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Uma Maheswari T
Department of Food Science and Nutrition, CSC&RI, TNAU, Madurai, Tamil Nadu, India

Selvi J
Department of Food Science and Nutrition, CSC&RI, TNAU, Madurai, Tamil Nadu, India

Corresponding Author:
Uma Maheswari T
Department of Food Science and Nutrition, CSC&RI, TNAU, Madurai, Tamil Nadu, India

Studies on improving the efficacy of eco-friendly chitosan based biodegradable antimicrobial films

Uma Maheswari T and Selvi J

Abstract
Due to increased demand in packaging films with more environmentally-friendly biodegradable materials. Recently, green packaging is gaining importance due to environmental and health reasons. Among various green packaging solutions, chitosan based films are becoming popular as it is commercially available seafood industry byproduct. It is non-toxic, biodegradable, has got strong antimicrobial and antifungal activities. It also has got good film forming property and has the ability to minimize respiration rate. But the main disadvantage is its relatively poor water vapour barrier characteristics. To improve the packaging property, composite coatings can be made using essential oils. Essential oils are gaining interest as natural preservatives which can reduce the risk of microbial attack especially fungi on perishables and also improves water barrier property of chitosan. Hence, chitosan and essential oil based composite packaging can be effectively used as biobased degradable packaging material. The antifungal activity of chitosan and four selected essential oils against *Fusarium* spp, *Aspergillus* spp, were tested and it was found that all essential oils @ 0.3% level completely inhibited the test organisms. Biodegradable packaging films were prepared using Chitosan @1% and 1.5% level with and without incorporating essential oil *viz*., tea tree oil, lemon grass oil, basil oil and thyme oil. It was found that films made with incorporating essential coil had good stability and strength. The addition of essential oil decreased the water vapour permeability, film solubility and swelling index which showed the improvement in moisture barrier properties of the films. The other physical properties of the films such as film thickness and water resistance got increased with addition of essential oil. Among the various essential oils used, tea tree oil and lemon grass oil were on par and recorded as best with improved physical properties. Hence, chitosan and essential oil incorporated films could be used in food packaging sector to replace the petrochemical based packaging material.

Keywords: chitosan, essential oils, physical and mechanical property, antimicrobial packaging film

Introduction
Since long time, synthetic petrochemical based packaging materials were dominated in food industry for storing foods. In spite of possessing many advantages such as ease of production, low cost, and superior barrier qualities of polythene based packaging materials, the disposal of these non-biodegradable materials elevated the environmental pollution. Because of this, there is need for natural eco-friendly packaging materials. These innovative films should be safe, sustainable, biocompatible, biodegradable and current trend towards green consumerism. Since spoilage occurs mainly on the food surface there is no need to add antimicrobial agents in the bulk of the food, but just to the headspace. Hence, a variety of biopolymers are explored in food packaging due to its simple film-forming ability, biodegradability, superior ability to block oxygen and water vapour, antimicrobial activity and strong mechanical strength [1]. Among these, chitosan, a byproduct obtained from shells of shrimp, crab is largely used for developing biobased films. However, the water barrier property, antioxidant and antibacterial potential needs to be improved for its successful use as active food packaging film. In order to improve mechanical properties of chitosan films, hydrophobic compounds, such as lipids and plant derived essential oils can be incorporated. And also essential oils are known to possess good spectrum of antimicrobial activity. The shelf life of food products can also be increased by adding essential oils to biopolymer films [2]. These films can also provide packaging systems with antioxidant or antibacterial qualities.

Materials and Methods
Materials used
Chitosan and all other chemicals of analytical grade were purchased from HiMedia chemicals, Mumbai. Essential oils *viz*., Tea Tree Oil, Lemon Grass Oil, Thyme Oil and Basil Oil were

purchased from a local organic farm stores, Madurai, India.

Antifungal activity of chitosan and essential oil

The antifungal activity of the chitosan (CH) solution, Lemon grass oil (LGO), Tea tree oil (TTO), Thyme oil (TO) and Basil oil (BO) were tested against fungal cultures *viz*., *Aspergillus flavus*, and *Fusarium* sp. The antifungal effect of lemon grass oil, tea tree oil, thyme oil, basil oil and chitosan at various concentrations were evaluated by poison plate technique [3]. Four different essential oils and chitosan solutions were filter (0.22 µm) sterilized. Experiments were carried out with different concentrations of chitosan (0.1%, 0.3% and 0.5%), essential oils (0.1%, 0.3%and 0.5%). The components were mixed with sterilized Potato Dextrose Agar (PDA) in separate flasks. It was then pour plated and allowed to solidify. Seven days old fungal culture disc of 0.5 cm diameter was taken and inoculated to the centre of petriplates containing various concentrations of essential oils and chitosan under aseptic condition. PDA medium with 0.3 M acetic acid and PDA medium alone served as control. All plates were incubated at 28±2 °C and radial growth of colony was measured on 0, 3, 5 and 7 days of incubation. Each test was performed in triplicates.

Film preparation with chitosan and essential oil

For preparing film, 1% chitosan solution was prepared by dissolving 1g chitosan in 100 ml of 1% acetic acid solution with constant stirring. The resultant chitosan solution was filtered using a Whatman No. 3 filter paper. The essential oil (EO) *viz*., Lemon grass oil, Tea tree oil, Basil oil and Thyme oil was added separately to the chitosan film forming solution (FFS) at three different concentrations of 0.1, 0.2 and 0.3% and one batch of solution without adding EO was kept as control. Tween 80 (0.05% v/v of the film forming solution) was used as an emulsifying agent and Glycerol was added at 0.1% v/v as plasticizer. The solution (250 ml) was then homogenized using a magnetic stirrer before casting. Then the solutions were casted into a tray and were air dried for 24-48 h. The films are peeled off and conditioned in a Dessicator until further use. Chitosan films prepared without essential oil served as control [4].

Film thickness measurement

The thickness of the sample was determined using a screw gauge equipment. Measurements were repeated in 5 different regions of each sample and then an average value was calculated.

Swelling index of films with chitosan and essential oil

Films were weighed and subsequently immersed in water for 10 minutes [5]. The mass of the swollen films was measured after the surface water was removed with filter paper. The swelling index (SI) of the film was calculated.

$$\text{Swelling index (\%)} = \frac{W_s - W_d}{W_d} \times 100$$

W_s ⟶ weight of the swollen film

W_d ⟶ weight of the film

This property predicts the maintenance of quality during packaging and storage of food products. In some cases, a higher swelling index can be desirable to absorb extra water from outer surface of food with high moisture.

Water resistance of films with chitosan and essential oil

The solubility was measured by immersing weighed pieces of chitosan film in 50 ml of distilled water by a constant using magnetic stirrer for 15 min at room temperature. Subsequently, the solutions were filtered at room temperature using whatman no.1 filter paper. The chitosan films and the wet filters were dried at 110 °C until constant weight [5].

$$\text{Water resistance of the film (\%)} = \frac{\text{Initial swollen wt. (mg)} - \text{Final dry wt. (mg)}}{\text{Initial swollen weight (mg)}} \times 100$$

Film solubility in water of films with chitosan and essential oil

A modified method from [5] was used to measure film solubility. Film was weighed at 50 mg and were dried at 110°C in a hot air oven for 24 h and then weighed for initial dry weight. Then films were placed in glass beaker with 50 ml of distilled water and shaken gently by using centrifuge at 25°C for 24 h. The solution was then filtered Whatman No.1 filter paper to recover the remaining undissolved films. The remaining pieces of film after immersion were dried at 110°C to constant weight (final dry weight). Solubility in water (%) was calculated by using,

$$\text{Solubility in water (\%)} = \frac{\text{Initial dry weight (mg)} - \text{Final dry weight (mg)}}{\text{Initial dry weight (mg)}} \times 100$$

Water vapour permeability of films with chitosan and essential oil

Water vapour permeability was determined by the method as described by [6]. Fifty ml water was measured and taken in 100ml beakers, each beaker is covered with different CH and EO based packaging material along with control (HDPE). The beakers covered with different packaging material was kept at room temperature for 24h and the loss in volume of water is measure which shows the water vapour permeability of the packaging material.

Moisture content of films with chitosan and essential oil

To determine the moisture content of the films, approximately 50 mg of film were dried at 105 °C for 24 h [5]. The weight of the sample was determined, and moisture content was calculated as the percentage of water removed from the film. The moisture content of the film pieces was determined measuring the weight loss of films, upon drying them in an oven until a constant weight was reached. The moisture content was calculated by using the formula given below.

$$\text{Moisture content of the film (\%)} = \frac{\text{Initial weight (mg)} - \text{Final dry weight (mg)}}{\text{Initial weight (mg)}} \times 100$$

Results and Discussion

Antifungal activity of chitosan and essential oil

Chitosan film @ 1.5% concentration exhibited maximum antifungal activity whereas essential oil incorporated PDA controlled the fungal growth completely even at 0.3% concentration. No growth of *Fusarium* sp. and *A.flavus* was observed till day 7 by poison plate technique irrespective of essential oils used.

Table 1: Antifungal effect of chitosan and essential oils

S. No	Treatment details	*Fusarium* spp. (mm)					*Aspergillus flavus* (mm)				
		Day 0	Day 1	Day 3	Day 5	Day 7	Day 0	Day 1	Day 3	Day 5	Day 7
1.	Control	0.5±0.0	1.23±0.05	6.2±0.1	9.26±0.05	9.76±0.11	0.5±0.0	1.43±0.05	5.23±0.05	8.2±0.1	9.43±0.05
2.	Acetic acid	0.5±0.0	1.26±0.05	6.06±0.11	9.1±0.17	9.53±0.05	0.5±0.0	1.3±0.1	5.06±0.05	8.3±0.2	9.16±0.2
3.	1% CH	0.5±0.0	0.76±0.05	0.93±0.05	1.93±0.05	2.43±0.05	0.5±0.0	0.83±0.05	1.13±0.05	1.83±0.05	2.3±0.05
4.	1.5% CH	0.5±0.0	0.5±0.0	0.83±0.05	1.46±0.11	2.13±0.15	0.5±0.0	0.56±.011	0.76±0.05	1.1±0.1	1.56±0.11
5.	0.1 TTO	0.5±0.0	0.5±0.05	1.3±1.0	1.86±0.15	2.46±0.15	0.5±0.0	0.5±0.5	2.1±0.2	2.3±0.2	2.86±0.15
6.	0.3 TTO	0.5±0.0	0.5±0.0	0.5±0.05	1.33±0.15	1.66±0.25	0.5±0.0	0.5±0.5	1.0±0.2	1.8±0.1	3.06±0.15
7.	0.5 TTO	0.5±0.0	0.5±0.0	0.5±0.0	0.56±0.05	0.66±0.05	0.5±0.0	0.5±0.5	0.53±0.05	0.53±0.05	0.6±0.1
8.	0.1 LGO	0.5±0.0	0.76±0.11	1.0±0.15	1.46±0.05	1.66±0.05	0.5±0.0	0.76±0.05	1.0±0.1	1.33±0.05	1.9±0.1
9.	0.3 LGO	0.5±0.0	0.53±0.05	0.6±0.1	0.76±0.05	1.26±0.05	0.5±0.0	0.56±0.05	0.53±0.05	0.76±0.05	1.33±0.15
10.	0.5 LGO	0.5±0.0	0.5±0.0	0.53±0.05	0.53±0.05	0.5±0.0	0.5±0.0	0.53±0.05	0.53±0.05	0.5±0.0	0.5±0.0
11.	0.1 TO	0.5±0.0	1.3±0.2	6.1±0.35	9.1±0.4	9.7±0.2	0.5±0.0	1.4±0.1	5.3±0.26	8.03±0.2	9.53±0.15
12.	0.3 TO	0.5±0.0	0.76±0.11	1.0±0.15	1.46±0.05	1.66±0.05	0.5±0.0	0.76±0.05	1.0±0.1	1.33±0.05	1.9±0.1
13.	0.5 TO	0.5±0.0	0.5±0.0	0.5±0.05	0.5±0.05	0.51±0.0	0.52±0.0	0.53±0.05	0.53±0.05	0.53±0.0	0.59±0.0
14.	0.1 BO	0.5±0.0	2.3±0.2	4.9±0.35	8.5±0.4	9.2±0.2	0.5±0.0	1.9±0.1	3.3±0.26	6.5±0.2	8.55±0.15
15.	0.3 BO	0.5±0.0	0.56±0.11	0.95±0.15	1.15±0.05	1.25±0.05	0.5±0.0	0.76±0.05	0.99±0.1	1.28±0.05	1.59±0.1
16.	0.5 BO	0.5±0.0	0.51±0.0	0.52±0.05	0.52±0.05	0.55±0.0	0.51±0.0	0.53±0.05	0.53±0.05	0.57±0.0	0.59±0.0

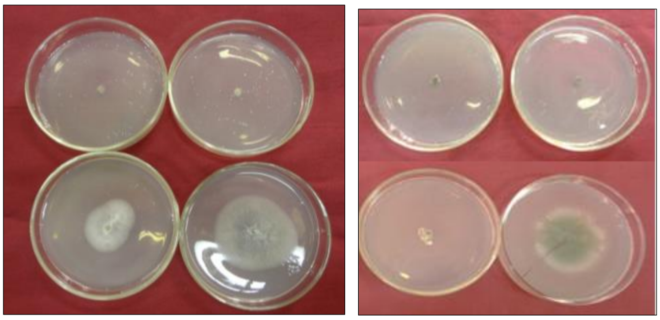
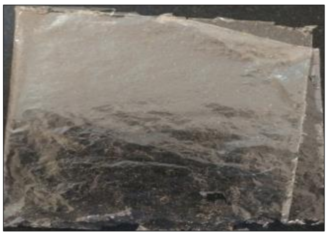


Fig 1 Inhibitory effect of essential oil on the growth of *Fusarium* spp. and *Aspergillus flavus*

Film preparation

Depending on the type of chitosan and the degree of deacetylation, the suitable chitosan concentration to be added to the film-forming solution will be different [7]. Chitosan films were prepared at three different concentrations *viz*, 0.5, 1.0 and 1.5% among which 0.5% chitosan films were very thin and has very poor mechanical property. Physical properties *viz*., film thickness, swelling index, film solubility, water resistance, water vapour permeability and moisture content.



Chitosan + Tea Tree Oil film

Chitosan + Basil oil film

Chitosan + Lemon grass oil film

Chitosan + Thyme oil film

Film thickness measurement

All of the films were transparent. The film thickness was dependent of the film composition and the thickness ranges from 0.90 to 0.98 mm, indicating that the thickness of the films was higher as the concentration of essential oils is increased (Table 2).

Table 2: Film thickness for chitosan films (expressed in mm)

S. No	Concentration of the oil	Tea tree oil		Lemon grass oil		Thyme oil		Basil oil	
		1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan
1.	0.1% oil	0.94	0.95	0.92	0.96	0.95	0.96	0.96	0.97
2.	0.2% oil	0.94	0.95	0.93	0.97	0.95	0.96	0.96	0.97
3.	0.3% oil	0.95	0.96	0.94	0.97	0.96	0.98	0.97	0.97

SED - 0.7580 CD(0.05) - 1.3495, CD(0.01) - 1.8772

Swelling index of films with chitosan and essential oil

The swelling degree, solubility in water were calculated according to the research of [8] with some modifications. By three factor ANOVA, among the treatments, lemon grass oil was found to be best superior in swelling index followed by tea tree oil, thyme oil and basil oil respectively at 0.3% concentration. Among 2 different chitosan concentration, 1.5% chitosan was observed to be superior in swelling index than 1.0% chitosan. The results shown in table 3 represents that CH+TT oil film and CH+LG oil decreases swelling index by the increased concentration of essential oil *viz.*, 0.1%, 0.2% and 0.3%.

Table 3: Swelling index for chitosan films (expressed in %)

S. No	Concentration of the oil	Tea tree oil		Lemon grass oil		Thyme oil		Basil oil	
		1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan
1.	0.1% oil	130	140	160	120	115	120	110	100
2.	0.2% oil	120	158	160	130	130	145	125	115
3.	0.3% oil	114	160	130	140	140	160	135	125

SED - 0.74365 CD(0.05) - 1.48359, CD(0.01) - 1.97025

Water resistance of films with chitosan and essential oil

Water resistance property of packaging material is important in food industry applications because the packaging must protect the food while it is in contact with water or foods with a high water activity. Chitosan film presented a low solubility. However, when the EOs were added to the film, a significant increase in water solubility was observed. Generally addition of essential oils improved the water resistance compared to control chitosan film. In specific, the LGO incorporated chitosan films had more water resistance than Tea tree, Thyme and basil oil. Table 4 shows that water resistance of the essential oil incorporated film increased in both 1% and 1.5% concentrations as compared to CH films alone in both 1% CH and 1.5%CH.

Table 4: Water resistance for chitosan films (expressed in %)

S. No	Concentration of the oil	Tea tree oil		Lemon grass oil		Thyme oil		Basil oil	
		1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan
1.	0.1% oil	18	20	16	28	15	18	16	18
2.	0.2% oil	20	26	26	36	18	28	20	25
3.	0.3% oil	30	30	32	40	24	32	26	34

SED - 0.7765 CD(0.05) - 1.3435, CD(0.01) - 1.9905

Film solubility in water of films with chitosan and essential oil

Film solubility plays an important role in food packaging, since they can affect the resistance of films to water. Film solubility significantly decreased with increase in essential oil incorporation compared to CH films. The solubility of films decreased above 0.1% incorporation essential oils.

Table 5: Film solubility for chitosan films (expressed in %)

S. No	Concentration of the oil	Tea tree oil		Lemon grass oil		Thyme oil		Basil oil	
		1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan
1.	0.1% oil	14.6	16.6	12.5	19.0	10.5	11.5	11.0	10.0
2.	0.2% oil	10.8	11.6	10	11.1	9.5	10.0	10.0	9.5
3.	0.3% oil	6.5	8.5	9.5	4.4	8.5	9.5	8.5	8.0

SED - 0.7656 CD(0.05) - 1.4125, CD(0.01) - 1.9898

Among different treatments, LGO incorporated chitosan films was superior than other Eos. The decrease in solubility and swelling degree could be attributed to the increasing cross linking interactions between chitosan and hydrophobic essential oil. The water solubility obtained for the chitosan film combinations agrees well with results reported in literature [9].

Water vapour permeability of films with chitosan and essential oil

Water vapour permeability (WVP) is an important parameter commonly considered in food packaging that comprises sorption, diffusion and adsorption. WVP should be as low as possible as it the property which retards moisture transfer between the food and the environment. Water vapour transfer generally occurs through the hydrophilic portion of the film; thus, WVP depends on the hydrophilic-hydrophobic ratio of the film components. Hence, the chemical nature of essential oil plays an important role in the barrier properties of food packaging films.

Table 6: Water vapour permeability for chitosan films (expressed in %)

S. No	Concentration of the oil	Tea tree oil		Lemon grass oil		Thyme oil		Basil oil	
		1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan
1.	0.1% oil	1.25	1.25	2.5	1.25	1.15	1.15	1.15	1.25
2.	0.2% oil	1.25	1.25	1.25	0.625	1.0	1.15	0.95	1.0
3.	0.3% oil	0.625	0.625	0.625	0.625	0.95	1.0	0.75	1.0

SED - 0.6765 CD(0.05) - 1.4934, CD(0.01) - 1.9225

Water vapour permeability (WVP) was very less in chitosan incorporated with 0.3% tea tree oil and lemon grass oil (0.625%) followed by basil and thyme oil. Whereas WVP was found to be maximum in chitosan based film (1.87%) which shows that incorporation of essential oil enhances the water barrier property. Rubilar *et al.* [10] reported that incorporation of Grape Seed Extract and carvacrol had a significant effect ($p < 0.05$) on WVP of chitosan films. This result may be explained by the hydrophobic nature of carvacrol, which affects the hydrophilic/hydrophobic balance of the film.

Moisture content of films with chitosan and essential oil

The addition of the essential oils decreased the moisture content value significantly, which is attributed to an increase in hydrophobicity of films. This result was shown in table 7 in which CH+LG oil recorded least moisture content followed by other essential oil than chitosan alone. It showed that the more hydrophilic films those that present the highest values of moisture content. These are in agreement with the results obtained for the same Chitosan concentrations reported in literature [11]. The essential oil inclusion causes the formation of covalent bonds between the functional groups of chitosan chains. This phenomenon leads to a decrease in the availability of hydroxyl and amino groups and limits the interactions between polysaccharide and water by hydrogen bonding.

Table 7: Moisture content for chitosan films (expressed in %)

S. No	Concentration of the oil	Tea tree oil		Lemon grass oil		Thyme oil		Basil oil	
		1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan	1% chitosan	1.5% chitosan
1.	0.1% oil	20	16	18	16	20	15	22	20
2.	0.2% oil	20	10	8	14	20	15	22	16
3.	0.3% oil	16	10	8	6	15	12	20	12

SED - 0.7645 CD(0.05) - 1.4459, CD(0.01) - 1.8925

Incorporation of carvacrol, Pomeranate Peel Extract (PPE) and carvacrol + PPE into the films decreased the transparency, but significantly increased the total phenol content and antioxidant activity [12]. Song *et al.*, [13] reported that the physical properties of the films *viz.*, water content, film solubility, water vapor permeability, thickness, and mechanical properties were known to be altered by addition of Tea Tree Oil and reduced the hydrophilicity of the films. Azadbakht *et al.* [14] characterized films with incorporation of Eucalyptus Essential Oil (0.5, 1.5, and 1.5%) in the chitosan biopolymer led to change in morphology, mechanical and barrier properties. They reported that addition of oil improved physical property and highest antimicrobial activity making it a suitable active film for packaging. Chitosan with sandalwood (*Santalum album*) essential oil (SEO) using malic acid as solvent were evaluated as an active packaging film by [15] and they reported that the films are completely water-soluble and can be easily removed from foodstuffs after use without generating solid wastes.

Conclusion

The use of natural products for food packaging and preservation gives a safer, comparatively effective and eco-friendly alternative to non-degradable materials. Seafood industry waste *viz*., chitosan with film forming ability when incorporated with plant derived essential oils (EOs) resulted in better packaging material with antimicrobial property too. as food preserving agents is being promoted given. Several essential oils (EO) have shown antioxidant properties as well as antimicrobial effects against mold, yeasts, bacteria, and viruses, mainly due to their bioactive components such as flavonoids, terpenes, carotenes, etc. Selecting mild odour EOs may help in reducing the inconvenience of strong flavours. The raw material for biopackaging should have similar functional characteristics for food packaging, so that they can be employed as a viable alternative to traditional petroleum-based plastics.

Conflict of interest: There is no conflict of interest

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