J Food Sci Technol (October 2014) 51(10):2425–2434
DOI 10.1007/s13197-012-0792-y

ORIGINAL ARTICLE

Synthesis, characterization and antibacterial activity of biodegradable films prepared from Schiff bases of zein

E. A. Soliman · A. A. Khalil · S. F. Deraz · G. El-Fawal · S. Abd Elrahman

Revised: 24 July 2012 / Accepted: 27 July 2012 / Published online: 14 August 2012

Abstract Pure zein is known to be very hydrophobic, but is still inappropriate for coating and film applications because of their brittle nature. In an attempt to improve the flexibility and the antimicrobial activity of these coatings and films, Chemical modification of zein through forming Schiff bases with different phenolic aldhydes was tried. Influence of this modifications on mechanical, topographical, wetting properties and antimicrobial activity of zein films were evaluated. The chemical structure of the Schiff bases films were characterized by ATR-FTIR spectroscopy. The results indicate an improvement in mechanical properties with chemically modification of zein to form Schiff bases leading to a reduction in the elastic modulus. An increase in the elongation at break has been observed, but with slight influence on tensile strength. Plasticized zein films have similar initial contact angle (~40°). An increase in reaction temperature and time increases film's affinity towards water. As shown by contact angle measurements, a noticeable relation was found between film composition and the hydrophilicity. Surface topography also varied by forming Schiff bases, becoming rougher than zein-based films. The antibacterial activities of zein and Schiff bases of zein-based films were investigated against gram-positive bacteria (*Listeria innocua*, *Listeria monocytogenes*, *Bacillus cereus* and *Clostridium sporogenes*) and gram-negative bacteria (*Escherichia coli, Yersinia enterocolitica and Salmonella enterica*). It was found that the antibacterial activity of the Schiff bases-based films was more effective than that of zein-based films.

Keywords Zein · Schiff bases · Cinnamaldehyde · Benzaldehyde · 4-dimethylaminobenzaldehyde · Bioactive biodegradable materials

E. A. Soliman (✉) · G. El-Fawal
Polymer Materials Research Department, Advanced Technology and New Materials Research Institute, CSRAT,
New Boarg El-Arab City 21934, Alexandria, Egypt
e-mail: emadsoliman@mucsat.sci.eg

A. A. Khalil · S. F. Deraz
Proteins Research Department, Genetic Engineering and Biotechnology Research Institute, CSRAT,
New Boarg El-Arab City 21934, Alexandria, Egypt

S. A. Elrahman
Department of Chemistry, Faculty of Science, Zagazig University,
Alzagazig 2455, Egypt

Present Address:
S. A. Elrahman
Faculty of Science, King Abdulaziz University,
Jeddah, Saudi Arabia

Introduction

Zein, the major storage protein of corn, is a prolamin by-product of the corn milling industry. It was used extensively in the fiber and coating industries through the early 1960's. Manufacturers lost interest when cheaper petroleum-based products became available (Shukla and Cheryan 2001; Lawton 2002). There has been a renewed interest in using zein as well as other renewable materials in consumer goods because of concerns about the impact that petroleum-based products have on the environment (Lawton 2002; Bhat and Karim 2012). Moreover, the development of new zein-based products that have broader applications is needed to improve the economics of the growing bioethanol industry. However, structural applications of zein such as biodegradable films, food coatings and drug tableting are limited because of their water insolubility and brittle nature (Shukla 1992; Soliman et al. 2009; Scramin et al. 2011; Ünalan et al. 2011). On the other hand, zein has reactive amino groups that leads to the possibility of several chemical modifications and subsequently alter its functional properties.

Formation of Schiff bases (–RC=N–) through the reaction of amino groups of protein with aldehydes and ketones can be considered one of the potential chemical modification routes to improve the mechanical properties with acquiring antimicrobial properties for the obtained modified zein-based coatings or films. Where, many biological activities of Schiff bases including; antibacterial, antifungal, antimicrobial activities have been previously reported (Raman et al. 2003; Zhong et al. 2009; Shi et al. 2010). On the other side, cinnamaldehyde (CA), benzaldehyde (BA) and 4-dimethylaminobenzaldehyde (DMABA) were know by their activities as antimicrobial agents (Ali et al. 2005; Gill and Holley 2004; Gende et al. 2008). Therefore, Schiff bases derived from zein with various aromatic aldehydes (CA, BA and DMABA) have been synthesized, structurally characterized and determined their antibacterial activity against gram-positive (*Listeria innocua*, *Listeria monocytogenes*, *Bacillus cereus*, and *Clostridium sporogenes)* and gram-negative bacteria (*Escherichia coli*, *Yersinia enterocolitica* and *Salmonella enterica*).

Materials and methods

Materials

Pure zein was purchased from Sigma-Aldrich. CA, BA and DMABA was obtained from Merck and Sigma-Aldrich. All other reagents of analytical grade were provided by Sigma-Aldrich. Gram-positive bacteria (*Listeria innocua* 33090, *Listeria monocytogenes* 19116, *Bacillus cereus* NRRLB 49064 and *Clostridium sporogenes* 3584) and gram-negative bacteria (*Escherichia coli*, *Yersinia enterocolitica* 23715 and *Salmonella enterica* EMCC 1038) were supplied by Microbiology Laboratory of the Ain Shams University of Egypt.

Film preparation

Zein formulations for films were prepared by dissolving a definite amount of zein (10 g) in 100 ml of an aqueous ethanol solution (88 %) with stirring at room temperature using a magnetic stirrer. Afterwards, pre-determined amounts of CA, BA or DMABA were added to zein solution, so their concentrations were 1.25, 1.25 and 1 %, respectively, based on the weight of zein. Finally, glacial acetic acid was used as catalyst (2 ml). These zein Schiff bases film forming solutions, along with a control one (zein film forming solution), were heated at different temperatures of 25, 40, 50, or 60 °C for 2, 4, 6, or 8 h with stirring. Then, PEG (400) was add at concentration of 35 % of zein amount throughout stirring and prior to pouring the film forming solutions onto polystyrene Petri dishes (9 cm diameter) by about 10 min., gently swirled, placed in a ventilated oven at 50 °C overnight to evaporate the solvent and produce a free standing films. After drying, the films were conditioned at 23±2 °C and 70±5 % relative humidity (RH) for 48 h at least till the films were further characterized and tested.

Film thickness measurement

Ten thickness measurements were taken along each specimen (2.5 cm×5 cm) with a hand-held electronic digital micrometer (Mitutoyo, Japan) and mean thickness was used in tensile strength calculations.

Infrared spectroscopic analysis

All samples were stored in a desiccators containing silica gel for at least 48 h at room temperature to ensure minimal moisture content before spectroscopic analysis. The following samples were examined: unmodified and chemically modified zein films. Shimadzu 8400S spectrometer (Shimadzu, Japan) with a Golden Gate diamond horizontal attenuated total reflectance (ATR) system was used. Film samples (10 mm×10 mm) was placed, on the ATR crystal to cover the crystal surface area. The sample was gently squeezed by a screw to promote contact with the crystal. The spectra (128 scans at 2 cm^{-1} resolution) were collected with the frequency range of 4000–500 cm^{-1}. The angle of incidence for the ATR crystal was 45°. The empty crystal was used as background. The FTIR spectra were Fourier-deconvoluted with a resolution enhancement factor of 1.5 and a bandwidth of 15 cm^{-1}.

Tensile testing

Film tensile strength (TS) and percentage elongation at break (E) were determined with an Shidmadzu universal testing machine (model AG-I, Shidmadzu, Japan). Initial grip separation was set at 10 cm, and cross-head speed was set at 30 mm/min. TS was calculated by dividing maximum load (force) by initial cross-sectional area of a specimen. E was expressed as percentage of change of initial gauge length of a specimen (5 cm) at the point of sample failure. Prior to tensile testing, film specimens were conditioned for 2 days in an environmental chamber at 23±2 °C and 70 % RH according to ASTM Standard Method D 882-91 (ASTM 2011). In a slight deviation from this ASTM method, tensile testing of film samples was conducted, as quickly as possible, in ambient conditions rather than in the recommended standard laboratory atmosphere of 23±2 °C and 70±5 % RH. TS and E values for each type of the obtained films were determined for individually prepared cast films as replicated experimental units. Each TS and E replicate value was the mean of five experimental units (specimens).

Color measurement

Color values of films were measured with a portable colorimeter (X-Rite, model SP 64, U.S.A.). Film specimens

(5 cm×5 cm) were placed on a white standard plate (calibration plate CR-A43) and the Hunter Lab color scale was used to measure color: L=0 (black) to L=100 (white); a=−80 (greenness) to a=100 (redness); and b=−80 (blueness) to b=70 (yellowness). The total color difference (ΔE) can be calculated by the following equation;

$$\Delta E = \left[\Delta L^2 + \Delta a^2 + \Delta b^2\right]^{1/2}$$

Where, ΔE are the difference between the reference and a sample. Five measurements were taken at different locations on each specimen, one at the center and four around the perimeter. Color measurements for each type of the prepared films were replicated four times with individually prepared films as the replicated experimental units and each replicate being the mean of two tested sampling units (specimens) taken from each type of the films.

Contact angle measurement

The contact angle means the inside angle between the surface of the film and the tangent to surface of the liquid droplets (water) that express on the surface hydrophobicity or wettability of the films. Contact angle measurements carried out with water droplet using a goniometer (Ramé-hart, model 500-F1, France). 4 μL droplet of de-ionized water was placed on the surface of the film with an automatic piston syringe and photographed. An image analyzer was used to measure the angle formed between the base, constituted of the surface of the film in contact with the droplet of water, and the tangent to the droplet of water. For each film specimen, at least three measurements on different positions of film surface were taken place, the average was calculated, however, each type of these films was replicated four times with individually prepared films.

Microstructure examination

The surface characteristic of zein films was examined by a Joel 6360LA scanning electron microscope (JEOL Ltd., Tokyo, Japan) operated at an acceleration voltage of 15 kV. Film specimen were mounted on stainless steel stubs with double sided tape, 10–20 nm thick layer of gold was sputtered on the samples by JFC-1100E sputter (JOEL Ltd., Tokyo, Japan).

Bacterial cultures and in vitro antibacterial assays

In order to study the antimicrobial activity of the films, seven pathogenic strains including four gram-positive bacteria (*Listeria innocua* 33090, *Listeria monocytogenes* 19116, *Bacillus cereus* NRRLB 49064 and *Clostridium sporogenes* 3584) and three gram-negative bacteria (*Escherichia coli* wild, *Yersinia enterocolitica* 23715 and *Salmonella enterica* EMCC 1038) were used as test microorganism.

Bacterial cultures Gram-positive bacteria (*L. innocua*, *L. monocytogenes*, *B. cereus*, and *C. sporogenes)* were cultured at 37 °C for 24 h. in BHI (Brain Heart Infusion, Difco Co., Detroit, MI, U.S.A.), nutrient (synthetic media), tryptone and soy broth media (Oxoide LTD, England), respectively. On the other hand gram-negative bacteria (*E. coli*, *S. enterica* and *Y. enterocolitica*) were cultured overnight at 37 °C in LB (Carl Roth GmbH Co. UK), nutrient (synthetic media) and Tryptone (synthetic media), respectively. By appropriately diluting with sterile normal saline (0.9 %) solution, the cultures of bacteria containing 10^7 CFU/mL were prepared and used for the antibacterial test.

In vitro antibacterial assay The antibacterial efficacy of the prepared zein- and zein Schiff bases-based films was assessed using the agar diffusion method. The peptone culture plates were prepared, in which 100 μl of bacterial suspension prepared in the previous section were added. After the agar solidified, a 35-mm specimen of zein- or zein Schiff bases-based films was placed on top of the agar medium. All the plates were incubated at 37 °C for 24 h. A blank without zein or modified zein films was prepared for comparison. Then the plates were taken out of the incubator, and the inhibition rate was calculated from the following equation:

$$\text{Inhibition rate}(\%) = N_1 - N_2/N_1 \times 100$$

Where,

N_1 and N_2 are the number of colonies on the blank plates.
N_2 the number of colonies on the plates of zein and modified zein films.

Analyses were realized in triplicate.

Statistical analysis

Analysis of variance and Duncan's multiple range tests with ($P \leq 0.05$) were performed to analyze the results statistically using a SAS program (SAS Institute, Inc., Cary, NC, USA).

Results and discussion

Infrared spectroscopic analysis

Figure 1a represents the IR spectrum of pure zein films that exhibited main characteristic bands between 2904.6 and 3122.5 cm^{-1} that are belonging to stretching of –COO– groups, at 3502.5 cm^{-1} for stretching of –N–H of amines and 1593.1 cm^{-1} (amide II) for bending of –N–H. Whereas,

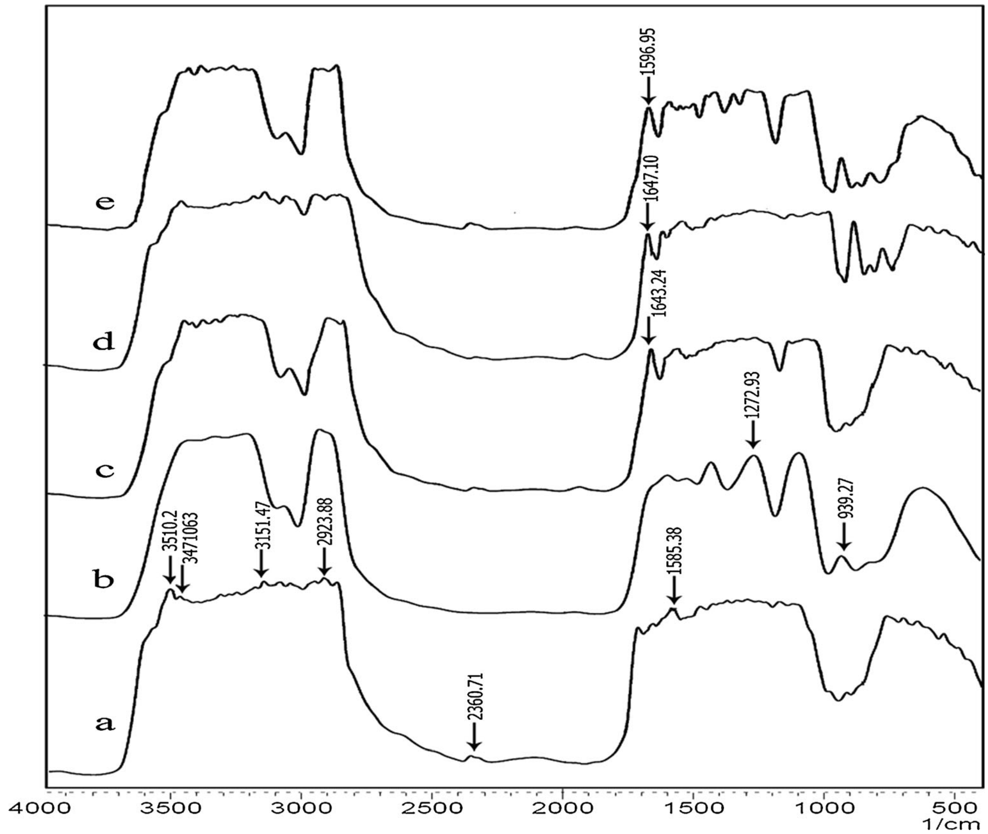

Fig. 1 ATR-FTIR spectra of zein- and Schiff bases-based films (*a*: pure zein; *b*: plasticized zein; *c*: Schiff bases based on cinnamaldehyde; *d*: Schiff bases based on benzaldehyde; *e*: Schiff bases based on 4-dimethylaminobenzaldehyde)

Fig. 1b, shows IR spectrum of zein-based films cross-linked with citric acid, exhibited peaks at 1272.9 cm^{-1} for –OH groups and peaks at 939.3 cm^{-1} due to C–O of polyethylene glycol (PEG_{400}). The IR spectra of Schiff bases of zein-based films (Fig. 1c, d, e) show a strong absorption band at 1643.2, 1647.1 and 1596.9 cm^{-1} attributed to C=N vibrations characteristic of imines for CA, BA or DMABA Schiff bases, respectively (Colthup et al. 1990; Guinesi and Cavalheiro 2006; Jin et al. 2009) which is not observed in pure zein and zein cross-linked with PEG. On the other hand, characteristic bands of the utilized free aldehydes (1678, 1703 and 1694 cm^{-1} for CA, BA or DMABA, respectively) didn't appeared. These results confirm formation of the Schiff bases through reacting zein with such aldehydes with no noticeable residues of ree aldehydes.

Tensile strength (TS) and elongation (E)

The tensile properties of zein-based films prepared from unmodified and chemically modified zein (Schiff bases of zein) were shown in Table 1. Zein-based films exhibited high strength with low percentage of elongation. Whereas, the opposite mechanical behavior was noticed for all Schiff bases-based films which showed lower tensile strength with higher elongation comparing with zein-based films. These results agree with those of Arcan and Yemenicioğlu (2010), when phenolic compounds incorporated within zein film matrices. Thus, such Schiff bases films based either on CA, BA or DMABA prepared at various temperatures for different times exhibited lower tensile strength and higher elongation percentage than zein based films. Whereas, Schiff base film based on CA, BA or DMABA prepared at 25 °C showed higher tensile strength comparing with those prepared at the other temperatures. The impact of temperature was more pronounced with extension time of reaction. However, Schiff bases based on CA films that prepared at 25 °C with reaction time of 8 h had the highest tensile strength value. On the other hand, Table 2 exhibited that elongation percentages of Schiff bases-based films were noticeably higher than those of zein based film. Thus, the effect of temperature and time of reaction on elongation values was indicated in the same figure. Whereas, elongation percentages increased with increasing temperature of reaction and extending reaction time, as elongation reach ~252 and 267 % for Schiff bases based films prepared from CA and BA, respectively, at 60 °C for 6 h Moreover, there was a noticeable difference between the elongation percentages for these three types of Schiff bases films depending on reaction condition, for example at 25 °C for 4 h Schiff base film based on CA exhibited higher elongation than the other films based on BA or DMABA. By comparing elongation of Schiff bases films at constant temperature, it was indicated that elongation percentages of CA and DMABA based films at 60 °C (2 h) reached ~237.7 and 259.2 %, respectively, while it reached ~263.7 % for BA.

Table 1 Influence of reaction temperature and time on tensile strength of Schiff bases-based films

Zein Schiff bases films	Tensile strength (MPa)				
	Temp.(°C)	Time (h)			
		2	4	6	8
CA	25	1.73±0.13 [ab]	1.87±0.17 [ab]	2.01±0.17 [a]	2.10±0.39 [a]
	40	1.04±0.10 [d]	1.30±0.32 [bc]	1.60±0.26 [b]	1.20±0.13 [cd]
	50	1.10±0.14 [cd]	0.64±0.48 [cd]	1.20±0.06 [cd]	1.97±0.17 [a]
	60	1.70±0.03 [b]	1.13±0.19 [d]	1.50±0.19 [bc]	1.50±0.03 [bc]
BA	25	1.42±0.12 [c]	1.31±0.13 [c]	1.53±0.03 [bc]	1.14±0.04 [d]
	40	0.90±0.46 [cd]	1.30±0.26 [c]	1.03±0.25 [d]	0.86±0.68 [d]
	50	1.52±0.08 [bc]	1.32±0.12 [c]	0.59±0.04 [e]	0.86±0.18 [de]
	60	1.49±0.03 [bc]	1.16±0.10 [cd]	0.79±0.06 [de]	0.45±0.05 [e]
DMABA	25	1.75±0.13 [b]	1.78±0.20 [b]	1.78±0.38 [ab]	1.32±0.12 [c]
	40	1.11±0.12 [cd]	1.27±0.20 [bc]	1.10±0. 31 [d]	1.17±0.26 [d]
	50	1.71±0.34 [b]	0.96±0.20 [d]	1.40±0.06 [c]	1.20±0.07 [cd]
	60	1.67±0.17 [b]	1.05±0.20 [d]	1.12±0.24 [cd]	2.14±0.12 [a]

n=5

Values not followed by the same letters are significantly different at $P \leq 0.05$

CA: Schiff bases based on cinnamaldehyde; BA: Schiff bases based on benzaldehyde; DMABA: Schiff bases based on 4-dimethylaminobenzaldehyde)

These mechanical properties of Schiff bases of zein films can be explained on the basis that structure of zein consists of a meshwork composed of doughnut structures formed by asymmetric rods joined to each other via hydrophobic interactions that subsequently maintain film integrity, while intensive hydrophobic interactions can be also responsible for the brittleness and lack of flexibility in zein films (Guo et al. 2005). On the other side, lutein (natural pigment) had an obvious role in maintaining the helical structure of zein polypeptides and subsequently affect formation of zein assemblies (Anderson and Lamsal 2011). Increase of the flexibility of zein films via its reaction with phenolic aldehydes by forming Schiff bases of zein can be attributed to form side phenolic and alkyl groups of aldehydes on zein polypeptide chains resulting to attenuate the hydrophobic interactions between these chains and subsequently increase of chain mobility and flexibility of the resultant film. Moreover, The Schiff bases reaction conditions can affect on lutein that has an obvious role in maintaining zein polypeptide conformation. Changes in this conformation can lead to change the exposing functional groups of these polypeptides and subsequently alter the nature and types of forces forming between these polypeptide chains (Soliman et al. 2009; Arcan and Yemenicioğlu 2010).

Contact angle

When a drop of liquid is placed on a solid surface, the liquid spread across the surface to form a thin, approximately uniform

Table 2 Influence of reaction temperature and time on elongation percentage of Schiff bases-based films

Zein Schiff bases films	Elongation (%)				
	Temp.(°C)	Time (h)			
		2	4	6	8
CA	25	193±23 [b]	145±50 [cd]	95±46 [d]	97±12 [d]
	40	242±32 [a]	238±20 [a]	222±30 [ab]	124±27 [d]
	50	245±36 [a]	183±11 [b]	170±22 [bc]	129±16 [d]
	60	238±41 [a]	239±19 [a]	252±30 [a]	225±13 [ab]
BA	25	158±21 [c]	213±14 [ab]	150±54 [c]	177±33 [bc]
	40	218±50 [ab]	239±21 [a]	212±46 [ab]	218±46 [ab]
	50	112±18 [d]	151±24 [c]	172±68 [bc]	192±14 [b]
	60	264±36 [a]	172±38 [bc]	267±14 [a]	168±12 [bc]
DMABA	25	213±25 [ab]	157±17 [c]	179±45 [bc]	203±14 [b]
	40	219±37 [ab]	224±38 [ab]	223±20 [ab]	233±15 [ab]
	50	165±27 [bc]	233±36 [ab]	175±32 [bc]	266±33 [a]
	60	229±30 [ab]	197±35 [b]	210±30 [b]	164±37 [bc]

n=5

Values not followed by the same letters are significantly different at $P \leq 0.05$

CA: Schiff bases based on cinnamaldehyde; BA: Schiff bases based on benzaldehyde; *DMABA: Schiff bases based on 4-dimethylaminobenzaldehyde)* film (duplex film) or spread to a limited extent but remain as a discrete drop on the surface. The contact angle (θ) is defined as the angle formed by the intersection of the two tangent lines to the liquid and solid surfaces at the perimeter of contact between the two phases and the third surrounding phase (mostly air or vapor) (Wong et al. 1992). This contact angle is taken as an indication of superficial hydrophilicity (hydrophobicity) of the films. Table 3 showed the contact angles of zein-based and zein Schiff bases-based films that indicate influence of temperature and time of the reaction on hydrophilicity of the prepared types of Schiff bases films. These data exhibited that zein based film has a relatively high contact angle (37.25°). The contact angles for all Schiff bases-based films prepared at 25 °C were lower than that value. While, contact angles of these Schiff bases films were increased with increasing the reaction temperature up to 40 °C, then these values were decreased again at higher reaction temperature. Moreover, extending reaction time lead to a significant increase in values of contact angles at different temperatures. However, extending the reaction time has lower significant effect on contact angle than that for temperature. It was observed that there is a noticeable difference in the hydrophilicity of the different types of obtained Schiff bases films prepared at 25 °C for 8 h, where, Schiff bases films based on CA exhibited higher contact angle (43.30°) than those based on DMABA and BA that were 38.15° and 38.44°, respectively. This difference was significantly reduced with increasing the reaction temperature and time. It was indicated from the aforementioned results that zein based films characterized with a relatively high contact angle because of being prolamin proteins having a high ratio of hydrophobic amino acids. Whereas, forming Schiff bases of zein decreased the hydrophobicity of the resultant films as a result to change the conformational structure of zein polypeptide chains leading to exposing the more hydrophilic functional groups. The impact of this chemical modification technique on conformation of zein polypeptide chain was increased with increasing both of temperature and time of reaction can be attributed to increase the substitution degree and introducing higher amount of aldehydes. This explanation can be proven from increasing the flexibility of the resultant films.

Color

Color is, to a great extent, relevant to the film functionality because of its great impact on the appearance (Mali et al. 2004). In order to further evaluate the color differences between zein-based films and Schiff bases of zein-based films, color coordinates were calculated from the spectral distribution of the films obtained on a standard white plate. These date are presented in Table 4. Whereas, *L, a, b* and ΔE values for zein-based film are 82.2, −0.98, +20.5 and 36.54, respectively. The lightness (*L*) values of Schiff bases of zein-based films were lower than those of zein-based films. However, slight differences were noticed between the values of lightness of different types of Schiff bases of zein-based films. Thus the red-green (*a*) values for Schiff bases films are generally higher than that of zein-based film. While, yellow-blue (*b*) values of such films were higher than zein ones. For the influence of reaction temperature and time on the color of Schiff bases films. Generally, Schiff bases-based films exhibited slight increases in *L* and ΔE values with increasing reaction temperature and time. However, extent of the impact of reaction temperature and time on color of the resultant films was varied based on type of Schiff base. Where, Schiff base films based on BA had lighter color than

Table 3 Influence of reaction temperature and time on contact angle of Schiff bases-based films

Zein Schiff bases films	Contact angle (θ)				
	Temp.(°C)	Time (h)			
		2	4	6	8
CA	25	29.4±5.00 c	36.3±1.62 b	30.8±0.12 bc	43.6±0.73 a
	40	39.2±4.52 ab	42.2±5.84 ab	37.5±4.90 ab	45.7±0.33 a
	50	34.3±5.14 b	28.1±0.62 c	26.8±0.94 c	36.4±1.60 b
	60	32.5±4.90 bc	25.1±1.31 c	23.4±1.82 cd	23.9±5.33 cd
BA	25	35.8±2.52 b	33.2±3.13 b	42.9±3.24 a	38.2±4.82 ab
	40	43.1±1.73 ab	47.0±1.80a	34.4±5.12 b	36.2±2.00 b
	50	32.7±4.42 bc	31.7±2.53 bc	25.3±1.41 c	39.7±3.63 ab
	60	28.1±1.83 c	42.2±1.94 a	27.2±0.94 c	37.7±2.94 ab
DMABA	25	33.5±4.30 bc	36.4±5.52 b	31.2±1.92 bc	38.9±2.06 ab
	40	40.2±1.14 a	37.8±0.94 ab	37.1±3.93 ab	35.8±2.80 b
	50	30.5±2.60 bc	25.7±1.33 c	28.8±1.82 c	36.6±1.34 b
	60	30.3±0.43 bc	32.4±2.50 b	35.7±2.20 b	36.8±2.40 b

n=5

Values not followed by the same letters are significantly different at $P \leq 0.05$

CA: Schiff bases based on cinnamaldehyde; BA: Schiff bases based on benzaldehyde; *DMABA: Schiff* bases based on 4-dimethylaminobenzaldehyde)

Table 4 Color coordinates values for Schiff bases-based films

Temp. (°C)	Schiff bases-based films												
	Time (hrs)	CA				BA				DMABA			
		L	*a*	*b*	Δ*E*	*L*	*a*	*b*	Δ*E*	*L*	*a*	*b*	Δ*E*
25	2	74.3±0.3^{e}	+ 4.4±0.6^{b}	+ 60.7±0.4^{cd}	47.5±0.4^{bc}	80.6±0.7^{c}	−2.2±0.1^{g}	+ 40.2±0.4^{f}	38.1±1.1^{d}	78.2±0.7^{bc}	+ 7.0±0.3^{ef}	+ 52.0±0.8^{d}	43.4±0.4^{de}
	4	73.3±0.4^{fe}	+ 2.8±0.2^{cd}	+ 55.8±0.6^{f}	42.8±0.3^{f}	78.9±0.8^{de}	+ 0.3±0.1^{d}	+ 46.8±1.2^{d}	40.2±1.2^{c}	78.0±0.6^{c}	+ 7.5±0.2^{e}	+ 51.3±0.5^{de}	42.8±0.6^{e}
	6	75.6±0.9^{d}	+ 2.9±0.3^{c}	+ 58.4±1.2^{de}	46.2±0.6^{d}	79.6±0.5^{d}	+ 0.2±0.1^{d}	+ 45.5±0.9^{d}	39.9±0.5^{cd}	74.1±1.0^{e}	+ 12.8±0.6^{bc}	+ 66.5±0.8^{ab}	53.3±0.7^{ab}
	8	77.6±1.1^{b}	+ 0.5±0.1^{f}	+ 60.2±1.0^{cd}	48.9±0.3^{b}	77.4±1.1^{de}	+ 0.7±0.2^{c}	+ 48.1±0.2^{c}	39.9±0.6^{cd}	67.1±0.9^{f}	+ 21.5±0.5^{a}	+ 67.5±0.7^{a}	53.6±0.6^{ab}
40	2	74.8±0.2^{de}	+ 3.9±0.3^{bc}	+ 61.0±0.3^{c}	39.6±0.2^{g}	80.9±0.2^{c}	−2.5±0.1^{gh}	+ 43.7±0.2^{de}	40.2±0.2^{c}	78.3±0.4^{bc}	+ 6.2±0.2^{f}	+ 54.0±0.4^{cd}	44.8±0.4^{d}
	4	76.1±1.1^{cd}	+ 3.2±0.2^{c}	+ 58.4±1.2^{de}	46.6±0.3^{cd}	80.8±0.2^{c}	−2.3±0.2^{g}	+ 42.1±0.6^{e}	39.2±0.7^{cd}	78.5±0.3^{bc}	+ 6.6±0.2^{f}	+ 53.8±0.5^{cd}	44.9±0.3^{d}
	6	72.7±0.3^{e}	+ 7.4±0.2^{a}	+ 65.4±0.9^{a}	50.8±0.4^{a}	83.7±0.3^{ab}	−3.6±0.4^{h}	+ 32.6±1.2^{h}	38.1±0.8^{d}	73.2±0.2^{ef}	+ 14.2±0.7^{b}	+ 67.9±0.2^{a}	54.4±0.5^{a}
	8	77.8±0.6^{b}	+ 1.5±0.1^{e}	+ 57.7±0.3^{e}	47.1±0.2^{c}	79.1±0.6^{cd}	+ 1.0±0.1^{b}	+ 52.1±0.3^{b}	44.1±0.3^{b}	76.9±0.7^{cd}	+ 8.9±0.4^{c}	+ 59.4±0.6^{bc}	48.4±0.6^{bc}
50	2	77.1±0.2^{bc}	+ 1.5±0.3^{e}	+ 54.0±0.6^{fg}	43.8±0.4^{e}	82.8±0.5^{bc}	−2.0±0.1^{g}	+ 34.3±0.1^{gh}	37.5±0.8^{e}	78.5±1.0^{bc}	+ 7.5±0.2^{e}	+ 58.1±0.7^{c}	46.9±0.6^{c}
	4	78.3±0.5^{ab}	+ 1.5±0.1^{e}	+ 52.3±0.2^{g}	43.4±0.3^{ef}	83.3±0.6^{b}	−2.2±0.1^{g}	+32.2±0.2^{h}	37.5±0.2^{e}	79.9±0.3^{b}	+ 3.9±0.3^{g}	+ 47.2±0.9^{e}	41.2±0.6^{ef}
	6	77.8±0.3^{bc}	+ 2.4±0.1^{d}	+ 52.5±1.3^{g}	43.2±0.6^{ef}	84.3±1.2^{a}	−1.6±0.1^{d}	+ 29.0±0.8^{i}	37.5±0.7^{e}	75.8±0.1^{de}	+ 9.4±0.3^{c}	+ 60.5±0.2^{b}	48.6±0.3^{bc}
	8	78.5±0.4^{ab}	−0.2±0.0^{f}	+ 61.0±0.4^{c}	50.1±0.2^{ab}	79.2±0.9^{cd}	+ 0.4±0.0^{f}	+ 26.5±0.3^{j}	41.1±0.1^{c}	78.0±0.2^{c}	+ 5.6±0.5^{f}	+ 56.1±0.5^{cd}	46.1±0.7^{c}
60	2	76.5±0.2^{c}	+ 1.2±0.1^{e}	+ 59.0±0.7^{d}	47.3±0.4^{bc}	82.9±0.9^{bc}	−3.7±0.5^{h}	+ 35.3±0.3^{g}	38.2±0.3^{d}	83.0±0.6^{a}	+ 0.1±0.0^{j}	+ 39.5±0.5^{e}	39.8±0.4^{f}
	4	76.9±0.4^{bc}	+ 2.2±0.1^{d}	+ 62.5±1.1^{b}	50.4±0.7^{ab}	80.4±1.1^{c}	−1.0±0.1^{e}	+ 44.8±1.4^{d}	40.3±1.3^{c}	82.2±0.4^{ab}	+ 1.0±0.2^{i}	+ 41.9±0.2^{f}	40.0±0.2^{f}
	6	74.9±0.1^{de}	+ 3.8±0.2^{bc}	+ 61.6±0.2^{bc}	48.6±0.2^{b}	80.6±0.2^{c}	−1.7±0.1^{f}	+ 45.7±0.9^{d}	41.0±0.7^{b}	76.5±0.7^{d}	+ 8.1±0.5^{d}	+ 61.3±0.6^{b}	49.5±0.4^{b}
	8	81.4±0.4^{a}	−3.2±0.2^{c}	+ 41.5±0.3^{h}	39.5±0.3^{g}	76.0±0.7^{e}	+ 3.7±0.2^{a}	+ 64.6±0.3^{a}	51.6±0.2^{a}	80.0±0.6^{b}	+ 2.2±0.1^{h}	+ 47.2±0.2^{e}	41.3±0.3^{e}

n=4

Values not followed by the same letters are significantly different at $P \leq 0.05$

L; lightness (black to white), *a*; greenness to redness, *b;* blueness to yellowness, and Δ*E*; total color difference

CA: Schiff bases based on cinnamaldehyde; BA: Schiff bases based on benzaldehyde; DMABA: Schiff bases based on 4-dimethylaminobenzaldehyde)

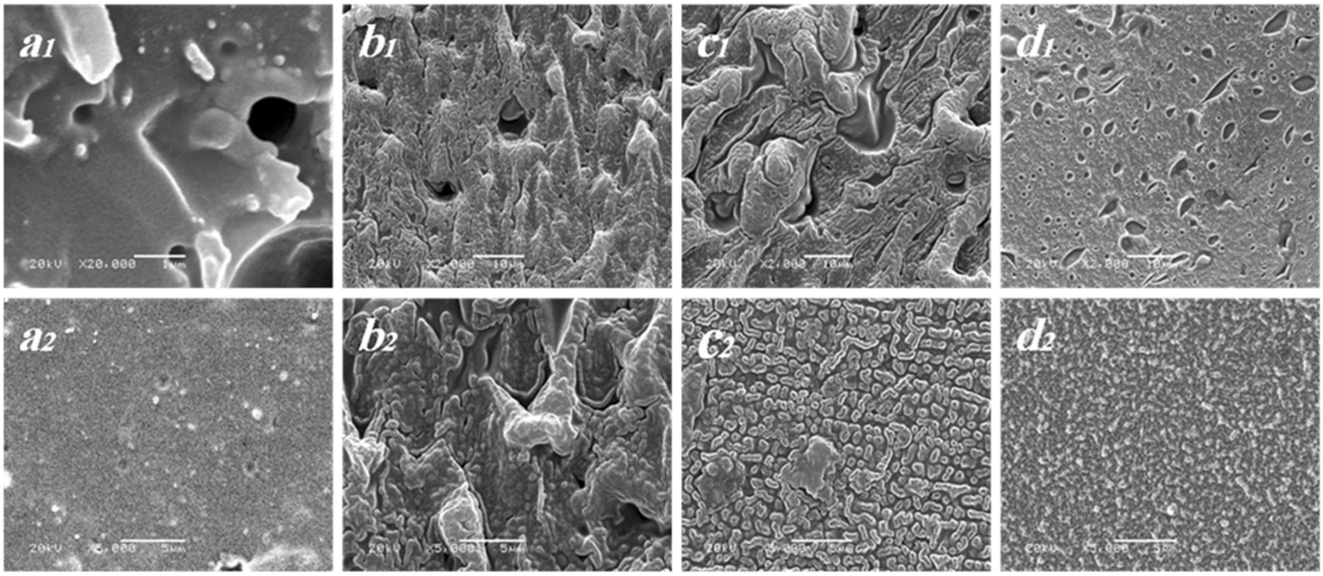

Fig. 2 Scanning electron micrographs of zein- and Schiff bases-based films (*a*, pure zein; *b*, Schiff bases based on cinnamaldehyde; *c* Schiff bases based on benzaldehyde; *d* Schiff bases based on 4-dimethylaminobenzaldehyde) at a magnification power of 2000× (1); and 5000× (2)

those based on DMABA. Whilst, the latter films were lighter than those based on CA. Change in color of Schiff bases of zein-based films can be attributed to color belonging to Schiff bases themselves, in addition to the natural pigment (lutien) that can be affected by the potential oxidation-reduction reactions through synthesis process (Soliman et al. 2009).

Microstructure

Differences in the film microstructure of the zein- and Schiff bases of zein-based films were investigated by SEM (Fig. 2). Zein has been described as a globular protein in many systems, while, Lai and Padua (1997) when studied the microstructure of the zein films and the zein casting films plasticized with oleic acid, they observed three types of structures in the films (in the same sample): (1) aligned protein fibrils embedded in a continuous material, (2) arrangements of oriented ribbon-like structures of zein, and (3) entanglements of ribbon-like strands of zein. In our works, we observed these three different structures in different films. In the plasticized zein films, compact and dense structures with little cavities and voids were detected (Fig. $2a_{1,2}$). These micrographs showed continuous even matrix because existing plasticizer increased association within protein polymer chains (Pereda et al. 2010). On the other hands, micrographs of the Schiff bases of zein films based on CA, BA and DMABA (Fig. $2b_{1,2}$, $c_{1,2}$, $d_{1,2}$) exhibited that topography of these films were clearly uneven and had rougher surface than the zein-based films. Moreover, more cavities and voids were observed in these films. However, the extent of wrinkling and compactness were different based on the type of aldehyde, where the films based on DMABA characterized with more dense structure with less wrinkles comparing with the other Schiff bases films. These noticeable topographic features of Schiff bases films owing to collapsing continuous structure and alteration of entanglements of ribbon-like strands were thought to be the result of change the evaporation rate of solvent. These changes in assembly of zein structures with forming Schiff bases can be interpreted with changing type and intensity of the linking forces between the zein Schiff bases

Table 5 Antibacterial activity of Schiff bases-based films

Bacteria	Growth inhibition rate (%)		
	Zein Schiff bases films		
	CA	BA	DMABA
L. monocytogenes	34±2.3 [d]	29±1.2 [d]	37±2.9 [cd]
L. innocua	36±3.4 [cd]	42±3.8 [c]	26±4.2 [de]
C. sporogenes	31±3.5 [d]	44±1.9 [c]	43±3.9 [c]
B. cereus	57±4.9 [b]	72±4.2 [b]	78±2.2 [ab]
S. enterica	62±3.8 [b]	84±5.8 [a]	92±2.8 [a]
Y. enterocolitica	18±6.1 [e]	43±1.8 [c]	29±4.3 [d]
E. coli	39±2.1 [cd]	41±1.9 [c]	25±1.0 [de]

n=3

Values not followed by the same letters are significantly different at $P \leq 0.05$

CA: Schiff bases based on cinnamaldehyde; BA: Schiff bases based on benzaldehyde; DMABA: Schiff bases based on 4-dimethylaminobenzaldehyde. All of these Schiff bases were prepared at 40 °C for 4 h molecules, plasticizer, and other components in the system particularly, hydrophobic interactions and hydrogen bonding that subsequently affect hydrophilic–lipophilic balance of the system, where, structures formation and zein self-assembly is considered a spontaneous processes controlled by the hydrophilic–lipophilic balance as reported in previous studies.

Antibacterial activity

The capabilities of the zein- and Schiff bases of zein-based films in inhibiting the growth of the tested bacteria, *L. monocytogenes*, *L. innocua, Clo. sporogenes, Y. enterocolitica* and *E. coli*, *B. cereus* and *S. enterica* were assessed. The obtained results of these biological assays were shown in Table 5. The obtained results revealed that the zein-based film have no antibacterial activity against these bacterial strains. These results are in agreement with previous works of Janes and his colleagues (2002) and Lungu and Johnson (2005), as they showed that zein films had no effect on suppressing the growth of *L. monocytogenes*. Whereas, Schiff bases films exhibited higher antibacterial effect on the tested strains comparing with zein film. This inhibitory effect was differed according to the type of aldehyde and type of tested bacterial strains. All Schiff bases films exhibited higher inhibitory effect on *B. cereus* and *S. enterica* comparing with their effects on other tested bacteria. Moreover, this effect was more pronounced on gram negative bacterium, *S. enterica* than that on gram positive bacterium, *B. cereus*. On the other side, the inhibitory effect for Schiff base films based on DMABA was significantly higher than those resulting from Schiff bases based on CA or BA. Nevertheless, the antibacterial activity of such synthesized Schiff bases of zein was lower than the free aldehyde as reported in many previous studies, where high activity of CA was reported against *L. monocytogenes and E. coli* (Friedman et al. 2002; Gill and Holley 2004; Ravishankar et al. 2009) and thus for BA against *E. coli*, *L. monocytogenes*, and *S. enterica* (Friedman et al. 2002; Ramos-Nino et al. 1996; Ramos-Nino et al. 1998).

The antibacterial action of these Schiff bases arises from side aromatic functional groups of aldehydes that affect the electrostatic interactions between these species and cell membrane. Besides, the decided structural properties of the Schiff bases films can affect on the adhesion of bacterial cells and consequently on their ability for inhibition of bacterial growth. The structural changes for such Schiff bases films can be attributed to the deformation of the strong interactions in the zein backbone by act of substitution of these aromatic groups on the N atoms of zein chains. These structural properties can be indicated from the aforementioned microstructure examination and change of hydrophilicity and wettability of the film surfaces that revealed from contact angle measurements.

On the other hand, the differences between the inhibitory effect of Schiff bases on both of gram positive and gram negative bacteria can be explained on the structural differences referring the molecular structures of the outer cell membrane. In gram-negative bacteria, phospholipids, lipopolysaccharides and proteins exist in the outer membrane. Therefore, electric interaction hardly influences the inner membrane. In contrast, in gram-positive bacteria, the electric charge of Schiff bases acts directly on the inner membrane of the cell. The electric interaction between Schiff bases and the cell membrane was one factor inducing bacterial growth inhibition, cell destruction, and activity depression. Moreover, extracellular protyoletic enzymes and metabolic products can be another factor affecting the capability of Schiff bases in inhibiting the bacterial growth through its impact on both of the physicochemical properties of the Schiff bases molecules and the medium.

Conclusions

Schiff bases of zein-based films were synthesized, structurally, optically, mechanically characterized and their antibacterial activity against *L. monocytogenes*, *L. innocua, C. sporogenes, Y. enterocolitica* and *E. coli*, *B. cereus* and *S. enterica* was assessed. The results exhibited that this chemical modification technique had a potential for modify the properties of zein-based films such as mechanical, solubility and antibacterial activity. Formation of Schiff bases of zein may hold practical significance in improving the flexibility, solubility and antibacterial activity. Therefore, these improvements can extend the potential applications of Schiff bases films in packaging and biomedical fields.

Acknowledgements This work was supported by a grant of Innovation Investment Initiative (III), under Research, Development and Innovation Programme (RDI), on behalf of the Scientific Research Ministry of Egypt.

References

Ali SM, Khan AA, Ahmad I, Musaddiq M, Ahmed K, Polase H, Rao LV, Habibullah CM, Sechi LA, Ahmed N (2005) Antimicrobial activities of eugenol and cinnamaldehyde against human gastric pathogen *Helicobacter pylori*. Annu Clin Microbiol Antimicrob 4:20–28

Anderson TJ, Lamsal BP (2011) Zein extraction from corn, corn products, and co-products and modifications for various applications: a review. Cereal Chem 2:159–173

Arcan I, Yemenicioğlu A (2010) Incorporating phenolic compounds opens a new perspective to use zein films as flexible bioactive packaging materials. Food Res Int 44:550–556

ASTM (2011) Annual book of ASTM standards. American Society for Testing and Materials, Philadelphia

Bhat R, Karim AA (2012) Towards producing novel fish gelatin films by combination treatments of ultraviolet radiation and sugars (ribose and lactose) as cross-linking agents. J Food Sci Technol. doi:10.1007/s13197-012-0652-9

Colthup NB, Daly LH, Wiberley SE (1990) Introduction to infrared and Raman spectroscopy, 3rd edn. Academic, San Diego

Friedman M, Henika PR, Mandrell RE (2002) Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes*, and *Salmonella enterica*. J Food Protect 65:1545–1560

Gende LB, Floris I, Fritz R, Eguaras MJ (2008) Antimicrobial activity of cinnamon (*Cinnamomum zeylanicum*) essential oil and its main components against *Paenibacillus larvae* from Argentine. B Insect 61:1–4

Gill AO, Holley RA (2004) Mechanisms of bactericidal action of Cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. Appl Environ Microbiol 70:5750–5755

Guinesi L, Cavalheiro E (2006) Influence of some reactional parameters on the substitution degree of biopolymeric Schiff bases prepared from chitosan and salicylaldehyde. Carbohyd Polym 65:557–561

Guo Y, Liu Z, An H, Li M, Hu J (2005) Nano-structure and properties of maize zein studied by atomic force microscopy. J Cereal Sci 3:277–281

Janes ME, Kooshesh S, Johnson MG (2002) Control of *Listeria monocytogenes* on the surface of refrigerated, ready-to-eat chicken coated with edible zein film coatings containing nisin and/or calcium propionate. J Food Sci 67:2754–2757

Jin X, Wang J, Bai J (2009) Synthesis and antimicrobial activity of the Schiff base from chitosan and citral. Carbohydr Res 344:825–829

Lai HM, Padua GW (1997) Properties and microstructure of plasticized zein films. Cereal Chem 74:771–775

Lawton JW (2002) Zein: a history of processing and use. Cereal Chem 79:1–18

Lungu B, Johnson MG (2005) Fate of *Listeria monocytogenes* inoculated onto the surface of model turkey frankfurter pieces treated with zein coatings containing nisin, sodium diacetate, and sodium lactate at 4°C. J Food Protect 68:855–859

Mali S, Grossmann MVE, Garcia MA, Martino MN, Zaritzky NE (2004) Barrier, mechanical and optical properties of plasticized yam starch films. Carbohydr Polym 56:129–135

Pereda M, Aranguren M, Marcovich N (2010) Caseinate films modified with Tung oil. Food Hydrocoll 24:800–808

Raman N, Kulandaisamy A, Thangaraja C, Jeyasubramanian K (2003) Redox and antimicrobial studies of transition metal (II) tetradentate Schiff base complexes. Transit Metal Chem 28:29–36

Ramos-Nino ME, Clifford MN, Adams MR (1996) Quantitative structure activity relationship for the effect of benzoic acids, cinnamic acids and benzaldehydes on *Listeria monocytogenes*. J Microbiol 80:303–310

Ramos-Nino ME, Ramirez-Rodriguez CA, Clifford MN, Adams MR (1998) QSARs for the effect of benzaldehydes on foodborne bacteria and the role of sulfhydryl groups as targets of their antibacterial activity. J Appl Microbiol 84:207–212

Ravishankar S, Zhu L, Olsen CW, McHugh TH, Friedman M (2009) Edible apple film wraps containing plant antimicrobials inactivate foodborne pathogens on meat and poultry products. J Food Sci 74:440–445

Scramin JA, de Britto D, Forato LA, Bernardes-Filho R, Colnago LA, Assis OBG (2011) Characterization of zein–oleic acid films and applications in fruit coating. J Food Sci Technol 46:2145–2152

Shi L, Fang RQ, Zhu ZW, Yang Y, Cheng K, Zhu HL (2010) Design and synthesis of potent inhibitors of γ-ketoacyl-acyl carrier protein synthase III (FabH) as potential antibacterial agents. Eur J Med Chem 45:4358–4364

Shukla TP (1992) Trends in zein research. Cereal Food World 37:225–232

Shukla R, Cheryan M (2001) Zein: the industrial protein from corn. Ind Crop Prod 13:171–192

Soliman EA, Mohy Eldin MS, Furuta M (2009) Biodegradable zein-based films: influence of γ-irradiation on structural and functional properties. J Agric Food Chem 57:2529–2535

Ünalan İU, Korel F, Yemenicioğlu A (2011) Active packaging of ground beef patties by edible zein films incorporated with partially purified lysozyme and Na_2EDTA. J Food Sci Technol 46:1289–1295

Wong DWS, Gastineau FA, Gregorski KS, Tillin SJ, Pavlath AE (1992) Chitosan-lipid films: microstructure and surface energy. J Agric Food Chem 40:540–544

Zhong Z, Li P, Xing R, Chen X, Liu S (2009) Preparation, characterization and antifungal properties of 2-(β-arylamino phosphonate)-chitosan. Int J Biol Macromol 45:255–259