Annals of Agricultural Science (2013) **58**(1), 33–41

Faculty of Agriculture, Ain Shams University

**Annals of Agricultural Science**

www.elsevier.com/locate/aoas

ORIGINAL ARTICLE

# Physicochemical, functional, antioxidant and antibacterial properties of chitosan extracted from shrimp wastes by microwave technique

**M. Mahdy Samar *, M.H. El-Kalyoubi, M.M. Khalaf, M.M. Abd El-Razik**

*Food Sci. Dept., Fac. Agric., Ain Shams Univ., Shoubra El-Kheima, Cairo, Egypt*

Received 18 November 2012; accepted 25 November 2012
Available online 1 March 2013

**KEYWORDS**

Chitin;
Chitosan;
Shrimp wastes;
Microwave radiation;
Deacetylation degree;
Antibacterial;
Antioxidant

**Abstract** Chitosan is produced from shrimp waste chitin at three particle sizes 20, 40 and 60 mesh by deacetylating with different concentrations of NaOH solution (30%, 40% and 50%) under microwave irradiation for 10 min. The process describes a rapid synthesis procedure in comparison to conventional methods. The microwave-synthesized chitosan was characterized and the experimental results showed that the degree of deacetylation increased with increasing concentration of deacetylation alkali solution. A degree of deacetylation of 95.19% was achieved after irradiating chitin at 60 mesh with 50% NaOH solution in a microwave for 10 min at 1400-W power. Microwave-synthesized chitosan exhibited antioxidant activities of 47.71–72.31% at 10 mg/ml and showed reducing powers of 2.094–2.367 at 10 mg/ml. On the other hand, at 10 mg/ml, the scavenging ability of chitosan on 1,1-diphenyl-2-picrylhydrazyl radicals ranged from 43.03% to 90.48%. The antibacterial activities of microwave-synthesized chitosan were examined against two gram-negative ( *Escherichia coli* and *Salmonella* typhimurium) and two gram-positive (*Staphylococcus aureus* and *Bacillus* cereus), tested chitosan markedly inhibited the growth of tested bacteria although inhibitory effects differed with molecular weight (Mw) of chitosan and the species of bacteria. Generally, the microwave technique can be very useful for synthesizing good functional properties chitosan with rapid and clean chemistry.



## Introduction

Chitin is the second most abundant organic compound in nature after cellulose, is widely distributed in marine invertebrates, insects, fungi, and yeast. Chitosan is a fiber-like substance derived from chitin, a homopolymer of β-(1→4)-linked-acetyl-D-glucosamine (Muzzarelli, 1997). Several methods have been reported for preparation of chitosan from chitin. The major procedure for obtaining chitosan is based

* Corresponding author. Tel.: +20 01225739418.
E-mail address: samar_mahdy@yahoo.com (M. Mahdy Samar).

Peer review under responsibility of Faculty of Agriculture, Ain-Shams University.

ELSEVIER Production and hosting by Elsevier

0570-1783 © 2013 Faculty of Agriculture, Ain Shams University. Production and hosting by Elsevier B.V. Open access under CC BY-NC-ND license.
http://dx.doi.org/10.1016/j.aoas.2013.01.006

on the alkaline deacetylation of chitin with strong alkaline solution at high temperature (Castelli et al., 1996), other procedures included alkali treatment at high temperature and high pressure (autoclave) (Abdou et al., 2008), and enzymatic N-deacetylation (Martinou et al., 1995). In recent years microwave chemistry has received much attention as it can speed up the reaction rate by orders of magnitude over conventional heating. Microwave heating, as an alternative to conventional heating techniques, has been proved to be more rapid and efficient for several chemical reactions e.g., N-phthaloylation of chitosan (Liu et al., 2004). The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin, leaving behind a complete amino group ($-NH_2$), chitosan versatility depends mainly on this high degree chemical reactive amino groups (Muzzarelli, 1997). This makes the degree of deacetylation an important property in chitosan production as it affects the physicochemical properties, hence determines its appropriate applications (Rout, 2001). Another important characteristic to consider for this polymer is the molecular weight; chitosan is a biopolymer of high molecular weight. Like its composition, the molecular weight of chitosan varies with the raw material sources and the method of preparation (Li et al., 1992). Chitosan has been of interest in the food industry since, besides its antimicrobial effect, it possesses other functional properties including intestinal lipid binding and serum cholesterol lowering effects (Razdan and Pettersson, 1994), water binding (Knorr, 1982), emulsifying, thickening and stabilizing agent in food industry (Shahidi et al., 1999), antioxidative and preservative effects in muscle foods (Darmadji and Izumimoto, 1994), and emulsifying capacity (Lee et al., 1996). Chitosan is Generally Recognized As Safe (GRAS) by the US FDA (2001). The aim of this study was to extract chitosan from shrimp wastes using microwave technique at different concentrations of sodium hydroxide solution, and investigate the physicochemical, functional, antioxidant and antibacterial characteristics of extracted chitosan.

## Materials and methods

### *Materials*

Shrimp waste (heads and scales) were collected from El-Obour market. Kalubia governorate, Egypt. The waste was packed in plastic bags and stored at −20 °C until using.

The bacterial strains including *Escherichia coli ATCC 25922*, *Salmonella typhimurium ATCC 14028, Bacillus cereus DSMZ 345 and Staphylococcus aureus ATCC 6528* were obtained from the Egyptian Microbial culture collection (EMCC) at the Microbial Resource Center (Cairo, MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt.

Commercial chitosan (300 kDa), from crab shells and butelated hydroxyl toluene (BHT) purchased from Sigma chemical company were used in this study.

### *Methods*

#### *Technological treatments*

*Preparation of shrimp wastes for extraction*. Shrimp wastes were washed, dried at 50 °C overnight, grinded, sieved to obtain coarse powder at three particle sizes 20, 40, and 60 mesh and stored in dry place at room temperature until extraction.

#### *Production of chitin from shrimp wastes*

The different extraction steps of chitin were done according to Synowiecki and Al-Khateeb (1997). Shrimp wastes with three particle sizes of 20, 40, and 60 mesh were demineralized with 2% (v/v) HCl solution (10:1 v/w, 30 °C, 12 h) to remove minerals, insoluble fraction was separated by centrifugation (4000 rpm, 15 min.), then it washed twice with distilled water. Deproteinization of washed insoluble fraction was done by using 4% (w/v) sodium hydroxide solution (10:1 v/w, 90 °C, 12 h) and centrifuged (4000 rpm, 15 min) for separation of alkali-insoluble fraction (AIF), it washed twice with distilled water then dried at 40 °C overnight. The obtained product after drying was designated as purified shrimp waste chitin at aforementioned three particle sizes (C20, C40, and C60).

#### *Production of chitosan by microwave technique*

The different production steps of chitosan were done according to Sahu et al. (2009). Ten grams of extracted chitin with three particle sizes of C20, C40, and C60 were transferred to ceramic mortar and 100 ml of NaOH solution at three concentrations of 30%, 40% and 50% (w/v) was added and mixed. The ceramic mortar was placed on the center of the turntable of the microwave oven (Sumsung microwave oven Model, MX145, 1400 W, Made in Korea) and irradiated for 10 min at 1400 W. The mixtures were filtered and the residues were washed with distilled water until neutralization, then dried in a hot air oven at 40 °C until constant dry weight and stored until further analysis.

#### *Proximate analysis*

Moisture, ash, lipid and protein contents of shrimp waste, crude chitin and chitosan samples were determined according to (AOAC, 2007).

#### *Physicochemical and functional properties*

*Degree of deacetylation*. The degree of deacetylation of extracted chitosan at three particular sizes was determined according to the method of Qin et al. (2004).

*Molecular weight*. Molecular weight of extracted chitosan at three particular sizes was determined according to the method of Fernandez-Kim (2004).

*Solubility*. Solubility of extracted chitosan was determined according to the method of Fernandez-Kim (2004). The solubility was calculated according to the following equation:

$$\%\text{Solubility} = \frac{(\text{Initial weight of tube} + \text{chitosan}) - (\text{Final weight of tube} + \text{chitosan}) \times 100}{(\text{Initial weight of tube} + \text{chitosan}) - (\text{Initial weight of tube})}$$

*Water and oil binding capacity*

Water binding capacity (WBC) and oil binding capacity (OBC) of extracted chitosan samples were measured using a modified method of Wang and Kinsella (1976). WBC and OBC were calculated as follows:

$$\text{WBC } (\%) = \text{water bound (g)/initial sample weight (g)}$$

$$\text{OBC } (\%) = \text{fat bound (g)/initial sample weight (g)}$$

*Emulsifying properties*

Emulsifying capacity of extracted chitosan samples was determined according to Sciarini et al. (2009). The emulsifying capacity (EC) was calculated as follows:

$$EC = (e_v/t_v)100$$

where $e_v$ is the emulsion volume and $t_v$ is the total volume.

The emulsion stability (Es) of different chitosan emulsions at 0.5% concentration against high temperatures was determined according to Sciarini et al. (2009). The Es was calculated as follows:

$$Es = (f_{ev}/i_{ev})100$$

where $f_{ev}$ is the final emulsion volume and $i_{ev}$ is the initial emulsion volume.

*Antioxidant properties of chitosan*

*Scavenging ability*. The scavenging ability of shrimp wastes chitosan at different concentrations (0.5, 1, 2, 4, 6, 8 and 10 mg/ml) on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals were determined according to Shimada et al. (1992). BHA and commercial chitosan at the same concentrations were used for comparison. The scavenging ability was calculated as follows:

$$\text{Scavenging ability } (\%) = [(A_{517}\text{of control} - A_{517}\text{of sample})/A_{517}\text{of control}] \times 100$$

*Antioxidant activity*

The antioxidant activity of shrimp wastes chitosan at different concentrations (0.5, 1, 2, 4, 6, 8 and 10 mg/ml) was determined by the conjugated diene method according to Lingnert et al. (1979). BHA and commercial chitosan at the same concentrations were used as a comparison against the produced chitosan. The antioxidant activity was calculated as follows:

$$\text{Antioxidant activity } (\%) = [(A_{234}\text{of control} - A_{234}\text{of sample})/A_{234}\text{of control}] \times 100$$

*Reducing power*

The reducing power was determined according to the method of Oyaizu (1986). BHA and commercial chitosan were used for comparison.

*Antibacterial properties of chitosan*

*Screening of the antibacterial activity*

Six concentrations of extracted various chitosan samples (0.05%, 0.1%, 0.2%, 0.4%, 0.6%, 0.8% and 1%) prepared in 1% acetic acid were prepared and sterilized at 121 °C/20 min. Paper disc diffusion method was used as screening method to determine the antimicrobial activity of chitosan types against bacterial strains according to (Li et al., 2010).

*Determination of the minimal inhibitory concentrations (MICs)*

The antimicrobial activity of natural antimicrobial agents against both gram-positive and gram-negative bacteria strains were examined by detecting of the minimum inhibitory concentrations (MICs), which defined as ''the lowest concentration required for complete inhibition of test organism after incubation time in broth media and resulting in large inhibition zones of visible growth'' (Li et al., 2010).

*Statistical analysis*

The experimental data were subjected to an analysis of variance for a completely random design using a Statistical Analysis System (SAS Institute, Inc., 2000). Duncan's multiple range tests were used to determine the difference among means at the level of 0.05.

## Results and discussion

*Yield of chitin and chitosan*

The yields of chitin extracted from shrimp wastes at different particle sizes increased significantly ($p \leqslant 0.05$) with increasing the particle size of shrimp wastes, the highest yield was obtained by using shrimp waste at particle size of 20 mesh (36.43% on dry weight of shrimp waste) as shown in Table 1. This result indicates that shrimp waste is an excellent source for chitin, which was higher than that obtained from crab shell or crayfish which yielded 10% and 32% on dry weight basis, respectively (Tolimate et al., 2000).

Yields of chitosan obtained by deacetylation of extracted chitin at different particle sizes using different concentrations of NaOH solutions (30%, 40% and 50%) and using microwave technique are shown in Table 2. It could be noticed that, the yields of chitosan increased significantly ($p \leqslant 0.05$) with decreasing of chitin particle size at each concentration of NaOH solution used in deacetylation, it was 72.09%, 85.72% and 88.15% for chitosan at 20, 40 and 60 mesh extracted by 30% NaOH ($C_{20/30}$), respectively.

The same trend was observed for different chitosan samples. On the other hand, yields of chitosan increased significantly ($p \leqslant 0.05$) by increasing the concentration of NaOH solution used in deacetylation, the significantly ($p \leqslant 0.05$) highest yield was obtained from chitin samples at particle size 60 mesh deacetylated by 50% NaOH solution using micro-

**Table 1** Yields of chitin extracted from shrimp wastes at different particle sizes.

| Shrimp waste | Chitin yield (%)[a] |
|---|---|
| 20 mesh | $36.43^{A} \pm 0.40$ |
| 40 mesh | $34.45^{AB} \pm 0.51$ |
| 60 mesh | $33.77^{B} \pm 0.17$ |

Data are the mean ± SD, $n = 3$.
Means with different letters in each column are significantly different ($p \leqslant 0.05$).
[a] On dry weight basis.

**Table 2** Yields (%) of chitosan samples at different particle sizes extracted from shrimp wastes chitin using microwave technique.

| Chitin treatment | | Yield (%)[b] |
|---|---|---|
| PS[a] | NaOH conc. (%) | |
| 20 mesh | 30 | $72.09^{H} \pm 1.01$ |
| | 40 | $75.32^{G} \pm 0.55$ |
| | 50 | $85.39^{D} \pm 0.62$ |
| 40 mesh | 30 | $85.72^{D} \pm 1.49$ |
| | 40 | $78.83^{F} \pm 0.29$ |
| | 50 | $83.84^{E} \pm 0.76$ |
| 60 mesh | 30 | $88.15^{B} \pm 0.31$ |
| | 40 | $82.85^{E} \pm 1.62$ |
| | 50 | $90.12^{A} \pm 0.55$ |

Data are the mean ± SD, $n = 3$.
Mean values in the same column bearing the same superscript do not differ significantly ($p \leqslant 0.05$).
[a] PS: Particle size of chitosan.
[b] On dry weight basis.

**Table 3** Mean values of moisture, ash and protein of fresh shrimp wastes and extracted chitin at different particle sizes.

| Samples | Parameters (%) | | |
|---|---|---|---|
| | Moisture | Ash[a] | Protein[a] |
| Shrimp waste | 45.65 ± 42.78 | 32.46 ± 2.08 | 32.77 ± 4.58 |
| Chitin 20 | $4.73^{a} \pm 0.22$ | $1.24^{b} \pm 0.51$ | $2.11^{b} \pm 0.10$ |
| Chitin 40 | $7.93^{b} \pm 0.15$ | $1.38^{a} \pm 0.13$ | $3.56^{a} \pm 0.06$ |
| Chitin 60 | $6.29^{ab} \pm 0.09$ | $1.27^{b} \pm 0.54$ | $3.38^{a} \pm 0.07$ |

Data are the mean ± SD, $n = 3$.
Means with different letters in each column are significantly different ($p \leqslant 0.05$).
[a] On dry weight basis.

wave for 10 min, which yielded 90.12% (on dry weight of chitin). The chitin content on dry basis of crab processing waste (13–26%) is lower than in the case of shrimp (14–42%) as reported by Naczk et al. (1981).

The chemical composition of fresh shrimp wastes and extracted chitin are shown in Table 3. It could be reported that, shrimp wastes had the values of moisture, ash and protein content being 45.65%, 32.46% and 32.77%; respectively. About the chemical compositions of extracted chitin, it could be noticed the effect of extraction method (demineralization and deprotenization) in decreasing the ash and protein contents of extracted chitin as its values ranged from 1.24% to 1.38% and 2.11% to 3.56% in comparison to 32.46% and 32.77% for shrimp waste.

Results in Table 4 demonstrated the chemical composition of extracted chitosan. There was significant ($p \leqslant 0.05$) differences in the moisture content of all chitosans prepared from chitin, the moisture content of chitosans was affected by particle size, the highest moisture content was observed for chitosans at the bigger particle size of 20 mesh (11.87–13.12%), followed by chitosans at particle size of 40 mesh (5.69–11.40%), and the lowest content was observed for chitosans at particle size of 60 mesh (2.60–9.43%). These results may be due to the effect of particle size of chitosan whereas the larger particle size may lead to more ability to maintain moisture (Bough et al., 1978), while the littler size, the increasingly of surface and the more getting rid of water during drying.

From the same table the ash and protein content of extracted chitosan were correlation with the particle size and concentration of NaOH used in deacetylation step. As the particle size was minimized the most the content of ash and protein. Crustacean exoskeletons contain large amounts of calcium carbonate, depending on the source (Abdou et al., 2008). From the same table, it could be noticed that, all samples had low ash and protein content, ranging from 0.27% to 0.71% and from 1.95% to 4.93%, respectively indicating the effectiveness of the demineralization and deproteinization steps in removing minerals and proteins. A high quality grade of chitosan should have less than 1% of ash content (No and Meyers, 1995).

According to the results of physicochemical characteristics of extracted chitosan listed in Table 5 it could be noticed that, the degrees of deacetylation (DDA) increased significantly ($P \leqslant 0.05$) with decreasing of chitin particle size at each concentration of NaOH solution used in deacetylation, whereas it was 67.58%, 76.89% and 88.39% for $C_{20/30}$, $C_{40/30}$ and

**Table 4** Mean values of moisture, ash and protein of chitosan samples extracted from shrimp wastes using microwave technique.

| Chitin treatment | | Parameters (%) | | |
|---|---|---|---|---|
| PS[a] | NaOH conc. (%) | Moisture | Ash[b] | Protein[b] |
| 20 mesh | 30 | $13.12^{A} \pm 0.22$ | $0.30^{DE} \pm 0.36$ | $1.95^{GH} \pm 0.22$ |
| | 40 | $12.70^{AB} \pm 0.20$ | $0.27^{E} \pm 0.06$ | $2.16^{FGH} \pm 0.21$ |
| | 50 | $11.87^{BC} \pm 0.95$ | $0.31^{D} \pm 0.60$ | $2.83^{EF} \pm 0.18$ |
| 40 mesh | 30 | $11.40^{C} \pm 0.95$ | $0.59^{B} \pm 0.15$ | $2.53^{EFGH} \pm 0.36$ |
| | 40 | $6.90^{F} \pm 0.40$ | $0.32^{D} \pm 0.17$ | $2.62^{EFGH} \pm 0.22$ |
| | 50 | $5.69^{G} \pm 0.78$ | $0.58^{B} \pm 0.10$ | $3.80^{BC} \pm 0.28$ |
| 60 mesh | 30 | $9.43^{D} \pm 0.85$ | $0.71^{A} \pm 0.53$ | $4.44^{AB} \pm 0.36$ |
| | 40 | $7.00^{EF} \pm 0.20$ | $0.49^{C} \pm 0.07$ | $4.93^{A} \pm 0.56$ |
| | 50 | $2.60^{H} \pm 0.98$ | $0.47^{C} \pm 0.76$ | $3.01^{DE} \pm 0.64$ |

Data are the mean ± SD, $n = 3$.
Mean values in the same column bearing the same superscript do not differ significantly ($P \leqslant 0.05$).
[a] PS: Particle size of chitosan.
[b] On dry weight basis.

**Table 5** Degree of deacetylation (DDA), molecular weight (Mw) and solubility of chitosan samples extracted from shrimp wastes using microwave technique.

| Chitin treatment | | DDA% | Mw (k Daltons) | Solubility (%) |
|---|---|---|---|---|
| PS[a] | NaOH conc. (%) | | | |
| 20 mesh | 30 | $67.58^{F} \pm 0.92$ | 2415.09 | $66.31^{H} \pm 0.35$ |
| | 40 | $75.77^{E} \pm 3.54$ | 1476.21 | $74.94^{G} \pm 0.79$ |
| | 50 | $78.83^{D} \pm 1.05$ | 1267.11 | $96.77^{BC} \pm 0.17$ |
| 40 mesh | 30 | $76.89^{DE} \pm 0.89$ | 866.03 | $99.05^{A} \pm 0.05$ |
| | 40 | $78.64^{D} \pm 0.86$ | 1107.50 | $92.79^{D} \pm 0.01$ |
| | 50 | $83.05^{C} \pm 0.29$ | 2160.88 | $95.60^{C} \pm 0.87$ |
| 60 mesh | 30 | $88.39^{B} \pm 0.49$ | 949.95 | $83.28^{F} \pm 0.87$ |
| | 40 | $89.17^{B} \pm 0.28$ | 1274.85 | $85.57^{E} \pm 1.37$ |
| | 50 | $95.19^{A} \pm 0.74$ | 4467.05 | $97.73^{AB} \pm 0.95$ |
| Commercial chitosan | | $85.00^{C} \pm 0.66$ | 300 | $99.00^{A} \pm 0.72$ |

Data are the mean ± SD, $n = 3$.
Mean values in the same column bearing the same superscript do not differ significantly ($P \leqslant 0.05$).
[a] PS: Particle size of chitosan.

**Table 6** Water binding capacity (WBC) and oil binding capacity (OBC) of chitosan samples extracted from shrimp wastes using microwave technique.

| Chitin treatment | | WBC[b] | OBC[c] |
|---|---|---|---|
| PS[a] | NaOH conc. (%) | | |
| 20 mesh | 30 | $11.58^{CD}$ | $9.61^{B}$ |
| | 40 | $12.10^{C}$ | $13.43^{A}$ |
| | 50 | $14.42^{B}$ | $7.11^{C}$ |
| 40 mesh | 30 | $13.22^{BC}$ | $13.54^{A}$ |
| | 40 | $6.24^{F}$ | $5.98^{C}$ |
| | 50 | $10.06^{DE}$ | $6.63^{C}$ |
| 60 mesh | 30 | $9.26^{E}$ | $5.36^{C}$ |
| | 40 | $17.76^{A}$ | $14.45^{A}$ |
| | 50 | $16.78^{A}$ | $13.48^{A}$ |
| Commercial chitosan | | $3.12^{G}$ | $1.56^{D}$ |

Data are the mean ± SD, $n = 3$.
Mean values in the same column bearing the same superscript do not differ significantly ($P \leqslant 0.05$).
[a] PS: Particle size of chitosan.
[b] WBC: water binding capacity (g water/g chitosan).
[c] OBC: oil binding capacity (g oil/g chitosan).

$C_{60/30}$, respectively. The same trend of DDA was observed for other chitosan samples with different particle size and by using different concentrations of NaOH in deacetylation step, reaching its maximum values of 78.83%, 83.05% and 95.19% for $C_{20/50}$, $C_{40/50}$ and $C_{60/50}$, respectively. The deacetylation degree and molecular weight are important parameters for chitosan, as they affect its functional properties (Rout, 2001).

The DDA of chitosan is important for its application in the industry. From this regard, certain researchers (Li et al., 1992) suggested that the term chitosan should be used when the DDA is above 70%. Particle size of chitosan has sparked controversial reports in its effect on its quality and applications in food technology. Bough et al. (1978), reported that small particle size is better than large particle size, thereby; smaller particle size (1 mm) results in a chitosan product of both higher DDA and molecular weight (MW) than that of longer particle size (2–6.4 mm); also the larger particle sizes require longer swelling time resulting in a slower deacetylation rate. From the same table, it could be noticed that, the DDA of chitosan increased significantly ($p \leqslant 0.05$) by increasing of concentration of NaOH solution used in deacetylation step, the highest ($p \leqslant 0.05$) DDA was obtained from chitin samples at particle size of 60 mesh deacetylated by 50% NaOH solution, it was 95.19% compared with 85% for commercial chitosan.

The MW of extracted chitosan ranged from 949.95 to 4467.05 k. Daltons (Table 5). This range is considered suitable for several commercial applications. It is very important to remember that, there are several factors during commercial production, including high temperature, concentration of alkali, reaction time, previous treatment of the chitin, and particle size may influence the MW of chitosans (No et al., 2000).

The percentage of solubility for all produced chitosans were affected significantly ($p \leqslant 0.05$) by both of particle size of chitosans and concentration of NaOH solution used in deacetylation step as shown in Table 5. All extracted chitosans demonstrated an excellent solubility ranged from 83.28% to 99.05%, except chitosans $C_{20/30}$ and $C_{20/40}$ as their solubility was 66.31% and 74.94%, respectively. This result may be due to a reaction time of 10 min with low concentration of NaOH (30 and 40%) not be enough for large chitin particles (20 mesh) to be sufficiently swollen (Bough et al., 1978). On the other hand, a decrease of the NaOH concentration to 40% required increased time of $>30$ min to obtain a soluble chitosan (No et al., 2000). The significantly ($p \leqslant 0.05$) highest value of solubility was reported for $C_{40/30}$, which recorded 99.05% similarly to commercial chitosan (99%); this implies that the deproteinization process on our samples might have been nearly complete.

Water binding capacity (WBC) and fat binding capacity (FBC) of extracted chitosans were determined and the results are shown in Table 6. WBC significantly ($p \leqslant 0.05$) differed depending on particle size of chitosans and different concentrations of NaOH solution used in deacetylation step. WBC

**Table 7** Emulsification capacity (EC) and Emulsification stability (ES) of chitosan samples extracted from shrimp wastes using microwave technique.

| Chitin treatment | | EC | ES |
|---|---|---|---|
| PS[a] | NaOH conc. (%) | | |
| 20 mesh | 30 | $42.6^{G}$ | $37.5^{F}$ |
| | 40 | $77.6^{CD}$ | $42.5^{E}$ |
| | 50 | $95.5^{B}$ | $75.7^{A}$ |
| 40 mesh | 30 | $62.0^{E}$ | $62.5^{B}$ |
| | 40 | $100^{A}$ | $50.0^{D}$ |
| | 50 | $100^{A}$ | $44.0^{E}$ |
| 60 mesh | 30 | $76.3^{D}$ | $52.5^{D}$ |
| | 40 | $48.5^{F}$ | $56.7^{C}$ |
| | 50 | $100^{A}$ | $37.9^{F}$ |
| Lecithin | | $80.0^{C}$ | $75.0^{A}$ |

Data are the mean ± SD, $n = 3$.
Mean values in the same column bearing the same superscript do not differ significantly ($P \leqslant 0.05$).
[a] PS: Particle size of chitosan.

of extracted chitosan ranged from 6.24% to 16.78% compared with 3.12% for commercial chitosan. The range of WBC found in our study was somewhat higher than that reported by Cho et al. (1998) and No et al. (2000), also FBC significantly ($p \leqslant 0.05$) differed considerably with chitosan particle sizes and concentration of NaOH solution used in deacetylation step, where it was ranged from 5.36% to 14.45% compared with 1.56% for commercial chitosan. Overall, FBC values observed by Cho et al. (1998) and No et al. (2000) were lowest in comparison to our results being 3.14–5.35% and 2.17–4.03%, respectively.

Emulsification capacity (EC) and emulsification stability (ES) of different extracted chitosan samples as compared to lecithin are tabulated in Table 7. It could be noticed that, all extracted chitosan samples had fully emulsifying capacity; this functional significantly ($p \leqslant 0.05$) affected by both of particle size and concentration of NaOH used in extraction method. From the same table it could be seen that, lecithin had EC value of 80.0%, where extracted chitosan samples had EC values significantly differed between them and lecithin ranging between 42.6 and 100.0.

Extracted chitosan samples $C_{40/40,}$ $C_{40/50}$ and $C_{60/50}$ had significantly higher EC value (100) in comparison to other extracted chitosan samples and lecithin, the nearest value of the aforementioned EC was observed by $C_{20/50}$ (95.5%). The lowest value of EC was observed by $C_{20/30}$ (42.6%), and then 48.5%, 62.0%, 76.3% and 77.6% for $C_{60/40}$, $C_{40/30}$, $C_{60/30}$ and $C_{20/40}$, respectively.

The highest value of EC observed by $C_{40/40,}$ $C_{40/50}$ and $C_{60/50,}$ this may be due to, that chitosan samples had surface activity at the oil–water interface and their ability to facilitate the formation and stabilization of final droplets during and after emulsification as described by Dickinson (2009). At the same time this highest value of EC may be also due to the high concentration of NaOH used in the deacetylation step which increase the $NH_2$ groups released during deacetylation step which had an important role in emulsification. Data indicate that, $C_{60/50}$ with EC value of 100 had an excellent functional value of DDA, WBC, FBC and solubility as previously described. These results are agreed well with Wydro et al. (2007). About the ES values of different extracted chitosan, the data presented in Table 7 revealed that, the highest value of ES was observed for lecithin and $C_{20/50}$ with no significant differences ($P \leqslant 0.05$) being 75.0% and 75.7%, respectively which reflects that, $C_{20/50}$ had ability to impart strength to emulsion for resistance to stress. Other extracted chitosan samples had ES differed significantly ($p \leqslant 0.05$) as observed in the same table. The differential in ES values of extracted chitosan samples could be due to the differential values of degree of deacetylation as aforementioned reported these results was agree with Mun et al. (2006).

The scavenging abilities of different extracted chitosan samples on 1,1-diphenyl-2-picrylhydrazyl DPPH radicals were increased with their concentration increased as shown in Table 8. The scavenging abilities of chitosan at particle size 20 and 60 mesh increased with increasing concentration of NaOH solution used in N-deacetylation, as the values were in the

**Table 8** Free radical scavenging activity of chitosan samples extracted from shrimp wastes using microwave technique on 1,1-diphenyl-2-picrylhydrazyl radicals.

| Chitin treatment | | Concentrations (mg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| PS[a] | NaOH conc. (%) | 0. 5 | 1 | 2 | 4 | 6 | 8 | 10 |
| 20 mesh | 30 | $17.85^{Gf}$ | $20.05^{Fe}$ | $28.85^{Ed}$ | $29.83^{Dcd}$ | $31.05^{Gc}$ | $41.81^{Hb}$ | $52.81^{Ga}$ |
| | 40 | $15.16^{He}$ | $16.14^{Ge}$ | $19.32^{Id}$ | $20.54^{Gd}$ | $31.30^{Gc}$ | $60.15^{Eb}$ | $65.60^{Fa}$ |
| | 50 | $18.58^{Gg}$ | $20.54^{Ff}$ | $22.49^{GHe}$ | $24.45^{Fd}$ | $30.56^{Gc}$ | $67.73^{Db}$ | $74.08^{Da}$ |
| 40 mesh | 30 | $24.45^{Df}$ | $29.58^{De}$ | $31.05^{De}$ | $35.70^{Cd}$ | $38.63^{Ec}$ | $48.41^{Gb}$ | $80.69^{Ca}$ |
| | 40 | $20.29^{Fe}$ | $21.27^{Fe}$ | $21.27^{He}$ | $23.96^{Fd}$ | $26.16^{Hc}$ | $33.74^{Jb}$ | $46.46^{Ha}$ |
| | 50 | $22.98^{Ee}$ | $25.18^{Ed}$ | $26.16^{Fcd}$ | $27.38^{Ec}$ | $35.21^{Fb}$ | $36.43^{Ib}$ | $43.03^{Ia}$ |
| 60 mesh | 30 | $21.03^{Fe}$ | $21.03^{Fe}$ | $23.72^{Gd}$ | $26.65^{Ec}$ | $26.90^{Hc}$ | $36.68^{Ib}$ | $53.79^{Ga}$ |
| | 40 | $24.94^{Df}$ | $26.65^{Eef}$ | $27.87^{Ede}$ | $29.10^{Dd}$ | $44.25^{Dc}$ | $54.52^{Fb}$ | $70.42^{Ea}$ |
| | 50 | $32.03^{Ce}$ | $32.76^{Ce}$ | $33.50^{Ce}$ | $36.43^{Cd}$ | $66.02^{Cc}$ | $76.53^{Cb}$ | $90.48^{Ba}$ |
| BHT | | $74.63^{Ag}$ | $81.10^{Af}$ | $84.36^{Ae}$ | $88.03^{Ad}$ | $92.95^{Ac}$ | $94.97^{Ab}$ | $95.92^{Aa}$ |
| Commercial chitosan | | $44.67^{Bf}$ | $56.87^{Be}$ | $64.24^{Bd}$ | $77.84^{Bc}$ | $88.73^{Bb}$ | $90.61^{Ba}$ | $91.24^{Ba}$ |

Data are the mean ± SD, $n = 3$.
Mean values in the same column bearing the same superscript do not differ significantly ($P \leqslant 0.05$).
[*] PS: Particle size of chitosan.

**Table 9** Antioxidant activity (%) of the chitosan samples extracted from shrimp wastes using microwave technique.

| Chitin treatment | | Concentrations (mg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| PS[a] | NaOH conc. (%) | 0. 5 | 1 | 2 | 4 | 6 | 8 | 10 |
| 20 mesh | 30 | $17.88^{If}$ | $32.40^{He}$ | $37.21^{Fd}$ | $46.25^{Fc}$ | $47.60^{Hc}$ | $57.21^{Db}$ | $63.27^{Ea}$ |
| | 40 | $35.28^{Dg}$ | $36.59^{Ff}$ | $52.09^{De}$ | $52.34^{Dd}$ | $55.30^{Dc}$ | $56.07^{Eb}$ | $64.82^{Da}$ |
| | 50 | $32.98^{EFf}$ | $36.45^{Fe}$ | $36.75^{Fe}$ | $46.19^{Fd}$ | $49.44^{Gc}$ | $54.41^{Fb}$ | $56.01^{Ga}$ |
| 40 mesh | 30 | $34.48^{DEcf}$ | $44.42^{De}$ | $51.92^{Dcd}$ | $52.52^{Dd}$ | $52.92^{Ec}$ | $60.39^{Gb}$ | $67.40^{Fa}$ |
| | 40 | $18.86^{Ig}$ | $19.73^{Jf}$ | $23.22^{He}$ | $48.29^{Ed}$ | $50.17^{FGc}$ | $52.54^{GHb}$ | $58.61^{Ha}$ |
| | 50 | $28.33^{Gf}$ | $38.90^{Ee}$ | $43.92^{Ed}$ | $44.42^{Gc}$ | $51.36^{EFb}$ | $51.83^{Hb}$ | $56.15^{Ha}$ |
| 60 mesh | 30 | $21.25^{Hf}$ | $26.84^{Ie}$ | $30.55^{Gd}$ | $33.59^{Hc}$ | $34.93^{Ic}$ | $43.62^{Ib}$ | $47.71^{Ia}$ |
| | 40 | $32.38^{Ff}$ | $35.59^{Ge}$ | $44.42^{Ed}$ | $48.60^{Ec}$ | $52.64^{Eb}$ | $53.58^{FGeb}$ | $57.45^{Fa}$ |
| | 50 | $46.50^{Cf}$ | $55.07^{Be}$ | $57.37^{Cd}$ | $60.52^{Cc}$ | $67.23^{Cb}$ | $68.32^{Cb}$ | $72.31^{Ca}$ |
| BHT | | $54.74^{Ag}$ | $58.71^{Af}$ | $74.1^{Ae}$ | $81.62^{Ad}$ | $85.43^{Ac}$ | $88.76^{Ab}$ | $90.81^{Aa}$ |
| Commercial chitosan | | $51.15^{Bg}$ | $54.14^{Cf}$ | $65.26^{Be}$ | $73.50^{Bd}$ | $78.75^{Bc}$ | $82.09^{Bb}$ | $85.25^{Ba}$ |

Data are the mean ± SD, $n = 3$.
Mean values in the same column bearing the same superscript do not differ significantly ($P \leqslant 0.05$).
* PS: Particle size of chitosan.

**Table 10** Reducing power of chitosan samples extracted from shrimp wastes using microwave technique.

| Chitin treatment | | Concentrations (mg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| PS[a] | NaOH conc. (%) | 0. 5 | 1 | 2 | 4 | 6 | 8 | 10 |
| 20 mesh | 30 | $1.60^{Fg}$ | $1.87^{Ef}$ | $2.01^{Ee}$ | $2.23^{Bd}$ | $2.26^{Bc}$ | $2.32^{Ab}$ | $2.35^{ABa}$ |
| | 40 | $2.28^{Ad}$ | $2.28^{Ad}$ | $2.31^{Ac}$ | $2.32^{Abc}$ | $2.35^{Abc}$ | $2.33^{Ab}$ | $2.34^{ABa}$ |
| | 50 | $1.94^{Ce}$ | $2.02^{Cd}$ | $2.20^{Cc}$ | $2.22^{Bb}$ | $2.22^{Cb}$ | $2.22^{Cb}$ | $2.25^{CDa}$ |
| 40 mesh | 30 | $1.91^{Cg}$ | $2.10^{Bf}$ | $2.19^{Ce}$ | $2.30^{Ad}$ | $2.31^{Ac}$ | $2.32^{Ab}$ | $2.33^{ABa}$ |
| | 40 | $1.85^{De}$ | $2.04^{Cd}$ | $2.17^{Dc}$ | $2.22^{Bb}$ | $2.31^{Aa}$ | $2.31^{Aa}$ | $2.31^{BCa}$ |
| | 50 | $1.91^{Bf}$ | $1.98^{De}$ | $2.23^{Bd}$ | $2.33^{Ac}$ | $2.33^{Abc}$ | $2.34^{Ab}$ | $2.36^{ABa}$ |
| 60 mesh | 30 | $0.95^{Gg}$ | $1.79^{Ff}$ | $1.93^{Ge}$ | $1.98^{Dd}$ | $2.11^{Dc}$ | $2.15^{Db}$ | $2.30^{BCa}$ |
| | 40 | $0.49^{Hf}$ | $0.62^{Ie}$ | $0.91^{Jd}$ | $1.37^{Fc}$ | $1.79^{Fb}$ | $1.79^{Fb}$ | $2.09^{Ea}$ |
| | 50 | $1.64^{Eg}$ | $1.89^{DEf}$ | $1.97^{Fe}$ | $2.09^{Cd}$ | $2.20^{Cc}$ | $2.28^{Bb}$ | $2.31^{BCa}$ |
| BHT | | $0.96^{Gg}$ | $1.52^{Gf}$ | $1.86^{He}$ | $2.07^{Cd}$ | $2.19^{Cc}$ | $2.26^{Bb}$ | $2.38^{Ag}$ |
| Commercial chitosan | | $0.17^{If}$ | $0.71^{He}$ | $0.92^{Id}$ | $1.89^{Ec}$ | $2.01^{Ebc}$ | $2.12^{Eab}$ | $2.21^{Da}$ |

Data are the mean ± SD, $n = 3$.
Mean values in the same column bearing the same superscript do not differ significantly ($P \leqslant 0.05$).
[a] PS: Particle size of chitosan.

range of 52.81–74.08% and 53.79–90.48% at 10 mg/ml, respectively. It seems that scavenging abilities of chitosan increased with increasing the degree of deacetylation. These results agree with Yen et al. (2008). On the contrary, about the scavenging ability of chitosan samples at particle size 40 mesh, it could be noticed that, its values were higher for samples deacetylated with NaOH solution at concentration of 30%, where it was 80.69% at concentration of 10 mg chitosan/ml as shown in Table 8, these results may be due to its higher solubility as previously described for the extracted chitosan samples.

Antioxidant activity of different extracted chitosan samples were determined by using the conjugated diene method and the results are presented in Table 9. It could be noticed that, the antioxidant activity of all chitosan samples were increased with increasing the concentration of chitosan solution. However, antioxidant activity of chitosan samples at particle sizes of 20 and 40 mesh decreased with increasing the concentration of NaOH solution used in N-deacetylation of chitin. On the other hand, antioxidant activity of chitosan samples at particle size 60 mesh increased with increasing the concentration of NaOH solution used in N-deacetylation of chitin, where the value of antioxidant activity increased from 47.71% to 72.31% as shown in Table 9.

At higher NaOH concentration, the obtained chitosans characterized by higher DDA and had more amino groups on C2 to inhibit the oxidation of linoleic acid, and thereby, increasing their antioxidant activities. However, at 0.5 mg/ml, the antioxidant activity was 54.74% and 51.15% for BHA and commercial chitosan, respectively.

The reducing power of all extracted chitosan samples increased as the concentration of tested chitosan increased as shown in Table 10. Data indicated that, the reducing power values of extracted chitosan samples were remained lower than those obtained for BHT and higher than the value of tested

**Table 11** Antibacterial activity (against gram negative and gram positive bacteria) of different concentrations of chitosan samples extracted from shrimp wastes using microwave technique.

| Chitin treatment | | Bacterial strains | MIC% (W/V) | Bacterial strains | MIC% (W/V) |
|---|---|---|---|---|---|
| PS[a] | NaOH conc. (%) | | | | |
| 20 mesh | 30 | *E. coli* | 0.6 | *St. aureus* | 1.0 |
| | | *S. typhimurium* | 0.4 | *B. cereus* | 0.05 |
| | 40 | *E. coli* | 0.2 | *St. aureus* | 0.1 |
| | | *S. typhimurium* | 0.1 | *B. cereus* | 0.05 |
| | 50 | *E. coli* | 0.4 | *St. aureus* | 0.05 |
| | | *S. typhimurium* | 0.8 | *B. cereus* | 0.05 |
| 40 mesh | 30 | *E. coli* | 0.2 | *St. aureus* | 0.2 |
| | | *S. typhimurium* | 0.05 | *B. cereus* | 0.2 |
| | 40 | *E. coli* | 0.2 | *St. aureus* | 0.1 |
| | | *S. typhimurium* | 0.4 | *B. cereus* | 0.4 |
| | 50 | *E. coli* | 1.0 | *St. aureus* | 0.2 |
| | | *S. typhimurium* | 0.2 | *B. cereus* | 0.6 |
| 60 mesh | 30 | *E. coli* | 0.4 | *St. aureus* | 0.05 |
| | | *S. typhimurium* | 0.8 | *B. cereus* | 0.4 |
| | 40 | *E. coli* | 0.6 | *St. aureus* | 0.6 |
| | | *S. typhimurium* | 0.2 | *B. cereus* | 0.1 |
| | 50 | *E. coli* | 0.1 | *St. aureus* | 0.1 |
| | | *S. typhimurium* | 0.1 | *B. cereus* | 0.2 |
| Commercial chitosan | | *E. coli* | 1.0 | *St. aureus* | 0.6 |
| | | *S. typhimurium* | 1.0 | *B. cereus* | 0.8 |

[a] PS: Particle size of chitosan.

commercial chitosan which their values were 2.384 and 2.215 at 10 mg/ml, respectively. From the same table, the reducing power of chitosan samples at concentration of 0.5 mg/ml showed moderate values (1.60–2.28, 1.85–1.91 and 0.49–1.46) for chitosan samples at the three particle sizes 20, 40 and 60 mesh, respectively.

On the other hand, at concentration of 10 mg/ml the same chitosan samples exhibited high reducing power (2.25–2.35, 2.31–2.36 and 2.09–2.31), the highest reducing power was observed for chitosan sample at particle size 40 mesh extracted with NaOH solution at 50% concentration. Yen et al. (2007) reported a slight reducing power (0.13–0.29) at 1 mg/ml for fungal chitosan from sittake stripes and moderate reducing power (0.42–0.57) at 10 mg/ml. It seems that reducing power of chitosan also correlated with its degree of deacetylation.

The antibacterial activity of the different extracted chitosan samples at different tested concentrations against gram negative and gram positive bacteria are shown in Table 11. Data indicated that, chitosans markedly inhibited the growth of most of gram negative bacteria tested; however, the inhibitory effects differed depending on the types of chitosan and the tested bacteria. It could be reported that, the minimum growth inhibitory concentrations (MIC) of tested chitosans against *E. coli* ATCC 25922 has been ranged from 1.0% to 0.4% (W/V), where it have been ranged from 0.8% to 0.05% (W/V) against *S. typhimurium* ATCC 14028.

On the other hand, MIC of different tested chitosans against gram positive bacteria ranged from 0.05% to 1.00% (W/V) against *B. cereus* DSMZ 345 and *St. aureus* ATCC 6528 (Table 11). About the antibacterial activity of commercial chitosan against the aforementioned tested bacteria as a comparable to tested chitosans in our study, the MIC of commercial chitosan was 1.0% (W/V) against *E. coli* ATCC 25922 and *S. typhimurium* ATCC 14028, where it was 0.8% and 1.0% (W/V) against *B. cereus* DSMZ 345 and *St. aureus* ATCC 6528; respectively. Generally it could be observed that, antibacterial activity of tested chitosans has been influenced by its molecular weight, degree of deacetlyation and concentration of solution. Other studies have also demonstrated that the MIC of chitosan against *E. coli* and *S. aureus* are from 0.005% to 1.5% (No et al., 2002). At the same time it could be noticed that, tested chitosans showed greater antimicrobial activity against gram positive bacteria than gram negative bacteria as recorded by previous studies (No et al., 2002; Takahashi et al., 2008).

## Conclusion

The previous results indicates that microwave irradiation has attracted a considerable amount of attention and is becoming an increasingly popular method for chemical reaction as it offers a clean, cheap, and convenient method of heating. For that, microwave could be used in the extraction step of chitosan from chitin extracted from shrimp wastes. Within the results in this work it could be concluded that, shrimp wastes is an excellent source for chitin, and the yields of chitosan increased with decreasing of chitin particle size and increasing the concentration of NaOH solution used in deacetylation step. The highest degree of deacetylation was obtained from chitin samples at particle size of 60 mesh deacetylated by 50% NaOH solution, it was 95.19% compared with 85% for commercial chitosan. Molecular weight of extracted chitosan ranged from 949.95 to 4467.05 k. Daltons. All extracted chitosans demonstrated an excellent solubility ranged from 83.28% to 99.05% and excellent functional properties of water and fat

binding capacities. In the same time, emulsification (EC and ES) and antioxidant properties of tested chitosans were depending on its molecular weight and degree of deacetylation. Different tested chitosans markedly inhibited growth of most bacteria tested; however, the inhibitory effects differed depending on the types of chitosan and the tested bacteria with greater antimicrobial activity against gram positive bacteria than gram negative bacteria.

## References


Abdou, E.S., Nagy, K.S., Elsabee, M.Z., 2008. Extraction and characterization of chitin and chitosan from local sources. Bioresour. Technol. 99, 1359–1367.

AOAC, 2007. Official Methods of Analysis of the Association of Official Analytical Chemistry (A.O.A.C.) International, 18th ed., Gaithersburg, Maryland, USA.

Bough, W.A., Salter, W.L., Wu, A.C., Perkins, B.E., 1978. Influence of manufacturing variables on the characteristics and effectiveness of chitosan products. 1. Chemical composition, viscosity, and molecular-weight distribution of chitosan products. Biotechnol. Bioeng. 20, 1931–1943.

Castelli, A., Bergamasco, L., Beltrame, P.L., Focher, B., 1996. Some insights into the kinetics of non-conventional alkaline deacetylation of chitin. In: Domard, A., Jeuniaux, C., Muzzarelli, R., Roberts, G. (Eds.), Advances in Chitin Science, vol. I. Jacques Andre, Lyon, pp. 198–203.

Cho, Y.I., No, H.K., Meyers, S.P., 1998. Physicochemical characteristics and functional properties of various commercial chitin and chitosan products. J. Agric. Food Chem. 46 (9), 3839–3843.

Darmadji, P., Izumimoto, M., 1994. Effect of chitosan in meat preservation. Meat Sci. 38 (2), 243–254.

Dickinson, E., 2009. Hydrocolloids as emulsifiers and emulsion stabilizers. Food Hydrocolloids 23 (6), 1473–1482.

Fernandez-Kim, S.O., 2004. Physicochemical and Functional Properties of Crawfish Chitosan as Affected by Different Processing Protocols. Dissertation, Louisiana State University, Baton Rouge, La, USA. <http://etd.lsu.edu/docs/available/etd-11102004 134343/unrestricted/Fernandez-Kim_thesis.pdf>.

Knorr, D., 1982. Functional properties of chitin and chitosan. J. Food Sci. 47, 593–595.

Lee, S.H., No, H.K., Jeong, Y.H., 1996. Effect of chitosan coating on quality of egg during storage. J. Korean Soc. Food Nutr. 25 (2), 288–293.

Lingnert, H., Vallentin, K., Eriksson, C.E., 1979. Measurement of antioxidative effect in model system. J. Food Process. Preserv. 3, 87–104.

Li, Q., Dunn, E.T., Grandmaison, E.W., Goosen, M.F., 1992. Applications and properties of chitosan. J. Bioact. Compat. Polym. 7, 370–397.

Liu, L., Li, Y., Li, Y., Fang, Y., 2004. Rapid N-phthaloylation of chitosan by microwave irradiation. Carbohyd. Polym. 57, 97–100.

Li, X.F., Feng, X.Q., Yang, S., Fu, G.Q., Wanga, T., Su, Z.X., 2010. Chitosan kills *Escherichia coli* through damage to be of cell membrane mechanism. Carbohyd. Polym. 79, 493–499.

Martinou, A., Kafetzopoulos, D., Bouriotis, V., 1995. Chitin deacetylation by enzymatic means: monitoring of deacetylation processes. Carbohyd. Res. 273, 235–242.

Mun, S., Decker, E.A., McClements, D.J., 2006. Effect of molecular weight and degree of deacetylation of chitosan on the formation of oil-in-water emulsions stabilized by surfactant–chitosan membranes. J. Colloid Interf. Sci. 296, 581–590.

Muzzarelli, R.A.A., 1997. Human enzymatic activities related to the therapeutic administration of chitin derivatives. Cell. Mol. Life Sci. 53, 131–140.

Naczk, M., Synowiecki, J., Sikorski, Z.E., 1981. The gross chemical composition of Antarctic krill shell waste. Food Chem. 7, 175–179.

No, H.K., Lee, K.S., Meyers, S.P., 2000. Correlation between physicochemical characteristics and binding capacities of chitosan products. J. Food Sci. 65, 1134–1137.

No, H.K., Park, N.Y., Lee, S.H., Meyers, S.P., 2002. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int. J. Food Microbiol. 74, 65–72.

Oyaizu, M., 1986. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44, 307–315.

Qin, C.Q., Zhou, B., Zeng, L.T., Zhang, Z.H., Liu, Y., Du, Y.M., 2004. The physicochemical properties and antitumor activity of cellulase-treated chitosan. Food Chem. 84, 107.

Razdan, A., Pettersson, D., 1994. Effect of chitin and chitosan on nutrient digestibility and plasma lipid concentrations in broiler chickens. Br. J. Nutr. 72 (2), 277–288.

Rout, S.K., 2001. Physicochemical, Functional, and Spectroscopic Analysis of Crawfish Chitin and Chitosan as Affected by Process Modification. Dissertation, Louisiana State University, Baton Rouge, LA, USA.

Sahu, A., Goswami, P., Bora, U., 2009. Microwave mediated rapid synthesis of chitosan. J. Mater. Sci.: Mater. Med. 20, 171–175.

SAS Program, 2000. SAS/STAT User's guide Release 6.12 Edition. Cary NC, USA: SAS Inst. Inc.

Sciarini, L.S., Maldonado, F., Ribotta, P.D., Pérez, G.T., León, A.E., 2009. Chemical composition and functional properties of *Gleditsia triacanthos* gum. Food Hydrocolloids 23, 306–313.

Shahidi, F., Arachchi, J.K.V., Jeon, Y.J., 1999. Food applications of chitin and chitosans. Trends Food Sci. Technol. 10, 37–51.

Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T., 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem. 40, 945–948.

Synowiecki, J., Al-Khateeb, N.A., 1997. Mycelia of *Mucor rouxii* as a source of chitin and chitosan. Food Chem. 60, 605–610.

Takahashi, T., Imai, M., Suzuki, I., Sawai, J., 2008. Growth inhibitory effect on bacteria of chitosan membranes regulated with deacetylation degree. Biochem. Eng. J. 40, 485–491.

Tolimate, A., Desbrieres, J., Rhazi, M., Alagui, A., Vincendon, M., Vottero, P., 2000. On the influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin. Polymer 41, 2463–2469.

US FDA (US Food and Drug Administration), 2001. Center for Food Safety and Applied Nutrition. Office of Premarket Approval. GRAS Notices received in 2001. <http://vm.cfsan.fda.gov>.

Wang, J.C., Kinsella, J.E., 1976. Functional properties of novel proteins: Alfalfa leaf protein. J. Food Sci. 41, 286–292.

Wydro, P., Krajewska, B., HącWydro, K., 2007. Chitosan as a lipid binder: a Langmuir monolayer study of chitosan–lipid interactions. Biomacromolecules 8, 2611–2617.

Yen, M.T., Yang, J.H., Mau, J.L., 2008. Antioxidant properties of chitosan from crab shells. Carbohyd. Polym. 74, 840–844.

Yen, M.T., Tsengb, Y.H., Lia, R.C., Mau, J.L., 2007. Antioxidant properties of fungal chitosan from shiitake stipes. Lebensm.-Wiss. Technol. 40, 255–261.