolecular interactions between polymer chains, resulting in more extensible and flexible films [32]. Furthermore, due to the reduction in droplet size, EO nanoemulsions have a plasticizing effect on edible films, reducing resistance while increasing elongation [13][17]. However, the EAB of methyl cellulose films only increased from 19.3 to 20.0% when incorporated with oregano and thyme EOs blend nanoemulsion [25].

When 30% (*v/v*) glycerol concentration was used in the film formulation, corn starch edible films incorporated with nutmeg EO nanoemulsions showed the highest percentage of EAB of 52.8% [22]. This implies that increasing the glycerol concentration tends to increase the film elongation. The addition of glycerol may reduce the intermolecular force of the edible film matrix structure, increase flexibility, and decrease the number of hydrogen bonds, reducing fragility and making it more difficult to break [22].

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