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Enhancing antimicrobial activity for chitosan by adding Jojoba liquid wax

Osiris W. Guirguis *, Mahmoud F.H. Abd Elkader, Andrew A. Nasrat

Biophysics Department, Faculty of Science, Cairo University, Giza, Egypt

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ABSTRACT

The purpose of the present study is to enhance the antimicrobial activity of chitosan. For this reason, thin films of chitosan incorporated with different concentrations of Jojoba liquid wax (JLW) were prepared by using the casting technique. The agar disc diffusion method was used to investigate the antimicrobial activity of the films against two different microorganisms namely *Staphylococcus aureus* and *Bacillis subtilis*. The results indicated that as the concentration of JLW increases the antimicrobial activity for *S. aureus* and *B. subtilis* increases. Moreover, it is found that the minimum inhibitory concentration (MIC) values for both systems decrease by increasing the amount of the antimicrobial agent Jojoba with chitosan. In conclusion, the data obtained reveal that chitosan has a great potential to improve its antimicrobial activity by incorporating with antimicrobial agent.

1. Introduction

Recently, increasing attention has been paid to develop and test films with antimicrobial properties in order to improve food safety, shelf life and biomedical applications [1]. Chitosan has a great potential for a wide range of applications due to its biodegradability, biocompatibility, non-toxicity, food preservation and versatile chemical and physical properties [2]. Chitosan has exhibited high antimicrobial activity against a wide variety of pathogenic and spoilage microorganisms including fungi, and Gram-positive and Gram-negative bacteria [3,4]. Jojoba liquid wax (JLW) possesses an anti-inflammatory activity [5]. JLW was used in folk remedies for renal colic, sunburn, chaffed skin, hair loss, headache, wounds and sore throat [5,6].

The aim of the present work is to study the antimicrobial activity for thin films of chitosan incorporated with different concentrations of JLW against two different microorganisms namely *Staphylococcus aureus* and *Bacillis subtili*, by using the agar disc diffusion method.

2. Materials and methods

Shrimpchitosan powder with average molecular weight of 900,000 to 1,000,000 Da and antimicrobial agent Jojoba liquid wax (JLW) were supplied from Sigma-Aldrich Chemie, China.

To prepare thin films of chitosan incorporated with JLW, 2 g of shrimpchitosan was dissolved in 100 mL of 2% acetic acid solution at ambient temperature using a magnetic stirrer over night. The solution was then filtered through a silk screen to remove undissolved materials [3]. Five different volumes (0.25, 0.50, 0.75, 1.00 and 1.50 mL) of the antimicrobial agent JLW were added to the chitosan solution and stirred for 8 h at ambient temperature using magnetic stirrer to form solutions of various levels. Then, the solutions were cast in Stainless-steel plates with diameter 12 cm and dried at 40 °C for 20 to 24 h. The dried films obtained were peeled off and stored in a chamber at 50% RH and 25 °C.

The antimicrobial activity test of the prepared thin films was carried out using the agar disc diffusion method. Well discs with diameter 0.8 cm were made using a circular knife on Mueller Hinton agar containing the indicator microorganisms. Three quantities of 100, 200, and 300 mg from chitosan/JLW films were dissolved each in 1 mL dimethylsulfoxide (DMSO) and then 100 μL from each solution was injected in the well. The agar plates were cooled and kept at 4 °C for 4 h to allow biocide diffusion and then incubated at optimum temperature (37 °C) for 48 h. All tests were performed under sterile conditions in duplicates and repeated three times.

3. Results and discussion

Inhibition zone: Inhibition zone is the clear zone surrounding the well discs on the agar surface that has no growth of microorganism, and that is due to the antimicrobial activity of chitosan/JLW films. The inhibition zone diameter of the clear zone behind the control well disc is measured with caliper in mm [7] (see Fig. 1).

* Corresponding author. Tel.: +202 35676833; fax: +202 35727556.
E-mail address: osiris_wgr@yahoo.com (O.W. Guirguis).

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The mean and S.D. of the antimicrobial activity of chitosan films incorporated with JLW against *Staphylococcus aureus* and *Bacillis subtilis* are illustrated in Table 1. It is noticed from the table that as the amount of JLW with chitosan increases the antimicrobial activity for *S. aureus* and *B. subtilis* increases.

Minimum inhibition concentrate: Minimum inhibition concentrate (MIC) is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after incubation. Bonev et al. [8] reported that the values of MIC are determined by using two different mathematical models: Free Diffusion Model (FDM) (quadratic) and Dissipative Absorptive Diffusion Model (DADM) (linear). The zero intercept of a linear regression of the squared size of the inhibition zone radii, x^2, and the zero intercept of a linear regression of the size of the inhibition zone radii, x, plotted against the natural logarithm of the antimicrobial concentration, ℓn (c), respectively, are calculated [8]. Fig. 2 shows the agar disc diffusion of antimicrobial agent results of chitosan/Jojoba by FDM and DADM of *S. aureus* and *B. subtilis*, as well as the best fits lines. Table 2 summarizes the values of MIC for each sample and the corresponding regression factor (R^2).

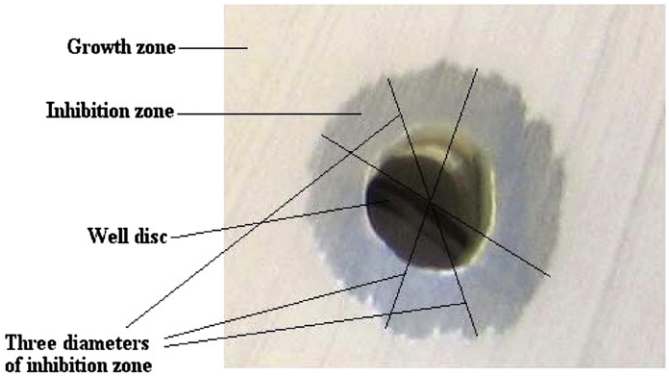

Fig. 1. Measurement of the average diameter of the inhibition zone.

Table 1
Antimicrobial activity of chitosan/JLW films against the microorganisms *S. aureus* and *B. subtilis*.

Antimicrobial agent	*S. aureus* Gram (+) inhibitory (mm)	Gram (+) inhibitory (mm)
Control (DMSO)	–	–
Pure chitosan		
100 mg/mL	0	0
200 mg/mL	14.67 ± 1.23*	15.15 ± 0.66*
300 mg/mL	20.83 ± 1.73*	17.75 ± 0.57*
Chitosan +0.25 mL JLW		
100 mg/mL	12.52 ± 0.64*	11.62 ± 0.79*
200 mg/mL	18.81 ± 0.64*	16.50 ± 0.49*
300 mg/mL	22.10 ± 1.04*	19.62 ± 0.97*
Chitosan +0.50 mL JLW		
100 mg/mL	16.63 ± 0.60*	18.63 ± 0.40*
200 mg/mL	20.97 ± 0.66*	19.99 ± 0.49*
300 mg/mL	22.58 ± 0.76*	21.38 ± 0.13*
Chitosan +0.75 mL JLW		
100 mg/mL	20.02 ± 0.56*	20.52 ± 0.35*
200 mg/mL	22.75 ± 0.81*	21.90 ± 0.14*
300 mg/mL	24.76 ± 0.35*	23.32 ± 0.41*
Chitosan +1.00 mL JLW		
100 mg/mL	23.44 ± 0.54*	22.41 ± 0.48*
200 mg/mL	24.53 ± 0.34*	23.81 ± 0.34*
300 mg/mL	26.95 ± 0.68*	25.26 ± 0.69*
Chitosan +1.50 mL JLW		
100 mg/mL	25.2 ± 0.54*	25.51 ± 0.54*
200 mg/mL	26.52 ± 0.19*	28.04 ± 1.43*
300 mg/mL	27.38 ± 0.87*	29.62 ± 1.03*

– Indicates no growth. Control is a well disc (100 μL DMSO) without antimicrobial agent.

* Significantly different ($p < 0.05$).

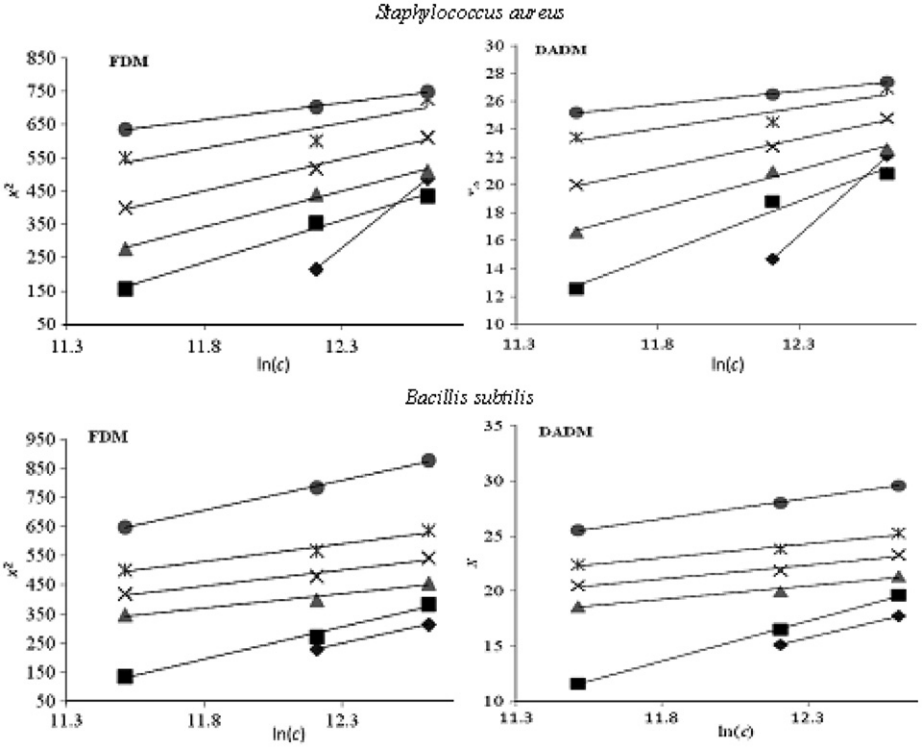

Fig. 2. Agar disc diffusion of antimicrobial agents against the microorganisms *Staphylococcus aureus* and *Bacillis subtilis*. (♦) Pure chitosan, (■) Chitosan +0.25 mL JLW, (▲) Chitosan +0.50 mL JLW, (*x*) Chitosan +0.75 mL JLW, (*) Chitosan +1.00 mL JLW and (●) Chitosan +1.50 mL JLW.

Table 2
Values of MIC (mg/L) for the microorganisms *S. aureus* and *B. subtilis* and the corresponding regression factor (R^2).

Sample	*Staphylococcus aureus*				Sample	*Bacillis subtilis*			
	FDM x^2/ℓn (c)		DADM x/ℓn (c)			FDM x^2/ℓn (c)		DADM x/ℓn (c)	
	MIC	R^2	MIC	R^2		MIC	R^2	MIC	R^2
Pure chitosan	145.31	1	89.79	1	Pure chitosan	67.38	1	18.83	1
Chitosan +0.25 mL JLW	46.77	0.992	19.15	0.981	Chitosan +0.25 mL JLW	55.97	0.991	20.27	0.999
Chitosan +0.50 mL JLW	27.12	0.994	4.76	0.988	Chitosan +0.50 mL JLW	2.97	0.969	0.05	0.976
Chitosan +0.75 mL JLW	12.38	0.991	0.92	0.996	Chitosan +0.75 mL JLW	2.17	0.969	0.03	0.975
Chitosan +1.00 mL JLW	2.94	0.861	0.05	0.872	Chitosan +1.00 mL JLW	1.61	0.969	0.02	0.974
Chitosan +1.50 mL JLW	0.22	0.998	0.00	0.999	Chitosan +1.50 mL JLW	0.01	0.999	0.11	0.999

It is clear from Table 2 for the obtained results that; chitosan has a great potential to improve its antimicrobial activity by incorporating JLW. For higher concentrations of JLW the inhibited zones are larger and the MIC is much lesser. Dissipative Absorptive Diffusion Model (DADM) for most samples attained relatively greater R^2 at equivalent concentrations than Free Diffusion Model (FDM), that is to say that DADM would be better for calculating the MIC mathematically for chitosan incorporated with Jojoba [8].

In general, chitosan film itself showed some antimicrobial effect even though it did not reveal inhibitory zone in any microorganisms tested. It was obviously revealed by the limited growth of *S. aureus* and *B. subtilis* underneath chitosan film discs. This is reasonable as chitosan has the innate characteristic of antimicrobial activity itself. According to Pranoto et al. [3] and Brody et al. [9], the antimicrobial effect of chitosan occurred without migration of active agents. The agar diffusion test is a method commonly used to examine antimicrobial activity regarding the diffusion of the compound tested through water-containing agar plate. The diffusion itself is dependent on the size, shape and polarity of the diffusing material. The chemical structure and the crosslinking level of the films also affect this phenomenon [3,10]. When antimicrobial agents are incorporated, there will be diffusing materials through agar gel, and furthermore, resulting clearing zone on the bacterial growth. Incorporating antimicrobial agents into chitosan edible film thus improves antimicrobial efficacy of chitosan, as diffused antimicrobial actively would add to nonmigrated antimicrobial potency of chitosan.

In conclusion, chitosan has great potential to improve its antimicrobial property by incorporating antimicrobial agents. Jojoba oil incorporated into chitosan film led to an increase in its antimicrobial efficacy. The biological activities of the investigated systems were tested against a representative number of pathogenic organisms using the minimum inhibitory concentration (MIC) method. It is found that the MIC values for both systems decrease by increasing the amount of the antimicrobial agent Jojoba with chitosan.

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