Edible films and coatings based on chitosan with essential oils for anti-microbial food packaging application

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The purpose of this paper is to provide an updated review of the emerging edible chitosan coatings in combination with essential oils providing an anti-microbial function for the food packaging systems. Chitosan is produced commercially by de-acetylating chitin obtained from shellfish waste, and is bio-degradable, and was approved as a food ingredient in the United States; hence chitosan-films that are clear, tough, and flexible and with good oxygen barriers can be formed by solution casting-method. Recently results from published studies suggested that chitosan films combined with essential oils prevent a broad spectrum of micro-organisms including Gram-positive and Gram-negative bacteria and fungi from growth and proliferation especially when there is a high concentration of the essential oil in the matrix of the natural polymer. Although recent studies showed that some essential oils, including Basil and Thyme essential oils, did not prevent the growth of certain species of fungi including *Aspergillus niger*, *Botrytis cinerea*, and *Rhizopus stolonifer* in an in vitro test. Despite the potentials of essential oils in active food packaging, its economic viability and economic sustainability must be looked into in light of the resource-intensive nature of its production, growing trend of 'green' consumerism, and the decline in the production output of aromatic plants.

Keywords: Anti-microbial, Chitosan, Coatings, Edible, Essential oils, Food packaging

1. INTRODUCTION

In recent times, there has been a growing interest in the development of sustainable coating materials that can effectively protect the quality and safety of different food products [1]. In this regards, bio-polymers based on proteins including gelatin, casein and whey, and polysaccharides, such as cellulose, locust bean gum, cassava starch or chitosan, are the most popular candidates for the fabrication of these coatings owing to their various advantages over synthetic materials, which include non-toxicity, bio-degradability, edibility, bio-compatibility, renewability and environmentally-friendly properties [2-5]. Among the polysaccharides used in the production of edible coatings, chitosan that is a natural biopolymer obtained through de-acetylation of chitin, a major component of crustacea's shells such as crab, shrimp, and crawfish, displays a viable potential for applications in the food industry [6, 7]. This is due to its desirable physicochemical properties, non-toxicity, bio-degradability, human tissues bio-compatibility, anti-microbial and anti-oxidant activities [8]. Chitosan is produced from renewable resources; and also it is being reported as the second most abundant polysaccharide found in nature, after cellulose [8].

The desirable characteristics of chitosan gave rise to its wide range of applications in pharmaceuticals, biotechnology, bio-medicine, agriculture, and food processing [4]. So far chitosan has been used successfully in the production of packaging material for the preservation of quality of various food products and has an incredibly significant potential in the food industry, given the contaminations that may be associated with food products. Moreover, chitosan is one of the most promising coating materials due to its film-forming properties and anti-microbial activity [8, 9]. Several studies have reported the strong anti-microbial activity of chitosan against microorganisms particularly the Gram-positive bacteria thereby making it suitable for the development of natural preservatives for applications in food processing and functional additives [8-11]. The bacteriostatic effect of chitosan against pathogenic bacteria including *Vibrio cholerae, Escherichia coli, Shigella dysenteriae, Bacillus fragilis*, and *Salmonella typhimurium* has been widely reported [11].

Chitosan-films and coatings are promising systems to be utilised as carriers of active ingredients in food packaging. Among the active ingredients that can be incorporated into chitosan-films and coatings, essential oils (EOs), volatiles and aromatic compounds from plants and spices with several biological properties have received much consideration as having a potential biological activity [12]. Certain EOs from spices including clove, oregano, thyme, basil, mustard, and cinnamon have been classified as Generally Regarded as Safe (GRAS) by the United States Department of Food and Agriculture (FDA) and they have gained popularity due to their volatility that facilitates the use of small concentrations that are safe for consumption [13]. Also, the hydrophobic properties of EOs are expected to help in reducing the water-vapour permeability of hydrophilic films; and additionally, they have been demonstrated to have some impact on other film properties including tensile strength, optical and structural properties in addition to providing anti-oxidant and anti-microbial effects [3,14]. This paper will review the use of chitosan with selected EOs in an active food packaging application with specific attention to the properties of emulsions/films and its anti-microbial and antioxidant functions.

2. PROPERTIES OF CHITOSAN AS FILM AND/COATING MATERIALS

Chitosan is derived from the deacetylation derivative of chitin. It is a cationic polysaccharide with 1,4-linked-2-amino-deoxy-b-D-glucan. Also, it has been approved by the FDA as a food additive, and it has been given the GRAS status [15]. Hence, pure chitosan is generally cohesive in nature and compact with a film surface that has a smooth contour without cracks or pores [16]. Moreover, chitosan has successfully gained recognition for use as an active packaging material and anti-microbial film due to its excellent film-forming, bio-compatibility, and bio-degradability properties [17]. The combination of its bio-degradability, good anti-microbial and anti-oxidant activities makes it more fascinating for use as a bio-degradable active packaging material as reported in some studies [16]. This is because the use of chitosan for films/packaging materials has been successfully experimented on several fresh and dairy products and was found to extend their shelf-life due to its excellent protection against microbial spoilage and contaminations owing to the fact that it (chitosan) forms a transparent film with an effective gas barrier (CO_2 and O_2) properties [17-19]. Another study has shown that chitosan-based coatings can protect foods from fungal decay and modify the atmospheres of fresh fruits; and chitosan-films also exhibit good mechanical properties such as durability, flexibility, strength, high break resistance and toughness [17].

2.1 MERITS AND DEMERITS OF CHITOSAN

Chitosan offers some advantages over many other biomolecule-based active polymers as packaging materials due to its anti-bacterial properties and the ability to chelate metal ions that are essential for microbial growth [20, 21]. Another reason for the anti-microbial property of chitosan is the possession of a positively charged amino group that interacts with the negatively charged microbial cells; and pure chitosan-film is an effective anti-microbial agent against bacteria including *Escherichia coli* and *Staphylococcus aureus*. Also, Vodnar *et al.* [22] reported that the chitosan nano-particles in the concentration of 0.1-0.7% w/v possess a higher anti-microbial effect when compared to the bulk chitosan due to the greater surface area and charge density, which could provide greater interaction with the positively charged surface of the bacterial cell. However, the major problem associated with chitosan-film is that it has relatively high water-vapour permeability and weaker mechanical properties when compared with the traditional plastic packaging materials since sufficient water-vapour barrier is required in many cases [23, 24]. However, few studies have suggested that the films can be improved through the addition of other ingredients such as lipids, plasticisers and essential oils [23, 24].

2.2 ESSENTIAL OILS

Essential oils (EO) are the secondary metabolites of aromatic flowers and plants. They have become very important in recent years due to their widely reported medicinal and nutritional characteristics. EOs are lipophilic materials which comprise composites of volatile and non-volatile compounds that are categorized into carotenoids, alkaloids, phenolic acids, flavonoids, monoterpenes, isoflavones, and aldehydes [25]. The functional properties of EOs are said to be linked with these components present in various proportions. Currently, they are mainly studied for their

able I - Studies on the properties of active packaging materials based on chitosan with some selected essential oils to act as antimicrobial and antioxidant agents in active food packaging

Essential oil	**Chitosan: EO ratios**	**Method**	**Properties of the film**	**Antimicrobial properties**	**Antioxidant properties**	**Application**	**References**
Cinnamon bark oil,	1%(w/w); 1-3%(w/w)	Casting Method	Increase T, WVP, decrease MC, SR, Opacity, TS, E%	Active against pathogens *S. enterica, L. Monocytogenes* *E. coli*	NA		[2]
Cinnamon Oil	1g: 0.5, 0.1g	Casting method	Increase T, WVP, Opacity, Lowered TS	Active against *E. coli* *L. monocytogenes,* *S. enteritidis,*	NA		[42]
Eucalyptus globulus	2%(w/v): 0%, 4% (v/v)	Casting method	Reduced transparency, Solvent-evaporation decreased lightness	*E. coli, S. aureus,* *C. albicans, C. parapsilosis* *P. aeruginosa*	Strong antioxidant activity		[32]
Basil or thyme Essential oil	1% (w/w): 0.25-1%(w/w)	Casting method	Increased WVP, E%, TS, stability	Do not exhibit antifungal effect against *A. niger,* *B. cinerea, R. stolonifera*	NA		[41]
Clove oil	1% (V/V): 0.5-2ml/l	Mixing	NA	Exhibit antifungal activity against *P. digitatum*	NA	Postharvest treatment against citrus green mold	[9]
Thyme and rosemary essential oils (TEO and REO)	2%(w/v): 1:1w/w (TEO: REO)	Mixing	Decrease thickness, moisture content and L* value. The b* value increases.	Inhibition of *S. enteritidis* strains	NA		[28]
Rosemary Essential oil	2%(w/v):1.5% v/v	Casting	Increase WVP, TS, E%	*L. monocytogenes,* *S. agalactiae*	Strong antioxidant activity	Citrus fruits	[20]
Lemon essential oil	1%:3%	Microfluidization	Decrease WVP	*Botrytis cinerea*	NA		[23]
Thyme essential oil	1%: 0%, 0.5%, 1%, and 2%	Mixing	Reduce water condensation in the package	Higher antimicrobial activity against yeast	NA	Ready-to-eat pork	[29]
Trachyspermum ammi EOs	2% w/w:1% and 2% w/w	Homogenization	NA	Highest inhibitory effects on total aerobic, total psychrophilic and coliform bacteria at 2% *T.* ammi EO.	NA		[15]

Continua Tabella I

Essential oil	**Chitosan: EO ratios**	**Method**	**Properties of the film**	**Antimicrobial properties**	**Antioxidant properties**	**Application**	**References**
Apricot kernel oil	1:1 (w/v)-1:0 (w/v)	Casting	Increased WVR, WVP, TS and E%.	Antifungal properties	Excellent antioxidant	Bread slices	[17]
Ginger essential oil	1.5% (w/v): 0.5%; 1% and 2% (v/v)	Casting method	NA	Antimicrobial activity against *Bacillus cereus (ATCC11778), Enterococcus faecalis (ATCC29212), Listeria monocytogenes (ATCC15313), Staphylococcus aureus (ATCC6538)); E. Coli (ATCC8739), Pseudomonas aeruginosa (ATCC9027), Salmonella enterica (ATCC10708));* and one yeast *Candida albicans (ATCC10231)*	Reduces lipid oxidation	Poultry meat	[24]
Ginger (*Zingiber officinale*) essential oil (GEO)	1%: 0.1,0.2 and 0.3%	Casting	No significant effect on mechanical properties of the film	Max. antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* at 0.3 % GEO	NA	Steak of barracuda (*Sphyraena jello*) Fish	[35]
Black pepper Essential Oil (BPEO)	2% (w/v): 1.5% (v/v)	Mixing	NA	Reduces the aerobic plate count, psychrophilic bacteria count, lactic acid bacteria count, and *Enterobacteriaceae* bacterial count	NA	Fish fillet	[36]

Note: T: Thickness, TS: tensile strength, WVP: water- vapour permeability, % E percentage elongation, MC: moisture content, SR: swelling ratio, L*: lightness, b*: yellowness/blueness different antioxidant, antimicrobial, anti-tumour, analgesic, insecticidal, anti-diabetic, and anti-inflammatory properties [12].

2.2.1 Economic viability and environmental sustainability aspects of using essential oils in active food packaging

Despite the numerous benefits associated with the use of EOs particularly in food safety and food preservation, studies looking at the economic viability and environmental sustainability aspects of EOs used in food packaging are scarce. We could only lay hands on a few references. Yeager [26] reported that the last decade has seen a boom in the sales of essential oils as Western consumers are searching for replacements to chemical-laden products that are potentially toxic to not only their bodies but to the planet. A market research study conducted by Grand View Research estimated that the global market value for essential oils stands at USD 7.03 billion in 2018 and is projected to rise to $11.67 billion by 2022 [27]. Hence, having such a high level of demand raises two vital questions: Where are all these essential oils coming from, and what is their impact on the environment?

From an economic point of view, essential oil production required a huge amount of raw materials. For example, the production of 1 kg of essential oil requires enormous quantities of plants: 9000 kg of rose petals, 200 kg of lavender, 5000 kg pounds of melissa plant, 1200 kg lemons, and so forth. Several factors necessitated the need for a large amount of product and this includes the fact that some oils are more difficult to extract because instead of being externally secreted by the plant, the oils are stored in tiny cavities or ducts within the plant. Other oils provide small yields in general [13]. The resource-intensive nature of essential oils production should be considered when using essential oils in active food packaging as this can significantly impact the final cost of the packaging material. Moreover, studies involving the use of EOs in active food packaging are still in the pilot scale and involves the use of smaller quantities (usually in millilitres) but a huge quantity will be required for large scale production. Several constraints will likely affect the future use of EOs especially with the fact that currently, 40% of all EOs produced globally goes into food and beverage application. Hence, the increasing trend of ‘green’ consumerism and the dwindling of supplies of aromatic plants possess a greater risk to the growth of the essential oils market and will surely affect its economic viability and environmental sustainability of active food packaging application.

Essential oils are here to stay and can be a more natural solution to common microbial contaminants in food products than traditional chemical and processing methods that are detrimental to the safety and quality of foods. However, good farming practices and proper disposal and recycling habits must be maintained to prevent this resource-intensive industry from falling into harmful ecological practices [26].

2.3 FORMULATION OF CHITOSAN-ESSENTIAL OIL FILMS/COATING

Advances in food packaging have recently focused on the inclusion of various compounds to enhance the properties of chitosan films. Several essentials oil has been used for this purpose as a replacement of synthetic anti-oxidant as reported in some studies [28, 29]. These EOs were found to have anti-oxidant and anti-microbial effects against bacteria, yeast, mould, and viruses primarily due to their rich phenolic or bio-active components such as carotenes, flavonoids and terpenes [30]. The inclusion of essential oils at a lower concentration of specifically 100 ug/g does not significantly affect the physical, mechanical and sensory properties of packaged foods [31]. Although EOs could be incorporated directly into foods for anti-microbial effect, high concentration is required to achieve this purpose, and this may cause in-appropriate flavours and odours in food products. To overcome this undesirable effect, EOs are rather incorporated into a bio-active film coating [32]. This type of packaging system where EOs are incorporated into the packaging material to improve the anti-microbial properties is referred to as anti-microbial active food packaging. Such packaging has been studied in the past few decades as a viable alternative to traditional packaging technologies for food preservation [24]. The application of EOs in films and coating with various bio-polymers has been extensively reviewed by some researchers [33, 34]. Chitosan-film is normally prepared by dissolving the chitosan in an aqueous solution (usually 1% v/v) of acetic acid to a concentration of 2% (w/v) while stirring on a hot plate. The solution is again stirred at a lower temperature of about 45°C, and the resultant solution is filtered, and the glycerol and Tween 80 are added during the film-forming to assist the dissolution of essential oil [15, 35, 36]. Chi, Zivanovic and Penfield [37] stated that the problem of water vapour permeability associated with the use of pure chitosan can be improved by the addition of EOs. The addition of EOs in a film or food coating also leads to a high concentration of active compounds on a product surface thereby preventing the microbial attack [37]. Hromiš *et al.* [16] have confirmed the anti-microbial activity of a chitosan film incorporated with oregano essential oil against *E. Coli*, and the same author also observed an increase in anti-oxidant activities of the chitosan film with the added black caraway essential oils from 11.64% to 33.47±4.77% after 2.5 h and to 81.08±0.64% after 24 h. This increase was even higher in the film with added oregano essential oil with an increase to 87.58±1.71% after 4 h and above 90% after 24 h.

The use of several types of EOs with chitosan for film or coating for anti-microbial activity has been reported in several studies such as cumin (*Cuminum cyminum L.*), clove (*Eugenia caryohyllata*), clecampane (*Inula helenium L.*), clove (*Syzgium aromaticum*), marjoram (*Origanum majorana*), cinnamon (*Cinnamomum zeylanicum*), and black pepper respectively [21, 36].

Mulla *et al.* [38] opined that the limitation regarding the incorporation of EOs for anti-microbial films and coatings is the volatility and thermal degradation during mechanical processing. Although, many types of research have demonstrated the feasibility of the EOs incorporation. Another setback while preparing a chitosan-based coating is the uniform dispersion of anti-microbial agents in the chitosan solution system. However, several techniques were reported by many researchers to solve this problem and help to develop complex coating with good anti-microbial activity [6]. Although, Abdollahi *et al.* [20] also stated that chitosan has a poor gas barrier, mechanical properties, and weak water resistance which limit its application especially in the presence of water and humidity. However, many techniques are employed to overcome such draw-backs which include the addition of salt and plasticisers; the addition of different polysaccharides, cross-linking of polysaccharides, chemical modification, use of a suitable solvent, change of pH and blending chitosan with other polymers; also lipid materials are sometimes added to chitosan-based films to improve the moisture barrier [23]. Also, Chi, Zivanovic and Penfield [37] have confirmed the diffusion of oregano essential oil from the chitosan film matrix to the processed meat. The moisture and high lipid content of the meat were seemed to contribute to the EOs diffusion. However, their results for the sensory evaluation has suggested that addition of about 45 ppm or lower concentration of oregano EOs could be more acceptable to the consumers.

Sánchez-González *et al.* [34] observed that the incorporation of bergamot essential oil in chitosan film has significantly improved the storage life of grapes by inhibiting microbial activity, inhibiting colour development, controlling respiration rate, and reducing water loss during storage. In contrast, the coating of the grapes with pure chitosan has resulted in increased fruit luminosity. Similarly, in another attempt to improve the properties of chitosan film by Shahbazi [10], incorporating *Ziziphora clinopodioides* essential oil has led to a significant improvement in terms of anti-bacterial and anti-oxidant activities, thickness and water vapour barrier property. Besides, the swelling index, tensile strength, puncture force and deformation of chitosan have also been improved, significantly as indicated by the report of Shahbazi [10].

In an *in vitro* study by Xing *et al.* [39], the effect of chitosan coating with cinnamon oil on anti-fungal properties against blue mould disease in Jujube fruit was investigated. The result has confirmed an improved anti-fungal activity against *P. citrinum* with increasing chitosan or cinnamon oil. The use of 2% cinnamon oil concentration has complete control of the *P. citrinum* growth as shown by an *in vivo* study. The result has also suggested that chitosan coating (1% chitosan with 0.75% cinnamon) on Jujube fruit could induce a fungi-toxic effect against pathogens and evoke a biochemical defence response in the fruit.

According to the findings of Wang *et al.* [7], the incorporation of natural essentials oils (clove bud oil, Star anise oil and cinnamon oil) has improved the chitosan-oil film properties such as water-vapour transmission rate, mechanical strength, moisture content and solubility. Additionally, the chitosan-oil solution incorporated with cinnamon oil had shown the best activity against *Escherichia coli, Staphylococcus aureus, Aspergillus oryzae, and Penicillium digitatum.* However, the film does not show a remarkable anti-microbial activity. In another study, the formulation of chitosan-thyme essential oil (0.5-2% w/w: 0.2 w/v) film was found to be an effective anti-microbial agent against two strains of *Listeria monocytogenes*, ATCC19115 and ATCC19112, in a fresh cabbage [40]. Hence, various methods have been employed by several studies for the production of chitosan-essential oil film which includes; homogenisation, a combination of homogenisation and micro-fluidisation [28].

2.4 PREPARATION OF FILM-FORMING DISPERSIONS (FFDS) AND PRODUCTION OF CHITOSAN-ESSENTIAL OIL

2.4.1 Preparation of Film-forming Dispersions (FFDs)

High molecular weight chitosan of 1% w/w can be dispersed in an aqueous solution of glacial acetic acid of 1% v/w at 25°C for 12 h, and selected essential oils or their mixture can be added at different concentrations as described in a study [41]. FFDs are usually prepared using a rotor-stator homogeniser at an average of 20,500 rotations per minute for 4 min. After homogenisation, the formulations are then degassed with a vacuum pump.

2.4.2 Production of Chitosan-Essential Oil

The essential oil during the film formation can be estimated from the difference between the weight of the initial solid extended in the casting plate and the final weight of the dried film. Results are usually expressed as wt % with respect to the initial amount of essential oil [41]. The films can be obtained by the casting technique. FFDs are to be poured onto a framed and levelled Teflon® plate (4¼ 15 cm) and then dried at room temperature for 48 h. Films are to be prepared by pouring the amount of FFDs that would provide a constant chitosan surface density in the dry films of 28 g/m^2. Dry films are to be peeled off the casting surface and preconditioned in desiccators at 5°C and 59% relative humidity, with an over-saturated $Mg(NO3)_2$ solution for one week before performing all the tests. Film thickness is to be measured using a Palmer digital micro-meter (± 0.001 mm) at a minimum of 5 different points of the same sample losses.

2.4.3 Properties of the emulsions/films

Over the years, several active packaging materials have been developed and studied using chitosan in combination with other biopolymers and their properties and potentials for the active food packaging application. Table I presents a summary of some of the recently conducted studies that give an overview of the different combinations of chitosan and EOs, the effect of incorporating an essential oil on the structure, physical and mechanical properties of edible films. Furthermore, the effects of EOs on the anti-microbial and anti-oxidative properties of the packaging material as well as potential food applications were investigated.

Nonetheless, chitosan displays a wide range of anti-microbial activities that are influenced by various determinants including microbial factors, intrinsic factors, and physical state of chitosan as well as environmental factors [9]. Ma et al. [42] formulated chitosan films with lauric arginate, cinnamon oil, and ethylene-di-amine-tetra-acetate (EDTA) and observed a significant increase in the thickness of the films and the yellowness of the film after incorporating the EOs. Also, the water solubility decreased with an increase in the concentration of cinnamon oil while the water-vapour permeability of films increased with the addition of the anti-microbial agents. The addition of the anti-microbial agents in chitosan films lowered its tensile strength but with no significant effect on the percentage elongation. There is a synergistic anti-microbial effect with the in-corporation of lauric arginate, cinnamon oil, and EDTA against Gram-positive *Listeria monocytogenes* and Gram-negative *Escherichia coli* O157: H7 and *Salmonella Enteritidis*. The incorporation of EDTA enhanced the activity of lauric arginate and overcame the antagonistic effect observed in lauric arginate-cinnamon oil combination [42].

In another study, Ma et al. [2] obtained a chitosan film with micro-emulsions of cinnamon bark oil and soybean oil; and the authors observed an increase in thickness and water-vapour permeability of films and a decrease in moisture content, the swelling ratio and opacity, respectively. The tensile strength decreased while the elongation percentage at break increased with an increase of micro-emulsions. The retention of cinnamon bark oil was improved for films prepared from micro-emulsions with 2% and 3% cinnamon bark oil immediately following film formation and after ambient storage when compared to control films prepared with emulsions with less Tween 80. Hence the study suggests that there was an increase in the anti-microbial effect of the films with observed large zones of inhibition against foodborne pathogens for film discs prepared with 2% and 3% cinnamon bark oil [2].

A chitosan-film incorporated with apricot kernel essential oil prepared by casting methods have been investigated by [17]. And an improved water resistance and enhanced water-vapour barrier property by 41% were observed when the chitosan-film to apricot kernel essential oil ratio of 1:1 was used during the study. The percentage elongation has also increased significantly for the chitosan film of ratio 1:0.125 of chitosan to apricot kernel essential oil. The authors also observed a continuous increase in tensile strength value with increasing the essential oil ratio equal to the chitosan. The film was found to have excellent anti-oxidant and anti-microbial properties and has successfully inhibited the fungal growth of packaged bread slices [17].

In another study, the incorporation of Eucalyptus globulus essential oil into chitosan films affects its physical, anti-microbial and anti-oxidant properties in a study conducted and reported by Hafsa et al. [42]. There is an observed decrease in moisture content and water solubility due to the incorporation of the EO. The antioxidant properties of the film have been significantly improved with increasing essential oil concentration. The antimicrobial sensitivity test indicated an excellent antimicrobial action of chitosan films containing Eucalyptus globulus essential oil against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans,* and *Candida parapsilosis* when compared with films containing chitosan only [43].

Ruiz-Navaja *et al.* [32] have studied the addition of two essential oil from Thymus species, *Thymus moroderi and Thymus piperella* at 2% concentration in the formulation of chitosan films for enhancing the safety and storage life of cooked cured ham for 21 days at 4°C. The result has demonstrated the ability of chitosan-EOs film to decrease the lactic and aerobic mesophilic bacteria count in coated samples when compared with the control uncoated sample. A lower degree of lipid oxidation was also observed in the cured ham samples coated with this film. Thus, the overall shelf life of the cured ham has significantly improved. In another study, Ruiz-Navaja *et al.* [44] investigated the bacterial growth inhibition, total phenolic content, and anti-oxidant activity of the chitosan edible film incorporated with *Thymus moroderi* and *Thymus piperella* EOs through disc diffusion assay, DPPH, FRAP, and FIC, respectively. The Chitosan film containing the *Thymus piperella* was found to be effective against *Serratia marcenscens and Listeria innocua* than the chitosan containing *Thymus* *moroderi*, and no significant difference was observed between the chitosan incorporated with both EOs against *Aeromonas hydrophila* and *Achromobacter denitrificans*. Also, the chitosan incorporated with *Thymus moroderi* essential oil has shown lower antioxidant activity than *Thymus piperella* essential oil at all concentrations [44].

Perdones, Chiralt and Vargas, [23] formulated emulsions and films based on chitosan with basil or thyme EOs and observed that the retention of essential oil in the chitosan films depends significantly on the stability of the film-forming emulsion during film formation. The addition of oleic acid to the chitosan-essential oil formulation improved the stability of emulsions and the retention of the EOs in the film while at the same time increasing the water-vapour barrier properties of the film. The addition of the essential oil also decreased the stretchability of the film which was later mitigated with the addition of oleic acid in the formulation. However, oleic acid significantly reduced the transparency of the film to a larger extent than pure essential oils [23]. Also, the chitosan: thyme and chitosan: basil oil films exhibited higher elasticity modulus and elongation at break than chitosan or chitosan: oleic acid films; and the result of the *in vitro* antifungal test indicated that the films with the either of the essentials oils did not inhibit the growth of *Aspergillus niger, Botritis cinerea,* and *Rhizopus stolonifera* [23].

In a different study, an *in vitro* and *in vivo* anti-fungal activity of chitosan incorporated with clove oil was tested against *Penicillium digitatum* which is the causative agent of citrus green mould in a study published by Shao et al. [9]. All treatments with chitosan and clove oil showed anti-fungal activity in the in vitro assay with 99.5% inhibition in a combined treatment with the EOs from the clove. Further, the result also indicated that a formulation based on chitosan with clove oil inhibited mycelial growth to a greater extent than the individual treatments from either the chitosan or clove oil, respectively. Chitosan combined with clove oil increased the leakage of the cell membrane which allowed the release of more cellular material to the supernatant than chitosan or clove oil alone. The results of the transmission electron microscopy examination of *P. digitatum* hyphae at 2 minimum inhibitory concentration revealed that fungal cells subjected to chitosan and/or clove oil treatment suffered structural damage, and the combined treatment showed greater damage than individual treatments [9]. These same authors (i.e. [9]) also stated that the synergistic anti-fungal activities of chitosan-clove oil observed in the *in vitro* tests were not found in the *in vivo* tests; consequently, the data suggested that a coating based on 1% chitosan alone, not in combination with clove oil, can contribute effectively to the control of green mould on citrus fruits.

3. CONCLUSION

Globally, consumers are continuously demanding safe and nutritious foods packaged in materials that are devoid of synthetic chemicals and other potentially unsafe materials. This necessitated the need to develop sustainable coating materials that can effectively protect the quality and safety of different food products and possessing myriads of advantages including non-toxicity, bio-degradability, edibility, bio-compatibility, renewability and environmentally-friendly properties. Among the biopolymers studied so far, chitosan, the second most abundant polysaccharide found in nature, after cellulose has shown a great promise owing to its desirable film-forming properties, physicochemical properties, non-toxicity, bio-degradability, human tissues bio-compatibility, anti-microbial and anti-oxidant activities. Chitosan-films incorporated with essential oils (EOs) have shown a great improvement in the physical and mechanical properties of the films and at the same time enhances the antioxidant and antimicrobial properties. Several studies suggested that chitosan and EOs can be incorporated to achieve modern food packaging that can enhance and ensure quality and safety of several food products including fresh fruits, vegetables, and meat as well as their products owing to their synergistic anti-microbial effect. However, future studies should also focus on the economic viability and environmental sustainability aspects of EOs use in active food packaging in light of the growing trend of ‘green’ consumerism, resource-intensive nature of EOs, and the declining production output of aromatic plants.

Contributors

All the authors wrote the first and final draft of this manuscript after detailed discussion and communication with one another. All the authors draft the revisions and approved the final version of this reviewed paper. Also, all authors have no conflict of interest associated with this review study.

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