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A Mini-Review on Essential oils, Chitosan, and Their Application in Preserving Fruits and Vegetables

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The promising eatable coatings, which were obtained by the combination of chitosan and essential oils (EO), attracted many researchers. This article summarizes recent studies on fruit and vegetable preservation using chitosan and EOs. CS-essential oils (clove, thyme, cinnamon, cymbopogon martinii, lemon oil, olive oil, cardamom oil, heracleum persicum oil) brings remarkable benefits such as: against many harmful microorganism (many strains of gram-positive and gram-negative bacteria: L. monocytogenes, S. aureus, S. Typhi, E. coli and the fungus Colletotrichum gloeosporioides). They also prevented weight loss, and maintained stable quality in color, firmness, and nutrient content, of many fruits and vegetables such as pomegranate arils, mango, cherry fruits, avocados, bell pepper, lettuce, and carrot under storage conditions.

1. Introduction

Essential oils (EOs) are common compounds of different aromatic plants (Maurya et al., 2021). They were concentrated in roots, peels, leaves, seeds, fruits, and barks. Plant essential oils include both polar and nonpolar compounds (Masango, 2005). And its main constituents are terpenes, aromatic compounds, oxygenated compounds, and terpenoids (Baldim et al., 2019), which provide many biological activities. Every year billion tons of chitin - a rich source of by-products derived from crustacean shells, are released into the environment. After chitin's acetyl groups are reduced, a product named chitosan (CS) is formed. CS's antimicrobial, antioxidant, and eco-friendly properties give it a potential biofilm-forming agent which prevents oxidation and bacteria growth. In recent years, packaging films made from biomaterials have been reported. Among them, CS emerged as a promising carrier system. Its combination with phenolic acids, plant extracts, or EOs has resulted in food packaging film and coating solutions that are both efficient and environmentally friendly (Torrier et al., 2015). The non-toxicity, antibacterial and bio-absorbable, film-forming, and soluble nature of CS, allow it can be obtained in various forms: nanoparticles, coated films, or gels (Muxika et al., 2017). Besides, the composition of EOs includes many natural compounds with outstanding biological activities such as antibacterial, antifungal, and volatile. EOs or nano EOs are often encapsulated by chitosan, to form coating solutions or films. This combination is intended to enhance antimicrobial resistance, and limit the evaporation of essential oils (Sotelo-Boyas et al., 2017). Nowadays, to ensure the quality of postharvest decay, many strategies have been applied such as cold storage or sterilizing the products. According to the goal of prolonging the shelf life and stabilizing the quality of vegetables and fruits, many studies have been published, on the use of microbial antagonists (Sharma et al., 2009), chemical substances (Spadoni et al., 2016), physical agents (Najafabadi et al., 2017), and also natural compounds (Chaves-López et al., 2012). In recent years, packaging films made out of bio-materials have been reported. Among them, CS emerges as a promising carrier system. Its combination with phenolic acids, plant extracts, or EOs resulted in coating solutions, and food packaging films that are both effective and environmentally friendly (Torrier et al., 2015). The non-toxicity, antibacterial and bio-absorbable, film-forming and soluble nature of CS, allow it can be obtained in various forms: nanoparticles, coated films or gels (Muxika et al., 2017). Besides, the composition of EOs includes many natural compounds with outstanding biological activities such as antibacterial, antifungal, and volatile. Therefore, EOs or nano EOs are often

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encapsulated by chitosan, to form coating solutions or films. This combination is intended to enhance antimicrobial resistance, and limit the evaporation of essential oils (Sotelo-Boyas et al., 2017).
Herein, this is a brief overview of producing and using CS films and coatings containing EOs to fruit preservation in the recent literature.

2. Essential oils (EOs)

2.1 EOs in plant

Essential oils - the secondary metabolites play important roles in many biochemistry processes of plants and relate them to their ecosystem. Pigments and aromatic compounds provide color and scent to flowers and fruits, support pollination and seed dispersal, while the volatile compounds protect plants against herbivores, insects, and microbial (Rios, 2016). Realizing the usefulness of these secondary compounds, humans have long used them as food and medicine.Among many phytochemicals in plants, EOs attracted a lot of attention. EOs form in oil cells, and because of EOs volatility, they can be collected by steam distillation method (Ríos, 2016). Based on the molecular structure of the constituent compounds, EOs can be grouped as follows (Morsy et al., 2017): terpenes, straight-chain compounds, phenylpropanoids, miscellaneous groups.

2.2 Anti-microbial property of essential oils

Over the past few decades, the emerging food safety potential of the EOS has encouraged scientists to investigate its antibacterial, antifungal, and antioxidant properties. Because of the diversity in the chemical composition of essential oils, as noted above, it provides effective mechanisms for scavenging free radicals, as well as against microbial growth (Chaudhari et al., 2021). The EOs' lipophilic property is related to their antibacterial efficacy. The interaction between EOs with microbial cell membrane components, allows them to attack cell. In the case of bacteria, the gram-negative cell membrane has a rather complex structure than the gram-positive one. This explains why EOs are capable easily of causing damage to gram-positive bacteria (Maurya et al., 2021). Thymol EOs react with lipids on bacterial cell membranes, causing disturbances and changes in membrane permeability (Sokolik et al., 2018). Similarly, the lipophilic EOs carvacrol causes membrane instability in various pathogenic bacteria (Rudramurthy et al., 2016). Furthermore, once EOs disrupt bacterial cell membranes, DNA, RNA, and proteins are also compromised (Han et al., 2020). The bacterial cells often exchange information with each other by forming biofilms (Snoussi et al., 2018). Therefore, inhibition of biofilm formation by EOs causes many harmful effects on bacteria (Bouyahya et al., 2017). For the same reason, EOs are used as effective agents in the prevention of antibiotic-resistant bacteria (Saxena et al., 2019).
The antifungal effect of EOs is similar to antibacterial mechanisms, and also depends on their components. The EOs is destructive to the fungal cell wall leading to the disintegration of mitochondrial membranes and also damaging the cytoplasm, resulting in fungal cell death. In yeast cells, ATP could not be synthesized by EOs' sabotage, leading to cell damage (Swamy et al., 2016).

3. Chitosan (CS)

3.1 Chitosan and methods of preparation

Chitosan is a polymer with chains *D*-glucosamine binds to *N*-acetyl-*D*-glucosamine. The *β*-1,4-*D*-glucosamine ratio is also known as the degree of deacetylation (DD). In the chitosan molecule, DD is directly proportional to the number of amino groups, the functional groups can form hydrogen bonds, bringing the water solubility and biological activity of CS. According to previous reports, there are two types of methods for the preparation of CS: chemical methods and biological methods (Figure 1a) (Kou et al., 2018).

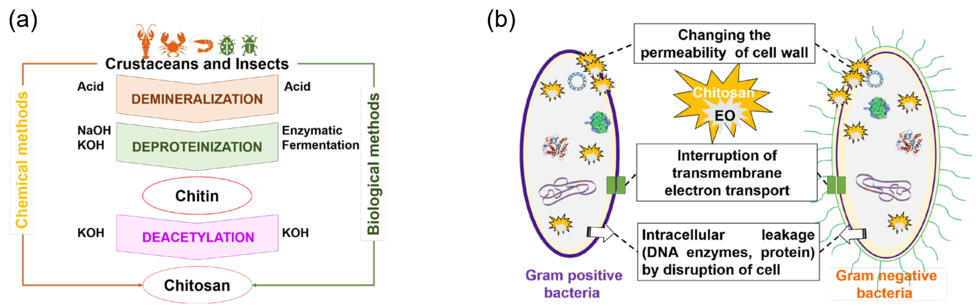

Figure 1: Methods of (a)chitosan preparation, and (b) the antibacterial plausible mechanism of CS-EOs

3.2 Chitosan's applications

According to the properties of CS: non-toxic, antibacterial, and bio-absorbed nature, easy gelation, CS offers many advantages in various fields such as pharmacy, cosmetics, wastewater treatment, and agriculture. Polyphenols is a precious compound found in tea, but it is easily oxidized and decomposed. Based on the properties of chitosan, Liang et al. (2017) have carried out encapsulation of polyphenols in tea with chitosan, to keep the stable nutritional and pharmacological of tea. Besides, the chitosan molecule contains a large number of functional groups, which are able to react with inorganic, organic compounds and absorb pollutants (Badawi et al., 2017). In the report of Boardman et al. (2018), CS hydrogel has proven effective in treating a wide range of colors in dye-containing wastewater.

In sustainable agriculture, minimizing pesticides and at the same time enhancing crop growth are urgent issues. And CS, a product of natural origin, is a suitable alternative to synthetic pesticides, reducing negative impacts on humans and the environment (Xing et al., 2015). Oliveira et al. (2016) used CS nanoparticles to encapsulate Nitric oxide. This is a method to control the adverse effects of salinity on maize growth. Deshpande et al. (2017) afforded crosslink CS with sodium tripolyphosphate and produced zinc-complexed CS nanoparticles as nanocarriers for micronutrient delivery. Using this complex in the growth processes of wheat plants, the results showed that zinc-complex CS is a slow-released form of fertilizer. It helps to control well the source of nutrients for plants, avoiding waste and environmental pollution. In cosmetic research and development, CS acts not only as a natural antibacterial compound but also as a material to encapsulate active ingredients (Morsy et al., 2017). CS acts as both an antibacterial active ingredient and a substrate in multifunctional sunscreens. Libio et al. (2016) used CS membranes for exfoliating applications on the skin.

4. Application of chitosan-essential oils (CS-EOs) on fruits and vegetables preservation

The combination of CS-EOs was reported in a study by Zhang et al. (2021). The coating dispersions and film-forming were synthesized and enhanced with antibacterial activity. According the advance in microbial resistant (Figure 1b), there are reports using of CS-EOs complexes for fruits and vegetables preservation (Table 1).

Table 1: Recent studies on the preservation of fruits and vegetables by CS-EOs

DDA of chitosan	Coating dispersion/ Film-forming	EOs	Fruits and vegetables	References
75-85 %	Coating dispersion	Clove	Pomegranate	Hadidi et al., 2020
90 %	Coating dispersion	Cinnamon	Mango	Hasheminejad et al., 2020
75-85 %	Coating dispersion	Lemon	Strawberries	Perdones et al., 2016
90 %	Coating dispersion	Eryngium campestre L.	Cherry fruits	Arabpoor et al., 2021
75–85 %	Coating dispersion	Thyme	Avocado cv. Hass	Correa-Pacheco et al., 2017
75-85 %	Coating dispersion	Cymbopogon martini	Maize grain	Kalagatur et al., 2018
75-85 %	Film-forming	Olive oil	Apple	Khalifa et al., 2017
75-85 %	Coating dispersion	Heracleum persicum fruit	Bell pepper	Taheri et al., 2020
75-85 %	Coating dispersion	Mandarin	Green beans	Donsì et al., 2015
_	Coating dispersion	Origanum majorana	Lettuce	Xylia et al., 2021
98 %	Coating dispersion	Thyme	Carrot	Viacava et al., 2022

Clove essential oil (CEO) has been studied and is known as an effective antibacterial and antifungal agent. Hasheminejad et al. (2019) investigated the antifungal properties of CS combined with clove oil on *Aspergillus niger*. Briefly, this EO was encapsulated by CS nanoparticles (ChNPs) using ionic gelation technique. The results show that the ratio of CS to TPP to CEO of 1:1:1 was performed as optimum oil–loaded ChNPs. Furthermore, the fungal growth was completely inhibited by CEO – ChNPs (1.5 mg/mL). Hadidi et al. (2020) encapsulated clove EO in CS nanoparticles to form the CEO-ChNPs. And many strains of gram-positive and gram-negative bacteria (*L. monocytogenes*, *S. aureus, S. Typhi* and *E. coli*) have been damaged by this CEO-ChNPs. In another research by Hasheminejad and Khodaiyan, similar method was applied to coating pomegranate arils and studied their shelf life and quality. The incorporation of CS with the CEO could provide the most effective in reducing the weight loss of pomegranate arils (Hasheminejad et al., 2020).

Cinnamon essential oil is a yellow aromatic oil and has the ability to inhibit many types of bacteria, molds, and yeasts. Its use is limited by its volatility and irritating nature. Yin et al. (2019) have studied the microencapsulation of cinnamon essential oils in CS to improve the quality of mangos. Compared with CS - EOs uncoated mangoes, the coated ones had a significant reduction in weight loss; reduce the loss of nutrients; inhibits cellular respiration; retains solidity at 25 °C storage conditions. CS nanoparticles and cinnamon essential oil was also applied on zein nanocomposite (Vahedikia et al., 2019) against both Gram-positive (*S. aureus*) and Gram-

negative bacteria (*E. coli*). The citrus group has long been studied to apply their EOs to life due to their excellent antimicrobial and antioxidant abilities. Filho et al. (2020) made incorporation between Citrus limonia into CS film. The result has coincided with previous reports that the antibacterial resistance of these films obtained corresponds to the EOs limonene content. In many cases, chitosan-essential oil coating can be considered a novel approach to vegetable preservation, food preservation, and bioactive packaging. Perdones et al. (2015) demonstrated that despite a good preservative effect on strawberries, CS-lemon oil coating contained many volatile constituents that affected the aroma of this fruit. It gave negative sensory prices to the product.
Correa-Pacheco et al. (2017) demonstrated that at concentrations of 3 % and 5 %, CS -thyme nanosystems (CSTEO - NPs) were effective against the fungus *Colletotrichum gloeosporioides*. In addition, this coating solution also improved the preservation quality of cv avocados Hass. In a study by Shetta, et al. (2018) with menthol (PO) and green tea oil (GTO), by using emulsion cross-linking - ion gelation, the antioxidant capacity of EO was improved about 2 times, GTO's is better than PO's. The antibacterial properties have been studied on *Staphylococcus aureus* and *Escherichia coli*. PO should not be used as an encapsulation because it reduces antibacterial activity, while coated GTO gives 4-5 times more resistance. Jamil et al. (2016) studies showed that CS nanoparticles, loaded with cardamom oil, were found to be able to inhibit ESBL (extended-spectrum *β*-lactamase) - a dangerous property of intestinal bacterial strains. Kalagatur et al. (2016) provided the minimum inhibitory and minimum fungicidal concentrations of Cymbopogon martinii essential oil (CMEO 421.7 ± 27.14, 618.3 ± 79.35 ppm. In addition, the Chitosan-CMEO-NPs complex shows great potential for post-harvest preservation of agricultural products. The results showed that CS-olive oil coating stabilized the quality of apples under low-temperature storage conditions. CS-olive coating- an edible film, maintains a low weight loss effect, delays many biochemical processes, and avoids slurry and rotting in ripe fruit (Khalifa et al., 2017).
Taheri et al. (2020) conducted research on the extension preservation of bell pepper by using the ionic gelation method to encapsulate Heracleum persicum fruit EOs in CS nanoparticles (HPEO – CSNPs). The study was keened on the ability of HPEO-CSNPs to resist strong oxidizing agents during the storage period of one month. The final results showed that bell peppers coated with HPEO-CSNPs maintained stable quality in color, firmness, and nutrient content after 24 d of storage, while those without treatment only maintained for 18 d. As a similar example, the CS-Eryngium campestre (CHNP - ECEO) nanosystem coated on cherries reduced fruit weight loss (Arabpoor et al., 2021).

5. Conclusion

To ensure the quality and prolong the shelf life of fruits and vegetables, derived micro-films are used more and more commonly. The combination of chitosan and essential oils could effectively fight against bacterial, and fungal strains and apply for the preservation of fruits and vegetables (pomegranate, mango, strawberries, green beans, avocado cv. Hass, maize grain, apple, bell pepper, cherry fruits, lettuce, carrot). Some EOs face their volatility, and strong scent, which degrades the sensory perception of food. Controlling the influence of EOs scent is an issue that needs further attention.

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