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ORIGINAL PAPER

Antimicrobial Activity of the Chitosan Extracted from *Metapenaeus stebbingi* Shell Wastes

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Abstract In the study, chitosan chemically extracted from *Metapenaeus stebbingi* shells obtained from shrimp processing factories and commercial chitosan were used as antimicrobial materials. Antimicrobial activities of the chitosans dissolved in acetic, lactic, formic and hydrochloric acid at different concentrations (1.00, 0.50, 0.25, 0.10 and 0.05%) were tested in vitro by using the disk diffusion method with standard microorganisms (*Pseudomonas putida*, *Pseudomonas fluorescens, Vibrio parahaemolyticus, Staphylococcus epidermidis*, *Staphylococcus aureus, Escherichia coli* 1, *Escherichia coli* 2, *Listeria monocytogenes, Enterecoccus faecalis, Aeromonas caviae*). On the other hand, gentamicin was used as positive control. Antimicrobial test results indicated that chitosan demonstrated different effects depending on the chitosan concentration, solvent material and bacteria type. In the present study, both the extracted and commercial chitosans were observed to have antimicrobial effects on nearly all types of the bacteria.

Keywords Chitosan · Antimicrobial activity · *Metapenaeus stebbingi* shell

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Introduction

Chitin (β-1,4-poly-*N*-acetyl-*D*-glucosamine) is a natural biopolymer and its production in biomass of up to 10^{12} tons/year makes it one of the most abundant polysaccharides on Earth [1]. It is the main component of cell walls of fungi, exoskeletons of arthropods such as crustaceans (e.g. crabs, lobsters and shrimps) and insects. Chitosan (poly-β-1.4-2-amino-2-deoksi-β–D-glucopyranose) is derived by deacetylation of chitin [2, 3]. Due to its biodegradability, biocompatibility, non toxic and wound healing properties and haemostatic activity; chitosan has received increased attention as one of the promising renewable polymeric materials for various applications [4].

Recently, many studies of chitosan have focused on its strong antimicrobial effects against different groups of microorganism such as bacteria, fungi and yeast [5–10]. In fact, a number of commercial applications of chitosan benefit from its antimicrobial activity, and it is widely used in food preservation, dentistry, ophthalmology, the manufacture of wound dressings and antimicrobial finished textiles. Therefore, investigations of the antimicrobial potential of chitosan and its derivatives have recently gained momentum [11]. In the present study, chitosan was primarily extracted through chemical method from *Metapenaeus stebbingi* shells which are not used and discarded as waste product in Turkey. Solutions of the obtained chitosan at different concentrations dissolved in different organic acids like acetic acid, lactic acid, formic acid and hydrochloric acid, and the antimicrobial activities of these solutions on standard bacteria (*Pseudomonas putida*, *Pseudomonas fluorescens, Vibrio parahaemolyticus, Staphylococcus epidermidis*, *Staphylococcus aureus, Escherichia coli* 1, *Escherichia coli* 2, *Listeria monocytogenes, Enterecoccus faecalis, Aeromonas caviae*) were

determined using disc diffusion method. Gentamicin, an aminoglycoside antibacterial, was used as positive control.

Materials and Methods

Materials

In the study, shell materials were obtained from the wastes of *M. stebbingi*. Fresh samples of shrimp processing discards, including intact cephalothorax and abdominal exoskeleton, were collected from a local shrimp factory. Shells were completely separated from the shrimp wastes in laboratory, washed in pure water at room temperature and dried at 60 °C. Commercial chitosan was extracted from crab shells (Sigma Chemical Co., St Louis, MO, USA) and used in the comparison with the extracted chitosan in the study.

Preparation of Chitosan and Evaluation of their Characteristics

Chitosan was extracted from shrimp processing discards, using a method of Chang et al. [12]. The moisture was determined after drying samples for 24 h at 105 °C, and ash content was determined by heating at 530 °C for 20 h. Apparent viscosity of a 1% (w/v) chitosan in a 1% (v/v) acetic acid solution at 25 °C was measured in triplicate using an AMVn Automated Micro Viscometer (Anton Paar, Graz, Austria) and values reported in centipoise (cP) units. Degree of deacetylation (DD) of chitosan was determined by a potentiometric titration method [13]. For the determination of viscosity-average molecular weight, eight chitosan solutions were prepared at different concentrations in 0.2 M NaCl/0.1 M AcOH. The efflux times of the solutions were measured in triplicate using an Ubbelohde capillary viscometer in a constant-temperature water bath at 25 °C. The running times of the solution and solvent material were recorded as seconds (s) and used to calculate intrinsic viscosity. The Mark-Houwink equation $[\eta] = KM^{\alpha}$ was used to calculate the molecular weight of chitosan. In this equation, $[\eta]$ defines the intrinsic viscosity, K and α are the constant values based on the nature of the polymer and solvent material as well as on temperature. M is relative molecular weight. Values of K and α were 1.64×10^{-30} X DD^{14} (mL/g) and 1.02×10^{-2} X DD + 1.82, respectively, and *DD* is the degree of the deacetylation of chitosan expressed as the percentage [14]. Water binding capacity (WBC) and fat binding capacities (FBC) of chitosan were measured using a modified method of Wang and Kinsella [15]. Water and fat absorptions were initially carried out by weighing a centrifuge tube containing 0.5 g of sample, adding 10 mL of water or soybean oil and mixing on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with shaking for 5 s every 10 min and centrifuged at 3,500 rpm for 25 min. After the supernatant was decanted, the tube was weighed again.

Bacterial Strains and Antimicrobial Susceptibility Testing

The disc diffusion method was employed for the determination of antimicrobial activities of the chitosan solutions [16]. *Escherichia coli* 1 (ATCC-25922), *P. putida* (NRRL-B-14875), *A. caviae* (NRRL-968), *V. parahaemolyticus* (ATCC-17802), *P. fluorescens* (NRRL-2641), *S. aureus* (ATCC-25923), *S. epidermidis* (NRRL-B-4268), *E. coli* 2 (NRRL-B-4269), *L. monocytogenes* (ATCC-7644), *E. faecalis* (ATCC-29212) were used as standard organisms. Bacterial strains were cultured on Luria–Bertani (LB) agar (Merck, MILLER 110283) plates and the plates were incubated for 24 h at 37 °C. After the incubation, bacterial concentration was adjusted to McFarland no standard 0.5 and swabbed on Mueller–Hinton agar (MHA) (Merck, 1.05437) plates. After swabbing the bacteria, antibiotic free paper test disks (Oxoid, 6 mm in diameter) were put on to agar plates. Chitosan solutions were dissolved in four different solvents (1% lactic acid; 1% acetic acid; 0.2% formic acid; 0.1 N hydrochloric acid) at five different concentrations (1.00, 0.50, 0.25, 0.10 and 0.05%). 20 μL chitosan solutions inoculated paper disk and all plates were incubated for 24 h at 37 °C. The antimicrobial activity was evaluated by measuring the zone diameter (mm). The acids without chitosan were used as solvent control. Gentamycin, an aminoglycoside antibiotic (20 μL), was used served as positive control. All tests were performed in duplicate.

Statistical Analysis

Analysis of variance was performed using SPSS statistical package program (SPSS 15.0 for Windows, SPSS Inc., Chicago, IL).

Results and Discussion

Physicochemical Properties of Chitosan

Physicochemical properties of chitosan extracted from *M. stebbingi* shells and commercial chitosan were investigated. The results of moisture, ash, deacetylation degree, molecular weight, apparent viscosity, water and fat binding capacities of chitosan extracted from *M. stebbingi* shells and commercial chitosan are shown in Table 1. Chitosan extracted from *M. stebbingi* shells had higher deacetylation

Table 1 The physicochemical characterization of extracted from *M. stebbingi* shells and commercial chitosan

	Extracted chitosan	Commercial chitosan
Moisture (%)	1.33 ± 0.08	1.07 ± 0.09
Ash (%)	0.61 ± 0.03	0.59 ± 0.07
Deacetylation degree (%)	92.19 ± 2.56	86.92 ± 1.62[a]
Molecular weight (kDa)	2.20 ± 0.03	3.52 ± 0.02[b]
Apparent viscosity	46.14 ± 0.07	31.88 ± 0.00[b]
Water binding capacity (%)	712.99 ± 11.98	492.67 ± 10.14[b]
Fat binding capacity (%)	531.15 ± 12.26	383.04 ± 10.02[b]

Mean ± standard deviation of triplicate determinations

[a] Significant difference between two groups ($p < 0.05$)

[b] Significant difference between two groups ($p < 0.01$)

degree ($p < 0.05$), viscosity, water and fat binding capacity and lower molecular weight compared to commercial chitosan ($p < 0.01$). These physicochemical properties of chitosan, especially the deacetylation degree and molecular weight, showed differences depending on the crustacean species and the extraction method [17–19].

Antimicrobial Activity of Chitosan Dissolved in Different Organic Acids

The antimicrobial activity results of chitosan extracted from *M. stebbingi* shells and commercial chitosan in different concentrations of acetic acid solution are shown in Table 2. Extracted chitosan dissolved in acetic acid demonstrated inhibition zone diameter between 7.00 and 13.90 mm in all types of bacteria except for *S. epidermidis, S. aureus, E. coli* 1 and *E. coli* 2 in 0.10% concentration and for *S. epidermidis, S. aureus, E. coli* 1, *E. coli* 2 and *L. monocytogenes* in 0.05% concentration.

Inhibition zone diameter changed with different extracted chitosan concentrations of acetic acid and it was determined between 11.00 and 13.90 in *P. fluorescens*, *S. epidermidis* and *S. aureus* in 0.50% concentration; *P. putida*, *P. fluorescens*, *E. coli* 2 and *A. caviae* in 0.25% concentration; *P. putida, P. fluorescens* and *E. faecalis* in 0.10% concentration; and *P. fluorescens* and *V. parahaemolyticus* in 0.05% concentration, while it was determined between 9.00 and 10.90 mm for all bacteria in 1.00% concentration; *P. putida*, *V. parahaemolyticus*, *E. coli* 1, *E. coli* 2, *L. monocytogenes*, *E. faecalis* and *A. caviae* in 0.50% concentration; *V. parahaemolyticus*, *S. epidermidis, S. aureus, E. coli* 1, *L. monocytogenes* and *E. faecalis* in 0.25% concentration; *V. parahaemolyticus* and *A. caviae* in 0.10% concentration; *P. putida* and *E. faecalis* in 0.05% concentration. The inhibition zone diameter was observed

Table 2 Antimicrobial activity of chitosan extracted from *M. stebbingi* shells and commercial chitosan in acetic acid

Sample	Microorganisms and inhibition zone (mm)										
	C (%)	Pp	Pf	Vp	Se	Sa	Ec1	Ec2	Lm	Ef	Ac
Extracted chitosan	1.00	++	++	++	++	++	++	++	++	++	++
	0.50	++	+++	++	+++	+++	++	++	++	++	++
	0.25	+++	+++	++	++	++	++	+++	++	++	+++
	0.10	+++	+++	++	–	–	–	–	+	+++	++
	0.05	++	+++	+++	–	–	–	–	–	++	+
Commercial chitosan	1.00	++	++	+	++	++	++	++	++	++	++
	0.50	++	++	++	++	++	++	++	++	++	+++
	0.25	++	++	+++	++	++	++	++	++	++	++
	0.10	++	+++	++	–	++	–	–	–	–	++
	0.05	++	+++	++	–	–	–	–	–	++	++
Control-acid		–	–	+	–	–	–	–	–	–	+
Gentamisine		+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
		(29.75 ± 0.50)	(32.37 ± 0.47)	(26.18 ± 2.44)	(28.62 ± 1.10)	(26.75 ± 0.64	(29.50 ± 2.30)	(30.32 ± 3.91)	(23.50 ± 0.70)	(24.12 ± 1.93)	(25.00 ± 2.12)

Results were interpreted in terms of the diameter of the inhibition zones: (–), <7.00 mm; (+), 7.00–8.90 mm; (++), 9.00–10.90 (+++), 11.00–13.90 mm; (++++), 14.00–16.00 mm. (+++++), >16.00. *Pp Pseudomonas putida* NRRL-B-14875, *Pf Pseudomonas fluorescens* NRRL-2641, *Vp Vibrio parahaemolyticus* ATCC-17802, *Se Staphylococcus epidermidis* NRRL-B-4268, *Sa Staphylococcus aureus* ATCC-25923, *Ec1 Escherichia coli* ATCC-25922, *Ec2 Escherichia coli* NRRL-B-4269, *Lm Listeria monocytogenes* ATCC-7644, *Ef Enterecoc faecalis* ATCC-29212, *Ac Aeromonas caviae* NRRL-968, *C* concentration

between 7.00 and 8.90 mm for *L. monocytogenes* in 0.10% concentration, and *A. caviae* in 0.05% concentration.

Inhibition zone diameters of commercial chitosan were observed between 7.00 and 13.90 mm in all bacteria except for *S. epidermidis, E. coli* 1, *E. coli* 2 *L. monocytogenes* and *E. faecalis* with 0.10% concentration and *S. epidermidis, S. aureus, E. coli* 1 and *E. coli* 2 with 0.05% concentration. Inhibition zone diameters demonstrated differences with different acetic acid concentrations, and inhibition zone diameter between 11.00 and 13.90 mm were determined for *P. fluorescens* in concentrations between 0.50 and 0.05%, *V. parahaemolyticus* in 0.25% concentration and *A. caviae* in 0.50% concentration. Inhibition zone diameter of 7.00 and 8.90 mm was only detected for *V. parahaemolyticus* in 1.00% concentration, while it was between 9.00 and 10.90 mm for all other groups.

Acetic acid used as control group had antimicrobial effects on *V. parahaemolyticus* and *A. caviae* (7.00–8.90 mm of zone diameter), while it had no effect on other bacteria. In the present study, gentamicin (an aminoglycoside antibiotic) was used to compare the antimicrobial effects of chitosan. In the disk diffusion assays, gentamicin demonstrated the highest antimicrobial effect (>16.00 mm) on all bacteria (Table 2).

The antimicrobial activity results of the extracted and commercial chitosan in lactic acid solutions are given in Table 3. An inhibition zone diameter of 7.00–16.00 mm was formed in all bacteria except for *E. coli* 1 with the 0.25, 0.10 and 0.05% concentrations of the extracted chitosan dissolved in lactic acid.

An inhibition zone diameter of 14.00–16.00 mm was observed in *P. fluorescens* with the 0.05% concentration of the extracted chitosan dissolved in lactic acid. Inhibition zone diameter of 11.00–13.90 mm was determined in *L. monocytogenes* with its 0.25% concentration; in *P. putida, P. fluorescens, L. monocytogenes* with 0.10% concentration; and in *V. parahaemolyticus* and *L. monocytogenes* with 0.05% concentration, while inhibition zone diameter of 9.00–10.90 mm was detected most of the other bacteria.

Both types of chitosan demonstrated similar antimicrobial effect for *P. fluoresecens*. Inhibition zone diameter changed between 7.00 and 16.00 mm in all bacteria except for *E. coli* 1 with 0.10 and 0.05% concentrations of commercial chitosan dissolved in lactic acid. Inhibition zone was determined between 11.00 and 13.90 mm in *P. fluorescens* with 0.25% concentration, and in *P. fluorescens, S. aureus* and *A. caviae* with 0.10% concentration. No antimicrobial zone was formed in any bacteria except for *E. coli* 1 (7.00–8.90 mm) in the control group using only lactic acid.

Antimicrobial test results of the extracted and commercial chitosan dissolved in formic acid are presented in Table 4. Inhibition zone diameter of chitosan 7.00–13.90 was observed in all the bacteria except for 0.10% concentration of extracted chitosan for *E. coli* 1 and 0.05% concentration for *S. aureus* and *E. coli* 1.

When the formic acid was used as dissolvent based on the concentration in the study, inhibition zone diameter was observed between 11.00 and 13.90 mm for 0.25% concentration in *L. monocytogenes*, 0.10% concentration in *P. fluorescens* and *S. aureus*, and 0.05% concentration in

Table 3 Antimicrobial activity of chitosan extracted from *M. stebbingi* shells and commercial chitosan in lactic acid

Sample	Microorganisms and inhibition zone (mm)										
	C (%)	Pp	Pf	Vp	Se	Sa	Ec1	Ec2	Lm	Ef	Ac
Extracted chitosan	1.00	++	++	++	++	+	++	++	+	++	+
	0.50	++	++	++	++	++	+	+	++	++	+
	0.25	++	++	++	++	++	−	++	+++	++	+
	0.10	+++	+++	++	++	++	−	++	+++	++	++
	0.05	++	++++	+++	+	++	−	++	+++	++	++
Commercial chitosan	1.00	+	++	++	+	++	+	++	++	++	+
	0.50	++	++	++	++	++	++	++	++	++	++
	0.25	++	+++	++	++	++	++	++	++	++	++
	0.10	++	+++	++	++	+++	−	++	++	+	+++
	0.05	++	++++	++	+	++	−	++	++	+	++
Control-acid		−	−	−	−	−	+	−	−	−	−

Results were interpreted in terms of the diameter of the inhibition zones: (−), <7.00 mm; (+), 7.00–8.90 mm; (++), 9.00–10.90 (+++), 11.00–13.90 mm; (++++), 14.00–16.00 mm. (+++++), >16.00. *Pp Pseudomonas putida* NRRL-B-14875, *Pf Pseudomonas fluorescens* NRRL-2641, *Vp Vibrio parahaemolyticus* ATCC-17802, *Se Staphylococcus epidermidis* NRRL-B-4268, *Sa Staphylococcus aureus* ATCC-25923, *Ec1 Escherichia coli* ATCC-25922, *Ec2 Escherichia coli* NRRL-B-4269, *Lm Listeria monocytogenes* ATCC-7644, *Ef Enterecoc faecalis* ATCC-29212, *Ac Aeromonas caviae* NRRL-968, *C* concentration

Table 4 Antimicrobial activity of chitosan extracted from *M. stebbingi* shells and commercial chitosan in formic acid

Sample	Microorganisms and inhibition zone (mm)										
	C (%)	Pp	Pf	Vp	Se	Sa	Ec1	Ec2	Lm	Ef	Ac
Extracted chitosan	1.00	++	++	++	++	++	++	++	++	++	++
	0.50	++	++	++	++	++	++	++	++	++	++
	0.25	++	++	++	++	++	++	++	+++	+	++
	0.10	++	+++	++	+	+++	−	++	+	++	++
	0.05	+++	+++	++	+	−	−	++	+	++	++
Commercial chitosan	1.00	++	++	++	++	++	++	++	++	++	++
	0.50	++	++	++	++	++	++	++	++	++	++
	0.25	++	++	++	++	++	+++	++	+++	++	++
	0.10	+++	−	++	−	−	−	−	++	−	++
	0.05	++	−	++	−	−	−	−	++	−	++
Control-acid		+	−	−	+	−	−	+	−	−	+

Results were interpreted in terms of the diameter of the inhibition zones: (−), <7.00 mm; (+), 7.00–8.90 mm; (++), 9.00–10.90 (+++), 11.00–13.90 mm; (++++), 14.00–16.00 mm. (+++++), >16.00. *Pp Pseudomonas putida* NRRL-B-14875, *Pf Pseudomonas fluorescens* NRRL-2641, *Vp Vibrio parahaemolyticus* ATCC-17802, *Se Staphylococcus epidermidis* NRRL-B-4268, *Sa Staphylococcus aureus* ATCC-25923, *Ec1 Escherichia coli* ATCC-25922, *Ec2 Escherichia coli* NRRL-B-4269, *Lm Listeria monocytogenes* ATCC-7644, *Ef Enterecoc faecalis* ATCC-29212, *Ac Aeromonas caviae* NRRL-968, *C* concentration

P. putida and *P. fluorescens*. All 1.00 and 0.50% concentrations of the extracted chitosan created inhibition zone of 9.00–10.90 mm in all the tested bacteria. There were increases in antimicrobial effects of chitosan for some strains and decreases for some others with the decreasing concentration of chitosan.

Antimicrobial zone diameter changed between 7.00 and 13.90 mm in all bacteria except for *P. fluoresecens, S. epidermidis, S. aureus, E. coli* 1, *E. coli* 2 and *E. faecalis* with 0.10 and 0.05% commercial chitosan concentrations. It changed between 11.00 and 13.90 mm in *E. coli* 1 and *L. monocytogenes* with 0.25% concentration, and between 9.00 and 10.90 mm in *P. putida* with 0.10% concentration, while it was between 9.00 and 10.90 mm for all bacteria with 1.00 and 0.50% concentrations. Antimicrobial zone was observed between 7.00 and 8.90 mm in the control groups of *P. putida, S. epidermidis* and *E coli* 2, and no zone was determined in the control groups of other bacteria.

Antimicrobial activity results of the extracted and commercial chitosans in hydrochloric acid solutions are shown in Table 5. Inhibition zone diameter of 7.00 and 13.90 mm was observed in all bacteria except *E. coli* 1 with 0.10 and 0.05% concentrations of the extracted chitosan dissolved in HCl.

Inhibition zone diameter changed between 11.00 and 13.90 mm in *P. fluorescens* with 0.50, 0.25, 0.10 and 0.05% concentrations, in *V. parahaemolyticus* with 0.25%, in *S. aureus* with 0.25 and 0.10% and lastly in *L. monocytogenes* with 0.25, 0.10 and 0.05% concentrations. As there was no antimicrobial zone in *E. coli* 1 with 0.10 and 0.05% concentrations, antimicrobial activity of extracted chitosan changed between 9.00 and 10.90 mm in all the selected types of bacteria with other levels of concentrations.

Antimicrobial activity was observed in all types of bacteria except *E. coli* 1 with 0.10 and 0.50% concentrations, and the zone diameter was between 7.00 and 13.90 mm. Zone diameter changed with different commercial chitosan concentrations in HCl, and antimicrobial activity zone was determined between 11.00 and 13.90 mm in *P. fluorescens* with 0.50% concentration, in *P. fluorescens, V. parahaemolyticus* and *L. monocytogenes* with 0.25%; in *P. fluorescens, S. aureus* and *L. monocytogenes* with 0.10% concentration; in *P. putida* and *P. fluorescens* with 0.05% concentration. The same concentrations of both types of chitosan dissolved in HCl demonstrated similar effects on bacteria used in the study. No zone was determined in the control groups of bacteria except for *V. parahaemolyticus* (7.00–8.90 mm).

There are many reports on the antimicrobial activity of different chitosans and chitosan derivatives from various sources which were tested under diverse conditions [8, 20–22]. The results of all these studies proved that chitosan inhibited the development of many bacteria. In fact, the majority of the concentrations of both types of chitosan used in the present study demonstrated antimicrobial activity on the selected bacteria. Darmadji and Izumimoto [23] stated that the antimicrobial activity of chitosan might be caused by its interaction with membranes or cell wall components, which increases the permeability of the membranes and leakage of cell material from tissue. It

Table 5 Antimicrobial activity of chitosan extracted from *M. stebbingi* shells and commercial chitosan in hydrochloric acid

Sample	Microorganisms and inhibition zone (mm)										
	C (%)	Pp	Pf	Vp	Se	Sa	Ec1	Ec2	Lm	Ef	Ac
Extracted chitosan	1.00	++	++	++	++	++	++	++	++	+	++
	0.50	++	+++	++	++	++	++	++	++	++	++
	0.25	++	+++	+++	++	+++	++	++	+++	++	++
	0.10	+++	+++	++	++	+++	−	++	+++	+	+
	0.05	++	+++	++	++	++	−	++	+++	+	++
Commercial chitosan	1.00	++	++	++	++	++	++	+	++	++	++
	0.50	++	+++	++	++	++	+	++	++	++	+
	0.25	++	+++	+++	++	++	++	++	+++	++	+
	0.10	++	+++	++	++	+++	−	++	+++	++	++
	0.05	+++	+++	++	++	++	−	++	++	+	++
Control-acid		−	−	+	−	−	−	−	−	−	−

Results were interpreted in terms of the diameter of the inhibition zones: (−), <7.00 mm; (+), 7.00–8.90 mm; (++), 9.00–10.90 (+++), 11.00–13.90 mm; (++++), 14.00–16.00 mm. (+++++), >16.00. *Pp Pseudomonas putida* NRRL-B-14875, *Pf Pseudomonas fluorescens* NRRL-2641, *Vp Vibrio parahaemolyticus* ATCC-17802, *Se Staphylococcus epidermidis* NRRL-B-4268, *Sa Staphylococcus aureus* ATCC-25923, *Ec1 Escherichia coli* ATCC-25922, *Ec2 Escherichia coli* NRRL-B-4269 *Lm Listeria monocytogenes* ATCC-7644, *Ef Enterecoc faecalis* ATCC-29212, *Ac Aeromonas caviae* NRRL-968, *C* concentration

could also be caused by its water-binding capacity and inhibition of various enzymes; in addition, chitosan could inhibit the bacteria development as it absorbs the nutrient of bacteria.

Antimicrobial activity of chitosan is affected by different intrinsic and extrinsic factors. Source of chitosan, molecular weight, deacetylation degree, viscosity and solvent material could be stated as intrinsic factors, while the extrinsic factors are related with pH, temperature, ionic strength, metal ions, presence of EDTA organic matters and bacteria cultures [24–26]. Deacetylation degree is highly important among these factors. Chitosans with a high degree of deacetylation were more effective in inhibiting bacterial growth, probably due to the higher percentage of protonated amine groups compared to those with lower degrees of deacetylation [26, 27]. Similarly, molecular weight of chitosan is another significant factor. A previous study implemented on some bacteria reported that antimicrobial effect is increased with the decreasing molecular weight [28]. Both types of chitosan used in the study had low deacetylation degree and molecular weight, which signified the increased antimicrobial activity. In addition to all these factors, chitosan concentration is also effective on antimicrobial activity. Seo et al. [29] were reported that the antibacterial activities of chitosan differed depending on concentration of chitosan. Previous studies reported that high concentration of chitosan increased the antimicrobial activity on certain bacteria (*E. coli* and *S. aureus*) [10, 30].

In conclusion, huge amounts of crab and shrimp shells have been discarded as wastes by worldwide seafood factories. This has led to considerable scientific and technological interest in chitosan as an attempt to use these renewable wastes. Present study has proved the antimicrobial effect of chitosan on selected bacteria. This property increased the importance of chitosan as an antimicrobial matter in many different sectors, especially in the food industry. The present study is considered to be pioneering for the use and extraction of chitosan from shrimp shells, which are not used and discarded as waste product in Turkey.

References

1. Jeuniaux C, Vossfoucart MF (1991) Biochem Syst Ecol 19:347
2. Ruiz-Herrera J (1978) The distribution and quantitative importance of chitin in fungi. In: Muzzarelli RAA, Pariser ER (eds.) Proceedings of the first international conference on chitin/chitosan. MIT Sea Grant Report MITSG78-7, Index No. 78-307-Dmb. Massachusetts Institute of Technology, Cambridge, pp. 11–21
3. Sandford PA (2003) Commercial sources of chitin and chitosan and their utilization, In Vårum KM, Domard A, Smidsrød O (eds.) Advances in chitin sciences, vol 6. NTNU Trondheim, Trondheim, p 35
4. Yen MT, Yang JH, Mau JL (2009) Carbohydr Polym 75:15
5. Hongpattarakere T, Riyaphan O (2008) Songklanakarin J Sci Technol 30:1
6. Jeon YJ, Kamil JYVA, Shahidi F (2002) J Agric Food Chem 50:5167
7. Rabea EI, Badawy MET, Stevens CV, Smagghe G, Steurbaut W (2003) Biomacromolecules 4:1457
8. Tsai GJ, Zhang SL, Shieh PL (2004) J Food Protect 67:396
9. Tikhonov VE, Stepnova EA, Babak VG, Yamskov IA, Palma-Guerrero J, Jansson HB, Lopez-Llorca LV, Salinas J, Gerasimenko

DV, Avdienko ID, Varlamov VP (2006) Carbohydr Polym 64:66
10. Zheng LY, Zhu JF (2003) Carbohydr Polym 54:527
11. Fouad DRG (2008) Chitosan as an antimicrobial compound: modes of action and resistance mechanisms. Mathematisch-Naturwissenschaftliche Fakultät, Universität Bonn
12. Chang KLB, Tsai G, Lee J, Fu WR (1997) Carbohydr Res 303:327
13. Tolaimate A, Desbrières J, Rhazi M, Alagui A, Vincendon M, Vottero P (2000) Polymer 41:2463
14. Wang QZ, Chen XG, Liu N, Wang SX, Liu CS, Meng XH, Liu CG (2006) Carbohydr Polym 65:194
15. Wang JC, Kinsella JE (1976) J Food Sci 41:286
16. Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Am J Clin Pathol 45:493
17. Li Q, Dunn ET, Grandmaison EW, Goosen MFA (1992) J Bioactive Comp Polym 7:370
18. Nemtsev SV, Gamzazade AI, Rogozhin SV, Bykova VM, Bykov VP (2002) Appl Biochem Microbiol 38:521
19. No HK, Meyers SP (1997) Preparation of chitin and chitosan. In: Muzzarelli RAA, Peter MG (eds). Chitin handbook. European Chitin Society, pp 475–489
20. Jeon YJ, Park PJ, Kim SK (2001) Carbohydr Polym 44(11):71
21. No HK, Kim SH, Lee SH, Park NY, Lee SH, Prinyawiwatku W (2006) Carbohydr Polym 65:174
22. Yang TC, Chou CC, Li CF (2005) Int J Food Microbiol 97:237
23. Darmadji P, Izumimoto M (1994) Meat Sci 38:243
24. Jia ZS, Shen DF, Xu WL (2001) Carbohydr Res 333:1
25. Jumaa M, Furkert FH, Muller BW (2002) Eur J Pharm Biopharm 53:115
26. Shigemasa Y, Minami S (1995) Biotechnol Genet Eng Rev 13:383
27. Liu WG, De Yao K (2002) J Control Release 83:1
28. Xia WS, Wu YN (1996) J Wuxi Univ Light Ind 15:297
29. Seo S, King JM, Prinyawiwatkul W, Janes M (2008) J Food Sci 73(8):400
30. Tajik H, Moradi M, Rohani SMR, Efrani AM, Jalali FSS (2008) Molecules 13:1263