



Carbohydrate Polymers 71 (2008) 187–195

# Carbohydrate Polymers

www.elsevier.com/locate/carbpol

# Surface modification of polypropylene films by chitosan and chitosan/pectin multilayer

Maher Z. Elsabee a,\*, Entsar S. Abdou b, Khaled S.A. Nagy b, Mohamed Eweis c

<sup>a</sup> Department of Chemistry, Faculty of Science, Cairo University, Cairo, Egypt
 <sup>b</sup> Food Technology Research Institute, Agriculture Research Center, Cairo, Egypt
 <sup>c</sup> Department of Botany, Faculty of Science, Cairo University, Cairo, Egypt

Received 25 April 2007; received in revised form 17 May 2007; accepted 17 May 2007 Available online 23 May 2007

#### Abstract

Polypropylene (PP) films were irradiated with corona discharge then dipped into acidic solutions of chitosan extracted from different sources. The films were examined as for their antifungal and antibacterial properties. Carboxymethyl chitosan and carboxymethyl chitin were found also to adhere to these corona treated PP films. The antifungal and antibacterial properties of these derivatives were also examined and found to be even superior to the chitosan itself. Chitosan forms complex compounds with pectin and this property was used to build up a stable multilayered structure on the PP film surfaces to produce a much better antimicrobial films which can be used to fabricate excellent packaging materials for post-harvest crop protection.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Polypropylene films; Corona discharge; Chitosan; Pectin; Multilayers; Antimicrobial properties

# 1. Introduction

Many chemical and physical processes have been developed to preserve food quality, among which, adequate packaging is a fundamental factor in the conservation and marketing phases (Debeaufort, Quezada-Gallo, & Voilley, 1998). Antimicrobial films and coating have innovated the concept of active packaging and have been developed to reduce, inhibit or delay the growth of microorganisms on the surface of food in contact with the packaged product (Appendini & Hotchkiss, 2002). In most fresh or processed food, microbial contamination occurs at a higher intensity on the food surface, thus requiring an effective microbial growth control (Padgett, Han, & Dawson, 1998).

The use of antimicrobial films or coatings may be efficient since the active ingredient may selectively and gradually migrate from the package onto the surface of the food, thereby high concentration being maintained when most necessary (Quattara, Simard, Piette, Begin, & Holley, 2000).

Polyethylene (PE) and polypropylene (PP) are widely used in many engineering and biomedical applications. By appropriate surface treatments PE and PP can be rendered biocompatible with antimicrobial properties. Different methods for polymer surface modifications have been attempted such as plasma-treatment techniques (Ferrero & Bongiovanni, 2006). Depositing chitosan onto a plasma pretreated PP surface could impart antibacterial and antifungal properties since chitosan has been shown to possess efficient antimicrobial ability (Abdou et al., in press-a). If PP films were subjected to corona discharge peroxide or carboxylic groups may develop over the surface. By dipping these films into chitosan solution probably a mono layer of chitosan may adhere onto the film. However, this small concentration of chitosan was found to induce antimicrobial characteristics to the material.

Chitosan forms with pectin stable alternating multilayers over solid surface and the binding of the biopoly-

<sup>\*</sup> Corresponding author. Tel.: +20 26352316. E-mail address: mzelsabee@yahoo.com (M.Z. Elsabee).

mer with surface was irreversible over the time scale of the experiments (Marudova, Lang, Brownsey, & Ring, 2005). One can exploit this experiment to fabricate analogous multilayer structure over a corona treated PP film, thus providing a greater concentration of chitosan onto the surface. Pectin is one of the most widely investigated polysaccharides in the colon-specific drug delivery (Atyabia, Majzooba, Imana, Masoud, & Dorkoosha, 2005). It is predominantly a linear polymer of mainly  $\alpha$ -(1 $\rightarrow$ 4)-linked D-polygalacturonic acid residues. It has been used in different dosage forms for colon-specific drug delivery. They are structurally complex and heterogeneous polyelectrolytes (Schols, Posthumus, & Voragen, 1990; Schols & Voragen, 1994) consisting of linear regions of  $(1\rightarrow 4)$ - $\alpha$ -D-galacturonosyl units and their methyl esters, interrupted in places by  $(1\rightarrow 2)$   $\alpha$ -Lrhamnopyranosyl units.

Chitosan has been proven to control numerous pre- and post-harvest diseases on various horticultural commodities. It has been reported that both soil and foliar plant pathogens fungal, bacterial and viral may be controlled by chitosan application. In addition to its direct microbial activity, other studies strongly suggest that chitosan induces a series of defense reactions correlated with enzymatic activities. Chitosan increased harvested yield due to its ability to form a semi-permeable coating, chitosan extends the shelf life of treated fruit and vegetables by minimizing the rate of respiration and reducing water loss. As a nontoxic biodegradable material, as well as an elicitor, chitosan has the potential to become a new class of plant protecting agent, assisting towards the goal of sustainable agriculture (Hirano & Nagao, 1989).

In chitosan-treated fruits such as apples, kiwifruit, pears and others significant reduction of storage rots has been recorded (Bautista-Banos et al., 2006). In strawberries and raspberries chitosan coatings (10 and 15 mg ml<sup>-1</sup>) reduced two of the main post-harvest diseases, gray mould and Rhizopus rot. Moreover, chitosan fungicidal performance was equivalent to that of the synthetic fungicides such as ipriodione and thiabendazole (TBZ), commonly used to reduce these diseases (El Ghaouth, Ponnampalam, Castaigne, & Arul, 1992; El Gha-1994; outh et al., Romanazzi, Nigro, Ippolito, DiVenere, & Salerno, 2002). Polypropylene films covered with such multilayer of chitosan/pectin could then be a promising material for packaging applications to extent the shelf life of agricultural produces; this is the main point of interest this work is dealing with.

# 2. Experimental

## 2.1. Corona treated PP films

Transparent polypropylene films obtained from the local market were treated with corona discharge machine model SHIAN-L1 (CD800) from Shian L1 electric co. with power 1.1 kW and voltage 380 V.

## 2.2. Chitosan

Chitosan was extracted from four different local sources

- (a) Crayfish (CF) Procambarus clarkii is a species of freshwater crayfish, native to the south-eastern United States, but found also on other continents, where it is often an invasive pest. It is known variously as the red swamp crawfish, red swamp crayfish, Louisiana crawfish or Louisiana crayfish. Recently it has been a disturbing pest in the River Nile causing serious damage to the local ecology.
- (b) Brown shrimp (BS).
- (c) Pink shrimp (PS) both were obtained from the red sea.
- (d) Cuttlefish (CT).

The exoskeleton of the crayfish, shrimp or the squid pen (cuttlefish) were crushed and treated in the usual way with HCl, NaOH 1–2 M then with 40% NaOH to extract the chitosan (Abdou & Elsabee, in press-b). The extracted chitin and chitosan were characterized using elemental analysis, IR and X-ray powder diffraction.

The chitosan used have the following characteristics depicted in Table 1. The DDA is the degree of deacety-lation as determined by potentiometric titration (Domard & Rinaudo, 1983). The intrinsic viscosity was determined by an Ubbelohde viscometer and it gives an indication for the molecular weight. It has been shown that both the DDA and the molecular weight of chitosan have a profound effect on its biological activity (Kato & Ikada, 1998). These chitosan samples not only are from different sources but also have variable DDA and molecular weights.

#### 2.3. Pectin

Apple pectin obtained from Roch Co Switzerland was used as received.

PP films were immersed into the acidic chitosan solution for different periods of time, 3–24 h. After removing from the chitosan solutions the films were either dried with slight pressing with a filter paper or washed several times with distilled water then dried or dipped into aqueous glutaral-dehyde, GA, solution at pH 7 in order to induce crosslinking of the chitosan layer to prevent its leakage easily from the surface.

Table 1
Physical characteristics of the different chitosan used

	DDA	[η]	
Squid	85.3	6.11	
Blue shrimp (BS)	73.2	6.94	
Pink shrimp (PS)	76.8	7.94	
Cray fish (CF)	83.9	7.01	

#### 2.4. Chitosan derivatives

Carboxymethyl chitosan (CM chitosan) and chitin (CM chitin) were prepared according to the method in reference (Chen & Park, 2003), both were water soluble. The degree of substitution was determined by a potentiometric titration according to (Ge & Luo, 2005) and was found to be 0.70.

The corona treated PP films were also treated with a chitosan-g-polyvinyl pyridine copolymer soluble in 1% acetic acid solution. This graft copolymer was prepared previously by the authors and was found to have antimicrobial activity by itself (Elkholy, Khalil, & Elsabee, 2006).

## 2.5. Multilayer formation

The corona pretreated PP film is immersed in 1% chitosan solution for 15 min; the film was withdrawn and dried in air for few minutes followed by immersion in 1% pectin solution for another 15 min.

This process was then repeated. By this technique we were able to deposit up to 15 multilayer of chitosan and pectin. The extent of deposition was followed by FTIR spectroscopy. The antimicrobial properties of complex PP structure were also studied.

*FTIR*. The FTIR spectra were measured in the range 400–4000 cm<sup>-1</sup> using Perkin–Elmer 2000 spectrophotometer.

## 2.6. Mechanical properties

Stress-strain curves of the PP films were measured using a Zwick Tensile Testing Machine 2010/TH2A. The load cell used was 1 kN. The test standard was ASTM D882-90. The reported values are the average of five measurements.

#### 2.7. Swelling properties of the multilayer pp films

The swelling behavior of the PP/multilayered films was studied at 25 °C as a function of time in distilled water.

An exact weight of pre-dried film sample was immersed in distilled water at 25 °C. After certain time, the swollen sample was taken out and hung up for 5 min in order to eliminate excess unabsorbed liquid and then weighed. The degree of swelling at time t was calculated using the following relation:

Swelling degree = 
$$\frac{(W_s - W_o)}{W_o}$$

where,  $W_{\rm s}$  and  $W_{\rm o}$ : weights of swollen and dry film, respectively. The equilibrium extent of swelling was measured after 48 h.

#### 2.8. Pathogenic microorganisms and culture conditions

All strains of fungi and bacteria used in this study were maintained as pure cultures. The fungi *Fusarium oxysporum* SCHL., f. sp. *lycopersici*, (SACC.) Snyder and Hansen

(ATCC 64987), causing *Fusarium* wilt and *Verticillium* albo-atrum Reinke and Berthold (NRRL 1204), causing *Verticillium* wilt, were maintained on sarcina agar (Gibriel, Magdoub, El-Nawawy, Rizk, & Fayed, 1987) *Alternaria* solani (A. alternate) (ELL. and MART.) Jones and Grout (NRRL 2168), causing early blight, was maintained on Czapek-Dox agar.

The pathogenic bacteria *Clavibacter michiganensis* ssp. *Michiganensis* (E.F. Smith) (NRRL B-33), causing bacterial canker and *Pseudomonas solanacearum* (E.F. Smith) (NRRL B-3312), causing bacterial wilt, was maintained on nutrient agar.

## 2.9. Bioassay for antifungal activity

The susceptibilities of the test fungal spores *F. oxysporum*, *V. albo-atrum* and *A. solani* (*A. alternata*) as seeded in Dox's medium on sterilized discs (6 mm) of different kinds of the polypropylene polymers, were determined according to the method proposed by (Olurinola, Ehinmidu, & Bonire, 1992). The sterilized membrane discs were placed on the surface of the seeded Dox medium in triplicates. Plates were allowed to stand for 2 h to allow for the diffusion. Then the plates were incubated at 28 °C for 48 h, after which the susceptibility of each organism to each membrane sheet was estimated by measuring the diameter of the inhibition zones.

# 2.10. Antibacterial assessment

The bactericidal activity was evaluated based on the killing rate by the viable cell counting technique (Park, Kim, Nho, & Kwon, 1998) against C. michiganensis and P. solanacearum. One loopful of the bacteria was inoculated into 10 ml of nutrient agar and incubated at 37 °C for 18–24 h, and then 20 ml of PBS (composed of 0.2 M Na<sub>2</sub>HPO<sub>4</sub>, 0.2 M NaH<sub>2</sub>PO<sub>4</sub>, 0.5 g NaCl and 2 g/l Tween-80 to 1000 ml) was added. After mixing, 1 ml of the solution was added to 9 ml of the nutrient broth and mixed with vortex mixer. The bacteria solution was further diluted with PBS to  $1.5 \times 10^5$  cells/ml, and placed in flasks (0.4 g/sample of each group). After incubating for 0-24 h at 37 °C, 20 ml of PBS were added and stirred for 30 s. Consecutive dilutions were repeated by taking 1 ml of the previous solution and mixing with 9 ml of PBS. From the diluted solution the surviving bacteria were determined by the spread plate method. After inoculation, the plates were kept at 28 °C and the colonies were counted after 18-24 h.

Statistics. All measurements are the mean of five replicates; the results obtained were processed by analysis of variance and the significance was determined at the least significant difference (LSD) levels of 1% and 5%.

#### 3. Results and discussion

The physical characteristics of the used chitosan are given in Table 1, both  $\alpha$  chitosan extracted from shrimps

and crayfish and  $\beta$  chitosan from squid (cuttlefish) were used; the samples have various DDA and intrinsic viscosity and that is to find out if there is a marked effect of these two parameters on the antimicrobial activity of the final material.

#### 3.1. Antibacterial behavior

Figs. 1 and 2 show plots of the logarithm of the number of viable cells of *C. michiganensis* and *P. solanacearum* (log(CFU/ml)) after contact with native, corona exposed and chitosan/corona treated polypropylene (plastic transparent films).

The corona treated PP films were immersed into chitosan solution (2% acetic acid solution) for 24 h then removed and padded with a filter paper and subjected to the biological assay.

The native and corona treated PP films did not show any bactericidal property at all the bacterial counts increased even with time, although the corona treatment lowered that number slightly. On the other hand, the chitosan extracted from different sources showed a pronounced decline of the bacterial cells with complete cells annihilation (ca 10<sup>5</sup> cells/ ml) after 22-23 h for both C. michiganensis and P. solanacearum. It seems that the chitosan obtained from different sources was found to have molecular weights and degrees of deacetylation lying within a narrow range which did not affect the antibacterial activity in a pronounced way, and it was found that all of them lead to complete annihilation of the bacterial cells in almost similar time intervals. A slight enhancement of the activity of the film was observed after treatment with GA in the case of P. solanacearum but not for C. michiganensi. Microscopical observations (Bautista-Banos et al., 2006) indicated that chitosan has direct effect on the morphology of the chitosan-treated microorganisms reflecting its bacteri-static or cidal poten-

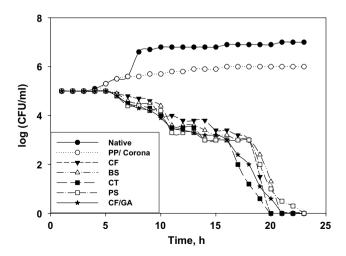


Fig. 1. Viable cell number of *Clavibacter michiganensis* as a function of time grown on PP films treated with corona and then dipped into solutions of chitosan extracted from different sources. One PP/chitosan film was treated further with glutaraldehyde (GA) solution at pH 7.

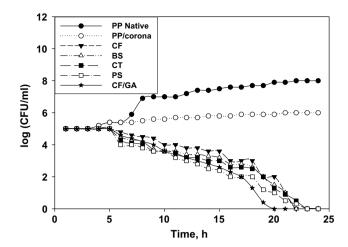


Fig. 2. Viable cell number of *Pseudomonas solanacearum* as a function of time, symbols are the same as in Fig. 1.

tial, in addition the antibacterial activity of chitosan strongly suggests that chitosan induces a series of defense reactions correlated with enzymatic activities.

The PP/corona films were further treated with different derivatives of chitin and chitosan namely carboxymethyl chitosan (CMC) and carboxymethyl chitin (CMch). The choice of these derivatives was based on the fact that both are water soluble polymers and one can thus avoid the use of acetic acid solution. Figs. 3 and 4 illustrate the viable cell number as a function of time for the two bacterial strains onto PP films dipped in CMC and CMch solutions. Other films were washed with sterilized distilled water and/or glutaraldehyde (1%), GA, at pH 7 after being in CMC and CMch solutions. Washing with water did not lead to dramatic reduction of the antibacterial activity which means that both the CMC and CMch are strongly bonded or adhered to the PP film surface. Treatment with GA led to a slight enhancement of the activity of the films which

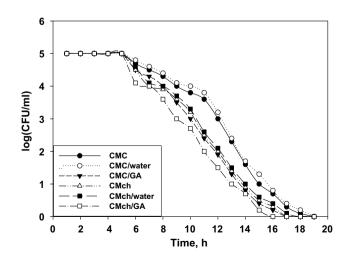


Fig. 3. Viable cell number of *Clavibacter michiganensis* as a function of time grown on PP films treated with corona and then dipped into solutions of carboxymethyl chitosan (CMC) and carboxymethyl chitin (CMch).

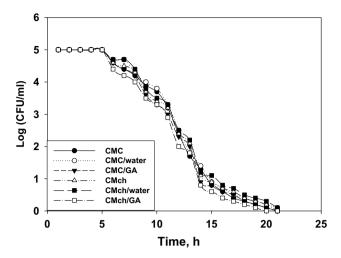


Fig. 4. Viable cell number of *Pseudomonas solanacearum* as a function of time, symbols are the same as in Fig. 3.

may be attributed to the increased hydrophobic nature of the crosslinked chitosan layer for both derivatives. Complete annihilation of the bacterial cells was achieved after 16–19 h for *C. michiganensis*, and 20 h for *P. solanacearum* indicating thus that the carboxylic derivatives have better antibacterial property than the original chitosan.

When the corona PP/chitosan films were padded with filter paper and their FTIR spectra were measured typical polypropylene spectrum with traces of chitosan on the surface was found (Fig. 5a), which means that nearly a chitosan monolayer has been adsorbed on the surface. The bactericidal effect should be proportional to the amount of chitosan on the surface therefore a trial has been made to increase this amount of the adsorbed chitosan. Marudova et al. (2005) have shown that chitosan forms with pectin stable alternating multi-layers over solid surface; bearing this in mind we have tried to build up an alternating multi layered structure of chitosan/pectin over the corona treated PP films using the strong association of pectin with chitosan. The alternating dipping of the corona treated PP film in 1% chitosan solution (2% acetic acid) followed by dipping in aqueous pectin solutions resulted in a build up of successive layered structure. The build up of layers was then followed and proven by FTIR, UV spectroscopy and even by measuring the thickness of the films by a micrometer. Another indirect method was also used by measuring the swelling extent of the different layers.

The FTIR spectra of the films with different layers, Fig. 5, showed that the intensity of the band at 1457 cm<sup>-1</sup> is independent on the presence of the chitosan layers so it was taken as a reference band and the intensities of the new bands characteristic for chitosan at 3380 cm<sup>-1</sup> which is due to the stretching vibration of the NH<sub>2</sub> and the OH, and 1744 and at 1603 cm<sup>-1</sup> which are due to the residual carbonyl groups were taken as an indication of the presence of chitosan.

The ratio of these bands was found to increase with increasing the number of the deposited layers as shown in Fig. 6.

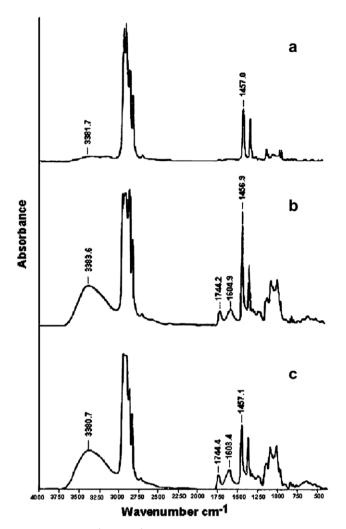


Fig. 5. FTIR of PP/chitosan/pectin multi layers: (a) 1 layer, (b) 6 layers, (c) 15 layers.

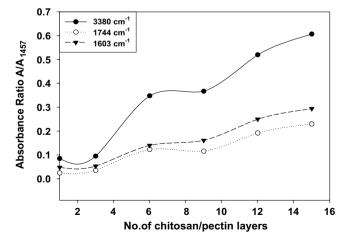


Fig. 6. Absorbance ratios of the bands at 3380, 1744 and 1603 cm<sup>-1</sup> over the reference band at 1457 cm<sup>-1</sup> as a function of the number of chitosan/pectin layers.

UV absorbance also indicated that the absorbance of the films at 209 and 218 nm increases with increasing the number of layers as shown in Fig. 7.

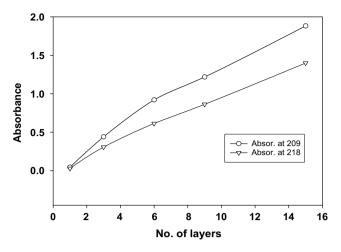


Fig. 7. UV absorbance of PP/chitosan/pectin films as a function of the number of layers.

The swelling of the films was measured by dipping them in aqueous solutions at pH 7 for 48 h. It was thought of as an indirect method for verifying the multilayer builds up. The swelling extent was found to increase with increasing the number of layers as shown in Fig. 8; the Figure also demonstrates that the adhered layers onto the films are stable up to 48 h in aqueous solution.

The ultimate goal for these PP/chitosan/pectin films is for the fabrication of a packaging means for the protection of agricultural products therefore it was of interest to check whether the antibacterial and antifungal behavior has improved after the multilayered formation. The two bacterial strains were used once more to measure the antibacterial property of the films; Fig. 9 illustrates the number of the viable cells as a function of the number of layers using CF chitosan with pectin. In order to enhance the stability of the layers the films with different number of layers were dipped in glutaraldehyde GA solution (1%) to crosslink the chitosan layers. The antibacterial behavior of the films is shown in Figs. 9 and 10 using the *C. michiganensis* and *P. solanacearum* strains. It can be seen that GA improves

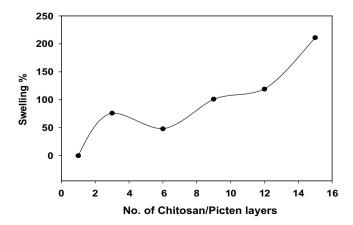


Fig. 8. Equilibrium swelling behavior (48 h) of the PP/chitosan/pectin film as a function of number of alternating layers.

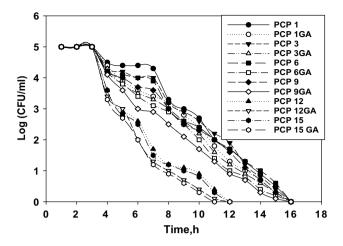


Fig. 9. Viable cell number of *Clavibacter michiganensis* as a function of time grown on polypropylene film covered with multi-layers of chitosan/pectin and treated with glutaraldehyde (GA).

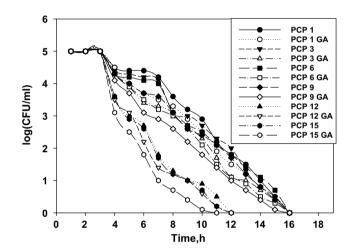


Fig. 10. Viable cell number of *Pseudomonas solanacearum* as a function of time grown on polypropylene films.

the antibacterial behavior in general as has been demonstrated earlier and that as the number of layers increases the bactericidal property improves, especially after 12 layers have deposited.

A dramatic reduction of the log(CFU/ml) has been achieved after 12–15 layers were deposited and treated with GA solution.

# 3.2. Antifungal behavior

The antifungal behavior of the prepared PP films was measured using fungal spores *F. oxysporum*, *V. albo-atrum* and *A. solani* (*A. alternata*) by measuring the diameter of the inhibition zones. The inhibition zone diameter is an indication of the antifungal capacity of the polymer, the bigger the zone the stronger the capacity. Table 2 contains the zone diameter for films covered with chitosan from dif-

Table 2 Antifungal efficacy of the polypropylene (native, corona exposed and chitosan/corona treated) films on the growth of *Fusarium oxysporum*, *Verticillium albo-atrum* and *Alternaria solani* (*A. alternata*) after 48 h of incubation at 28 °C by a disc plate method (mean values of the diameter of the inhibition zones in mm)

Samples	Fusarium oxysporum	Verticillium albo-atrum	Alternario solani
Control, native	0.0	0.0	0.0
Corona treated	12.1	13.0	15.5
Coated with/CFCS	34.6	37.2	44.9
Coated with/BS CS	31.7	34.4	40.8
Coated with/Squid CS	28.6	31.2	34.3
Coated with/RS CS	27.1	29.3	32.1
Coated with RS then immersed in water	3.6	5.7	9.8
RS/GA (1% solution pH 7)	27.1	29.1	32.1
CM chitosan	3.8	5.9	10.1
CM chitosan/H <sub>2</sub> O	3.6	5.8	10.0
CM CS/GA	10.6	11.5	15.6
CM Chitin	3.9	6.0	10.3
CM chitin/H2O	3.7	5.9	10.1
CM chitin/GA	10.8	11.8	15.9
Chitosan-g-PVP 75%	24.2	37.4	45.0
Chitosan-g-PVP 95%	24.9	38.1	45.9
Chitosan-g-PVP 160%	25.3	38.6	46.6
LSD			
5%	1.7	1.5	1.9
1%	3.2	3.1	3.5

ferent sources and chitosan and chitin derivatives, CMC and CMch.

Chitosan-g-PVP is a chitosan/vinyl pyridine graft copolymer with various degrees of grafting prepared in a previous work (Elkholy et al., 2006). Chitosan extracted from CF is shown to have a higher antifungal capacity for all fungal strains also the *A. solani* is more responsive to the chitosan and its derivatives than the other two fungi.

The multilayered structures were also tested for their antifungal activity as seen in Table 3.

A dramatic increase of the inhibition zones was achieved with increasing the number of layers and using GA afterward. It is also clear that *A. solani* is more sensitive to the chitosan/pectin layers than the other two pathogens.

It is now important to check whether the mechanical properties of these multilayered structures have deteriorated after such build up so the tensile strength, elongation and the Young's modulus were measured for several samples with different number of layers and the modulus of these films as a function of the number of layers is shown in Fig. 11.

Although the curve in Fig. 11 is not a smooth regular increase in the modulus still it demonstrates that the mechanical property of the films did not suffer deterioration and can thus be used for the fabrication of useful packaging materials with commercial applications.

Numerous reports indicate that chitosan effectively controls the post-harvest rots during storage, delays the onset of infection and slow down the infection process.

Table 3
Antifungal efficacy of the polypropylene (native, corona exposed and covered with an alternating multilayer of pectin and chitosan) on the growth of *Fusarium oxysporum*, *Verticillium albo-atrum* and *Alternatia solani* (A. alternata) after 48 h of incubation at 28 °C by a disc plate method (mean values of the diameter of the inhibition zones in mm)

Samples	Fusarium oxysporum	Verticillium albo-atrum	Alternaria solani
Control, native	0.0	0.0	0.0
Corona treated	12.1	13.0	15.5
Coated with/CFCS	34.6	37.2	44.9
Coated with/P/CS 1	34.9	38.0	45.6
Coated with/P/CS 1 G	35.9	40.4	46.1
Coated with/P/CS 3	35.2	39.2	46.2
Coated with/P/CS 3 G	36.7	41.9	48.3
Coated with/ P/CS 6	36.3	40.8	47.3
Coated with/ P/CS 6 G	38.4	43.2	50.4
Coated with/ P/CS 9	37.7	41.9	48.6
Coated with/ P/CS 9 G	42.3	46.8	53.8
Coated with P/CS 12	38.6	44.8	52.7
Coated with P/CS 12 G	44.6	49.9	55.9
Coated with P/CS 15	40.2	46.9	54.9
Coated with P/CS 15 G	46.9	51.7	61.8
LSD			
5%	1.9	1.8	2.3
1%	3.4	3.4	4.5

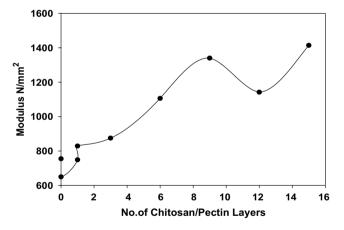


Fig. 11. Modulus (Stress/strain) of PP/chitosan/pectin films as a function of the number of layers.

In general, the reduction of rots increases with the increase in chitosan concentration (Bautista-Banos et al., 2006; El Ghaouth et al., 1992, 1994). In order to test the prepared multilayered films as a packaging device for storing and increasing the shelf life of tomato as an example we fabricated bags  $20 \times 20$  cm using the 12 layered films. A fresh tomato fruits of regular size (50 g) were collected cleaned and stored in this bag. Another similar fruit was stored in regular PP bag (B) and a third one was kept in air (C). The three species were kept in a refrigerator at 4 °C. The above experiment was done in triplicate. The samples were investigated at intervals, and after 13 days the samples were compared and

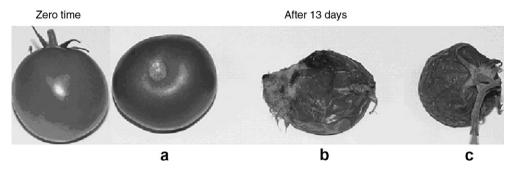


Fig. 12. Tomato fruit at zero time, (a) similar fruit kept for 13 days into a polypropylene bag fabricated from PP film covered by 12 alternating chitosan/pectin layers in comparison to similar fruit kept for the same period of time in untreated PP bag (b), and another one kept in open air (c).

Fig. 12 shows the state of the three samples. It can bee seen that samples B and C deteriorated completely while the sample in the treated bag was kept almost intact with no apparent rotting infection. In this way an environmental-friendly nonpolluting way of controlling post-harvest fruit-rots during storage and delaying the onset of infection was developed. More work is underway now to explore further these multilayered PP films in post-harvest crop protection.

#### 4. Conclusions

Coating the corona treated polypropylene films with the extracted chitosan and its derivatives imparts a biocidal activity onto them. It was found that the native PP had no biocidal activity, the corona treated ones showed slight activity however the films coated with chitosan and its derivatives showed much higher antifungal, as well as, antibacterial activity. Increasing the amount of chitosan on the surface of corona treated PP films by multilayer formation increase the antimicrobial effect of them.

The mechanical properties of these multilayered structures were measured and we found that the films did not suffer deterioration and can thus be used for the fabrication of useful packaging materials. Using these films for tomato packaging kept it almost intact with no apparent rotting infection for 13 days. This test indicates the potential of this treatment of PP films for the production of antimicrobial packaging.

#### References

- Abdou E. S., Elkholy S. S., Elsabee M. Z., & Eweis M. (in press-a). Improved antimicrobial activity of polypropylene and cotton nonwoven fabrics by plasma treatment and modification with chitosan. *Journal of Applied Polymer Science*.
- Abdou, E., Nagy, K., & Elsabee, M. Z. (in press-b). Extraction and characterization of chitin and chitosan from local sources. *Bioresource Technology*.
- Appendini, P., & Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. *Innovative Food Science and Emerging Technologies*, 3, 11–126.
- Atyabia, F., Majzooba, S., Imana, M., Masoud, M., & Dorkoosha, F. (2005). In vitro evaluation and modification of pectinate gel beads

- containing trimethyl chitosan, as a multi-particulate system for delivery of water-soluble macromolecules to colon. *Carbohydrate Polymers*, 61, 39–51.
- Bautista-Banos, S., Hernandez-Lauzardoa, A. N., Velazquez-del Vallea, M. G., Hernandez-Lopez, M., Ait Barka, E., Bosquez-Molinac, E., et al. (2006). Chitosan as a potential natural compound to control pre and post-harvest diseases of horticultural commodities. *Crop Protection*, 25, 108–118.
- Chen, X. G., & Park, H. J. (2003). Chemical characteristics of Ocarboxymethyl chitosan related to the preparation conditions. Carbohydrate Polymers, 53, 355–359.
- Debeaufort, F., Quezada-Gallo, J. A., & Voilley, A. (1998). Edible films and coatings: Tomorrow packaging: A review. *Critical Reviews in Food Science*, 38(4), 299–313.
- Domard, A., & Rinaudo, M. (1983). Preparation and characterization of fully deacetylated chitosan. *International Journal of Biological Macro*molecules, 5, 49–52.
- El Ghaouth, A., Ponnampalam, R., Castaigne, F., & Arul, J. (1992). Chitosan coating to extend the storage life of tomatoes. *Hortscience*, 27(9), 1016–1018.
- ElGhaouth, A., Arul, J., Grenier, J., Benhamou, N., Asselin, A., & Belanger, R. (1994). Effect of chitosan on cucumber plants: Suppression of *Pythium aphanidernatum* and induction of defense reaction. *Phytopathology*, 84, 313–320.
- Elkholy, S. S., Khalil, K. D., & Elsabee, M. Z. (2006). Homogeneous and heterogeneous grafting of vinylpyridne onto chitosan. *Journal of Applied Polymer Science*, 99, 3308–3317.
- Ferrero, F., & Bongiovanni, R. (2006). Improving surface properties of cellophane by air plasma treatment. *Surface and Coatings Technology*, 200, 4770–4776.
- Ge, H. C., & Luo, D. K. (2005). Preparation of craboxymethyl chitosan in aqueous solution under microwave irradiation. *Carbohydrate Research*, 340, 1351–1356.
- Gibriel, A. Y., Magdoub, M. N. I., El-Nawawy, M. A., Rizk, I. R. S., Fayed, E. O. (1987). *Catalogue of strains (CAIM)* (2nd ed.). Egypt: MIRCEN, Faculty of Agriculture, Ain-Shams University.
- Hirano, S., & Nagao, N. (1989). Effect of chitosan, pectic acid, lysozyme, and chitinase on the growth of several Phytopathogens. Agricultural and Biological Chemistry, 53, 3065–3066.
- Kato, K., & Ikada, Y. (1998). Immobilization of DNA onto a polymer support and its potentiality as immuno-adsorbent. *Biotechnology and Bioengineering*, 51, 581.
- Marudova, Mariya, Lang, S., Brownsey, G. J., & Ring, S. G. (2005). Pectin–chitosan multilayer formation. *Carbohydrate Research*, 340, 2144–2149.
- Olurinola, P. F., Ehinmidu, J. O., & Bonire, J. J. (1992). Antifungal activity of *n*-tributyl tin acetate against some common yam rot fungi. *Applied and Environmental Microbiology*, 58, 758–760.
- Padgett, T., Han, L. Y., & Dawson, P. L. (1998). Incorporation of food grade antimicrobial compounds into biodegradable packaging films. *Journal of Food Protection*, 61(10), 1330–1335.

- Park, J. S., Kim, J. H., Nho, Y. C., & Kwon, O. H. (1998). Antibacterial activities of acrylic acid-grafted polypropylene fabric and its metallic salt. *Journal of Applied Polymer Science*, 69, 221.
- Quattara, B., Simard, R., Piette, G., Begin, A., & Holley, R. A. (2000). Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. *International Journal of Food Microbiology*, 62, 139–148.
- Romanazzi, G., Nigro, F., Ippolito, A., DiVenere, D., & Salerno, M. (2002). Effect of pre and post harvest chitosan treatments to control
- storage grey mold of table grapes. *Journal of Food Science*, 67(5), 1852–1866.
- Schols, H. A., Posthumus, M. A., & Voragen, A. G. (1990). Structural features of hairy regions of pectin's isolated from apple juice produced by the liquefaction process. *Carbohydrate Research*, 206, 117–129.
- Schols, H. A., & Voragen, A. G. J. (1994). Occurrence of pectic hairy regions in various plant cell wall materials and their degradability by rhamnogalacturonase. *Carbohydrate Research*, 256, 83–95.