The Effect of Different Chemical and Physical Processing on the Physicochemical and Functional Characterization of Chitosan Extracted from Shrimp Waste Species of Indian White Shrimp

Marjan Nouri a, Faramarz Khodaiyan a*, Seyed Hadi Razavi b, and Mohammad Ali Mousavi b

aBioprocessing and Biodetection Laboratory, Department of Food Science, Engineering and Technology, Faculty of Agricultural Engineering and Technology, Campus of Agriculture and Natural Resource, University of Tehran, P.O. Box 4111, Karaj 31587-77871, Iran

bDepartment of Food Science, Engineering and Technology, Faculty of Agricultural Engineering and Technology, Campus of Agriculture and Natural Resources, University of Tehran

Received: 14 October 2014, Accepted: 31 March 2015

SUMMARY

The preparation and characterization of chitosan, water soluble polysaccharide, from waste of Indian white shrimp (penaeus indicus) are reported. The effect of different conditions of chemical and physical methods on extraction, physicochemical (proximate composition, solubility, degree of deacetylation and molecular weight) and functional properties (fat binding capacity, water binding capacity and color attributes) of biopolymer chitosan was examined. The results suggested that optimal condition preparation involves alkali concentration of 50%, a power of microwave of 720 W and a reaction time of 20 S. The end product had a white color, high degree of deacetylation, solubility and low molecular weight, ash, protein and moisture content.

Keywords: Chitosan, Chitin, Shrimp wastes, Microwave, Physicochemical

*Corresponding author: khodaiyan@ut.ac.ir
©Smithers Information Ltd., 2016
INTRODUCTION

Seafood productions consist of a large variety of species and have significant importance in the food industry and human nutrition [1]. In modern countries improvement of hygiene, food safety and public health issue are very important and most of the governments consider destroying certain hygienic and toxicological wastes of food, to overcome the problem of shrimp waste a solution was proposed not only to solve industrial problems but also to change the biowaste to useful component [2-4].

Chitosan, β- (1 → 4) D-glucosamine, is a cationic amino polysaccharide which is a partly deacetylated form of chitin (extracting from the waste of exoskeleton of shrimp) [5]. Chitosan has been interested in medicine, pharmaceutical, biomedical, biological, agriculture, environment and in food technology such as, food formulations, binding, thickening, gelling, stabilizing, clarifying, antimicrobial and antioxidant agent [6-8]. Over the years from 1936 until now, various procedures have been applied to extract chitin and the roles of various factors were considered in functional properties of chitosan, but here we focus on the extraction basis of the processes for industrial production of chitosan [9-12]. However, there is no existing report on the extraction of chitosan has just been waste species of Indian white shrimp (penaeus indicus).

The present study is an attempt to investigate the improved extraction method of deacetylated waste of Indian white shrimp by using mild conditions such as using organic acid like acetic acid instead of acid sulfuric, reducing the amount of alkali and acid required, and also heat treatment and irradiation time below 100ºC and 180 S in whole chemical and microwave process, respectively for short denaturation which did not degrade the structure of chitosan. Furthermore, the effect of different reaction conditions on physicochemical and functional properties of biopolymer chitosan was examined.

EXPERIMENTAL

Extraction and Purification of Chitosan

The dried waste of Indian white shrimp were grained and packaged to be stored at ambient temperature for further analysis. The process of extraction involved deproteinization (2% (w/w) sodium hydroxide solution 30:1 (w/v) for 2 h at 80ºC) with constant stirring then shells were demineralized (10% (w/w) acetic acid 40:1 (w/v) for 4 h at 50ºC) and mixed thoroughly to form insoluble particles. After each step separation was done by centrifugation (4,000 g, 15 min), dissolution chitosan was produced by precipitation of
The Effect of Different Chemical and Physical Processing on the Physicochemical and Functional Characterization of Chitosan Extracted from Shrimp Waste Species of Indian White Shrimp

sodium hydroxide solution. In physical method all reaction under microwave irradiation was carried out in the microwave device Butane microwave (M 245, Iran) at room temperature. The parameters employed at deacetylation of chitin in this article were as follows in Table 1. The samples were filtered and washed with distilled water until neutralized and finally, samples were washed with ethanol and then acetone to improve purity. The residue obtained was dried in a hot air oven at 50°C and used for further analysis.

### Table 1. Levels of various independent variables at coded values of RSM experimental design

<table>
<thead>
<tr>
<th>Variables</th>
<th>Units</th>
<th>Chemical method</th>
<th>NaOH concentration (%) by weight</th>
<th>Reaction temperature (Celsius)</th>
<th>Microwave method</th>
<th>Power of microwave irradiation (W)</th>
<th>Reaction time (min)</th>
<th>Run order</th>
<th>Second (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction time (min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction temperature (Celsius)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium hydroxide solution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Run order</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td></td>
<td>60</td>
<td>60</td>
<td>30</td>
<td>100</td>
<td>300</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td></td>
<td>60</td>
<td>60</td>
<td>50</td>
<td>100</td>
<td>300</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td></td>
<td>100</td>
<td>100</td>
<td>30</td>
<td>100</td>
<td>900</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td></td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>900</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td></td>
<td>80</td>
<td>90</td>
<td>30</td>
<td>20</td>
<td>600</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td></td>
<td>80</td>
<td>90</td>
<td>50</td>
<td>20</td>
<td>600</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td></td>
<td>300</td>
<td>300</td>
<td>30</td>
<td>20</td>
<td>600</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td></td>
<td>300</td>
<td>300</td>
<td>50</td>
<td>20</td>
<td>600</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>9</td>
<td>40</td>
<td></td>
<td>90</td>
<td>90</td>
<td>40</td>
<td>20</td>
<td>300</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td></td>
<td>100</td>
<td>100</td>
<td>40</td>
<td>20</td>
<td>300</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>11</td>
<td>40</td>
<td></td>
<td>100</td>
<td>100</td>
<td>40</td>
<td>20</td>
<td>300</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>40</td>
<td></td>
<td>80</td>
<td>80</td>
<td>40</td>
<td>20</td>
<td>300</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>13</td>
<td>40</td>
<td></td>
<td>80</td>
<td>80</td>
<td>40</td>
<td>20</td>
<td>300</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>14</td>
<td>40</td>
<td></td>
<td>195</td>
<td>195</td>
<td>40</td>
<td>20</td>
<td>300</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>15</td>
<td>40</td>
<td></td>
<td>195</td>
<td>195</td>
<td>40</td>
<td>20</td>
<td>300</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>
Analytical Procedures

FTIR Spectroscopy Analyses of Chitin and Chitosan

Fourier transform infrared (FTIR) Spectroscopy of samples was measured on KBr discs in the transmission mode in the range 400-4000 cm⁻¹ by using Bruker FT-IR spectrophotometer (model Vector 22, Germany).

Physicochemical Characteristics

Proximate Composition of Chitosan

According to the AOAC (2006) methods, moisture content of chitosan samples were indicated by drying the samples in oven at 105°C until reaches constant weigh for about 24 h (AOAC 950.46), crud ash content of samples was dedicated by heating the samples to 550°C for 20 h (AOAC 920.153), crude protein was determined by achieving the content of total nitrogen by using the micro- Kjeldahl procedure (AOAC 928.08), and total lipids were extracted described by the Bligh and Dyer method (1959) as some modification by Manirakiza et al. (2001) [13-15].

Solubility of Chitosan

Dissolving 0.1 g of chitosan samples with 10 ml of 1% acetic acid in a centrifuge tube (30 min), the solution was immersed in a boiling water bath (10 min), cooled to room temperature and centrifuged (10,000 g, 10 min) and the supernatant was decanted. The undissolved particles were washed in distilled water then centrifuged the supernatant was removed and undissolved pellets dried (60°C, 24 h), finally, the percentage solubility was determined through the following Equation (1) [16]:

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

Degree of Deacetylation (DDA) of Chitosan

The degree of deacetylation was measured by the acid-base titration method through some modification [17]. Chitosan (0.125 g) was dissolved in aqueous solution of HCL (30 mL, 0.1 mol/L), methyl orange was added as indicator and stirred (30 min) until totally dissolved at room temperature. The solution was titrated with 0.1 mol/L NaOH solution until it turned orange. The acetyl content of chitosan was measured from the amount of titrate used.
**Viscosity Average Molecular Weight (Mw) of Chitosan**

The intrinsic viscosity was measured according to Weska et al. (2007) by some alteration [18]. Chitosan samples were dissolved in a solvent consist of 0.1 M acetic acid, 0.2 M sodium chloride and water. The viscosity of samples in different concentration (0.001, 0.004, 0.007 and 0.010 g/mL) was measured by flowing through a capillary in a Cannon-Fenske capillary viscometer (model 9721-B53, USA) at 25ºC. The viscosity average molecular weight of chitosan for each sample was calculated by taking the values of $K= 1.81 \times 10^{-3}$ Ml/g and $\alpha= 0.93$ [19].

**Functional Characteristics**

**Fat Binding Capacity and Water-binding Capacity of Chitosan**

Fat binding capacity (FBC) and water-binding capacity (WBC) of chitosan samples were determined according to Wang and Kinsella (1976) method with some modifications [20]. FBC and WBC properties were determined by adding 10 mL of vegetable oil (for determined FBC) or water (for determined FBC) to centrifuge tube (50 mL) containing 0.5 g of samples (initial samples weight) then mixing on a vortex mixer (1 min). Samples were left at ambient temperature (30 min) with shaking (5 S) every 10 min and centrifuged (3,200 g, 25 min). Then the supernatant was decanted (fat bound weight or water bound weight), these properties were calculated as follows by the formula (4) and (5):

\[
\text{FBC}\% = \left[\frac{\text{fat bound weight (g)}}{\text{initial sample weight (g)}}\right] \times 100
\]  

(4)

\[
\text{WBC}\% = \left[\frac{\text{water bound weight (g)}}{\text{initial sample weight (g)}}\right] \times 100
\]  

(5)

**Colorimetric Properties of Chitosan**

The colorimetric characteristics of samples were determined using a Hunterlab colorimeter (D25 DP9000, Hunter Associates Laboratory, Inc., Reston, USA). Measurements of $L^*$ (lightness), $a^*$ (greenness–redness), $b^*$ (yellowness), total color difference ($\Delta E$) and whiteness index (WI) were calculated using Eqs. (6) and (7) respectively [21].

\[
\Delta E = \left[\left( L^* - L_{0*}\right)^2 + \left( a^* - a_{0*}\right)^2 + \left( b^* - b_{0*}\right)^2\right]^{0.5}
\]  

(6)

\[
\text{WI} = 100 - \left[\left(100 - L^*\right) + a^* + b^*\right]^{0.5}
\]  

(7)
Experimental Design and Statistical Analysis

Box–Behnken Design (BBD), which is well suited for accommodating a quadratic surface and action well for the process optimization, was offered for the experimental design. The variables, Units, Symbol code and levels were shown in Table 1. All experiments were carried out in triplicate and the results were showed as mean ± SD, (significant level p<0.05). Statistical analyses were performed using the statistical program Minitab 16 statistical software (Minitab Inc., PA, USA) and SPSS 16.0 (SPSS INC., Chicago, IL, USA).

RESULTS AND DISCUSSION

Response surface methodology (RSM) was the most advantageous statistical technique to investigate the effect of major independent factors on the yield, Mw and DDA of chitosan to choose the best treatment of chitosan extraction from waste species of Indian white shrimp with high deacetylation and low molecular weight. The results of a set of 30 experiments suggested that the optimal condition for chitosan extraction in chemical and microwave methods were 50% NaOH solution, 86°C reaction temperature, 174 min reaction time and, 50% NaOH solution, 720 W microwave power and a 20 S reaction time, respectively [22]. In the present article samples of chitosan (ChitosanCh and ChitosanM) were extracted under optimization conditions and also samples among a set of 30 experiments were selected randomly, shown in Table 2, and the physicochemical and functional properties of the experiments trial were compared.

FTIR Analysis

FTIR investigations showed the characteristic of chitin (A), chitosanCh (B) and chitosanM (C) in Figure 1a-b. The successful conversion of chitin to chitosan was confirmed with FTIR analysis. Main characteristics of chitin has the picks absorption bounds at 3300, 3152, 1701 and 1604 cm⁻¹ corresponds to the stretching NH(-NHCOCCH₃), CH(CH₃), C=O(-NHCOCCH₃) and CN(-NHCOCCH₃) respectively. In chitosan samples (B and C) the weak band at 1697 and 1700 cm⁻¹ (amide I) was observed because hydrogen interactions are less accentuated and the hydroxyl groups exist due to removal of acetyl group [23]. Further the new absorption achieved (compared with chitin sample) nearly 3400 and 3410 cm⁻¹ NH(-NH₂), 1502 and 1501 cm⁻¹ (N-H bend of R-NH₂) indicates an increasing in DDA and bending at nearly 1408 and 1406 cm⁻¹ in chitosan samples indicated the molecular weight.
Table 2. Parameters used for deacetylation under chemical and microwave methods and means (±SD) of physicochemical composition of samples

<table>
<thead>
<tr>
<th>Run order</th>
<th>Independent variables</th>
<th>Physicochemical characteristics (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical method</td>
<td>NaOH concentration (%) by weight</td>
<td>Reaction temperature (Centigrade)</td>
</tr>
<tr>
<td>Chitosan</td>
<td>50</td>
<td>86</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>15</td>
<td>40</td>
<td>80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microwave method</th>
<th>NaOH concentration (%) by weight</th>
<th>Power of microwave (Watt)</th>
<th>Irradiation time (Second)</th>
<th>Moisture</th>
<th>Ash</th>
<th>Protein</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>50</td>
<td>720</td>
<td>20</td>
<td>4.44±0.10</td>
<td>0.43±0.09</td>
<td>0.58±0.14</td>
<td>91.97±0.42</td>
</tr>
<tr>
<td>16</td>
<td>30</td>
<td>300</td>
<td>100</td>
<td>4.34±0.07</td>
<td>0.42±0.04</td>
<td>0.68±0.10</td>
<td>81.06±0.19</td>
</tr>
<tr>
<td>19</td>
<td>50</td>
<td>900</td>
<td>100</td>
<td>4.47±0.20</td>
<td>0.47±0.00</td>
<td>0.57±0.06</td>
<td>94.83±0.44</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>600</td>
<td>20</td>
<td>3.98±0.26</td>
<td>0.46±0.05</td>
<td>0.66±0.08</td>
<td>79.80±0.70</td>
</tr>
<tr>
<td>22</td>
<td>30</td>
<td>600</td>
<td>180</td>
<td>4.50±0.19</td>
<td>0.45±0.08</td>
<td>0.64±0.02</td>
<td>84.00±0.96</td>
</tr>
<tr>
<td>25</td>
<td>40</td>
<td>900</td>
<td>20</td>
<td>4.06±0.17</td>
<td>0.49±0.02</td>
<td>0.70±0.01</td>
<td>87.85±0.35</td>
</tr>
<tr>
<td>26</td>
<td>40</td>
<td>300</td>
<td>180</td>
<td>4.35±0.20</td>
<td>0.40±0.03</td>
<td>0.63±0.05</td>
<td>83.42±0.70</td>
</tr>
<tr>
<td>30</td>
<td>40</td>
<td>600</td>
<td>100</td>
<td>3.86±0.32</td>
<td>0.45±0.00</td>
<td>0.58±0.08</td>
<td>86.75±0.36</td>
</tr>
</tbody>
</table>

\(a, c\) Means within the same column with different superscript significantly \((p<0.05)\)
Figure 1. Fourier transform infrared (FTIR) spectrum of chitin (A) and sample of chitosan from Persian Gulf shrimp waste under optimized preparation conditions (chitosan_{Ch}: B, chitosan_{M}: C)

**Proximate Composition of Chitosan**

Results in Table 2 demonstrated the physicochemical composition of extracted chitosan, such as moisture, minerals (ash), proteins and lipids given as percentage of the dry weight of shrimp shells.

The parentage of content of moisture varied between 3.65 to 4.64% of the dry weight and there were non-significantly different (P \leq 0.05). The lower
moisture content of chitosan leads to shelf stability and quality. According to Li (1992), commercial chitosan products contain less than 10% moisture content [24], and all of these data were in the range (Table 2).

The statistical analysis was used to test whether the treatment difference in the ash content (0.40 to 0.49%) was significant, predicting a non-significant effect on ash content in our data. Ash is a mark of the effectiveness of the demineralization for removal of minerals specially calcium carbonate. The ash content of chitosan is an important parameter because residual ash could have an undesirable affect on functional properties such as solubility of the final product. A high quality grade of chitosan must have less than 1% of ash content [16].

The percentage of protein (0.56 to 0.70%) indicates the effectiveness of deproteinization steps and achieving to 100% deproteinization is so difficult because in the stable complex, protein is bound by covalent bonds with chitin and chitosan. At Table 2, it can be seen that the protein content of chitosan produced by the lower alkali percentage (30%) was higher than that of other treatments and chitosan with the lowest content of protein was detected in the samples with more concentration of alkali solution (50%). It could be observed because NaOH solution allows the easily breakdown of the matrix of protein and removal of it from the structure and more percentage of NaOH solution maybe more effective in opening structure of protein. From the same Table, our results show low contents of lipids less than 0.1% of the dry weight, with no significant variation of treatment in the lipid content of chitosan (data not shown).

Solubility Analysis

The percentages of solubility for samples were between 79.80 to 95.61%, and demonstrated a significant different among data (p ≤ 0.05), (Table 2). Solubility of chitosan is a crucial characteristic for application in medicine and food sciences [25, 26]. In the present study most of the samples had a perfect solubility ranged especially from 91.97 to 95.61% that the deproteinization process on samples might have been nearly complete. Existing significant different in samples could be due to low concentration of NaOH solution (30 and 40%) used in deacetylation which is not enough to be sufficiently swollen and still had some protein remaining or other impurities. These results showed that there was significant correlation between protein content and solubility ($Y_{Solubility} = 1688.9X^2_{Protein} - 2282.6X_{Protein} + 853.37, R^2 = 0.92$).
**Optimisation Preparation Conditions for Physicochemical and Functional Characteristics of Chitosan**

The degree of deacetylation, molecular weight, water binding capacity and fat binding capacity content are the four main parameters affecting solubility, chemical reactivity, biodegradability and application of chitosan [27]. In present study samples were deacetylated within range of 73.64 to 88.60% and the results for the water binding capacity ranged from 588.71 to 670.72%. DDA increased generally by increasing NaOH concentration (30 up to 50%), temperature (60 up to 88ºC), power of microwave (300 up to 700 W), time of chemical reaction (90 up to 200 min) and irradiation time (20 up to 40 S). Although temperature upper than 88ºC and power of microwave higher than 700 W did not have significant effects on the DDA and expanded reaction time resulted in DDA reduction in both methods and the same pattern was observed for WBC. It seemed that reduction in DDA and WBC was because of the inhibition of the deacetylation reaction of chitosan at higher temperature and longer time reaction (Figure 2a-f).

![Figure 2(a-f)](image-url) -- Effect of different reaction conditions on the DDA (%) and WBC (%)

*Figure 2(a-f). Effect of different reaction conditions on the DDA (%) and WBC (%)*
In Figure 3a-f the results indicated that the molecular weight and fat binding capacity of extracted chitosan from waste of Indian white shrimp at different conductions was in the range of 1105.032 to 806.931 (kilodalton: kDa) and 398.74 to 434.11%, respectively. Data revealed that the Mw and FBC of chitosan decreased and increased respectively by increasing of both NaOH concentration and temperature of mechanism, however with higher reaction time (more than 180 min in chemical and 30 S in microwave methods) changed the behavior of Mw and FBC of samples to the worst, also power of microwave upper than 700 W not significant effects on Mw and FBC. It was noticed that minimum and maximum of Mw and FBC of chitosan respectively was observed at 50% NaOHaq on microwave technique (Figure 3a-f).

The results showed that the microwave manner was more useful method for improving physiochemical and functional properties of chitosan biopolymer. The study noted that the DDA, WBC and FBC significantly increased and Mw of chitosan decreased by increasing NaOH concentration, which is due to adding

![Figure 3(a-f). Effect of different reaction conditions on the Mw (kDa) and FBC (%)](image-url)
of alkali concentration up to 50%, the content of acetyl group and molecular weight reduced and deacetylation of the chitosan biopolymer increased. Our data showed that the pattern recorded for water binding capacity was nearly observed for fat binding capacity. The reaction needed high temperature, power of microwave and time to achieve suitable properties, but if these factors increase dramatically, they will have a negative effect on behavior of chitosan. Results of the DDA, WBC, FBC and Mw of chitosan acquired from this study are in agreement with those factors from other studies of researchers, some of them were reported here like, Hwang (2002) observed that the rate of Mw (1106 to 106 kDa) decrease and DDA (67.3 to 95.7%) increase of chitosan gradually decreased with prolonged reaction time (2-34 hours) at different alkali concentration (32 to 58%) and temperature reaction (40 to 120ºC) laboratory scale reactors [29]. Rout (2001) said that WBC for chitosan ranges from 581 to 1150% with an average of 702% and the FBC average of commercial crab chitosan for soybean oil was 587%. [28]. Zhang et al. (2011) researched the effect of different factors on the yield, DDA, viscosity and ash content of chitosan obtained from housefly larvae and these results suggest that optimal conditions for chitosan preparation a reaction temperature of 125ºC and a reaction time of 6 hours [17]. Mahdy samar (2013) produced chitosan at three particle size with different concentration of NaOH solution under microwave irradiation. The results showed the highest degree of deacetylation and the excellent functional properties like WBC and FBC was obtained from chitin sample deacetylated by 50% NaOH solution [29].

In our present study it is noticed that WBC and FBC were significantly correlated with DDA and Mw of chitosan \(Y_{\text{DDA}} = 0.1576X_{\text{WBC}} – 17.78, R^2 = 0.89\) and the highest WBC and FBC were achieved with sample having the highest DDA and lowest Mw and a similar result has been reported by Ocloo et al. (2011) [30]. Commercial chitosan usually has a deacetylation degree varying from 70 to 95%, molecular weight 50 to 2000 kDa, WBC 458 to 805% and FBC 314 to 535%, variation of data serenely depending on the source and preparation procedure [16, 18, 26] and the results of these factors in this present were in the range of above.

**Color Attributes**

The parameters of \(L^*\) (lightness), \(a^*\) (green-red), \(b^*\) (blue-yellow), \(\Delta E\) (difference between color of white plate and samples), and WI (whiteness index) values are shown in Table 3. In fact the increase in lightness (\(L^*\) value) leads to decrease in factor of yellowness (\(b^*\) value) which was observed in different conditions,, however, response of redness (\(a^*\) value) was not very significantly different, which can be due to the hydrophilic property of chitosan biopolymer. The
whiteness characteristic of chitosan powder is so important for commercial production and customer satisfaction. The chitosan samples prepared by less deacetylation exhibited yellow color more than the other samples, because the natural colors of the starting material (chitin) was more yellow than white. The data showed that when the reaction time decreases the obtained chitosan shows lighter appearance (Microwave in compare with chemical process), it may be due to microwave rays oxidizing the existing pigment in shrimp waste such as astaxantin that contain double bonds thus improve the color of powder. The comparison between samples revealed that less deacetylation of chitosan increases total color difference ($\Delta E$) and causes imbalance in color.

Table 3. Means (±SD) of Hunter color values ($L^*$, $a^*$, and $b^*$), total color difference ($\Delta E$) and whiteness index (WI) of chitosan samples

<table>
<thead>
<tr>
<th>Run order</th>
<th>Color attributes</th>
<th>Chitosan$_{Ch}$</th>
<th>1</th>
<th>4</th>
<th>5</th>
<th>7</th>
<th>10</th>
<th>11</th>
<th>15</th>
<th>16</th>
<th>19</th>
<th>20</th>
<th>22</th>
<th>25</th>
<th>26</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L*</td>
<td>78.60±0.50</td>
<td>74.31±1.21</td>
<td>78.50±0.72</td>
<td>77.00±0.33</td>
<td>73.15±1.42</td>
<td>77.31±0.28</td>
<td>75.39±1.55</td>
<td>78.18±0.34</td>
<td>85.02±1.44</td>
<td>74.47±1.80</td>
<td>81.64±0.73</td>
<td>75.04±0.39</td>
<td>78.37±0.81</td>
<td>80.02±1.19</td>
<td>81.24±1.42</td>
</tr>
<tr>
<td></td>
<td>$a^*$</td>
<td>0.75±0.73</td>
<td>-0.20±0.14</td>
<td>0.25±0.69</td>
<td>0.10±0.25</td>
<td>0.01±0.20</td>
<td>0.30±0.50</td>
<td>-0.10±0.41</td>
<td>0.15±0.33</td>
<td>0.56±0.22</td>
<td>0.23±0.31</td>
<td>-0.25±0.23</td>
<td>0.40±0.30</td>
<td>0.39±0.51</td>
<td>-0.11±0.14</td>
<td>0.15±0.51</td>
</tr>
<tr>
<td></td>
<td>$b^*$</td>
<td>10.86±1.20</td>
<td>12.47±0.73</td>
<td>10.85±0.53</td>
<td>12.22±0.66</td>
<td>10.36±0.51</td>
<td>11.09±0.1</td>
<td>12.85±1.04</td>
<td>12.31±0.84</td>
<td>10.34±0.2</td>
<td>12.32±0.2</td>
<td>12.10±0.4</td>
<td>10.02±0.1</td>
<td>11.00±0.9</td>
<td>12.44±0.2</td>
<td>12.03±0.7</td>
</tr>
<tr>
<td></td>
<td>$\Delta E$</td>
<td>11.80±0.48</td>
<td>15.94±0.90</td>
<td>11.73±0.38</td>
<td>13.43±1.22</td>
<td>16.64±1.40</td>
<td>12.90±0.14</td>
<td>15.09±1.20</td>
<td>12.44±0.36</td>
<td>6.31±1.02</td>
<td>15.81±0.90</td>
<td>9.25±0.62</td>
<td>1.40±0.43</td>
<td>11.93±0.26</td>
<td>10.80±1.6</td>
<td>9.69±0.3</td>
</tr>
<tr>
<td></td>
<td>WI</td>
<td>94.25±0.17</td>
<td>93.84±1.24</td>
<td>94.29±0.30</td>
<td>94.06±1.08</td>
<td>93.89±1.19</td>
<td>94.16±0.12</td>
<td>93.88±0.22</td>
<td>94.14±0.49</td>
<td>94.91±0.22</td>
<td>93.84±0.26</td>
<td>94.50±0.14</td>
<td>94.05±0.10</td>
<td>94.25±0.16</td>
<td>94.31±0.20</td>
<td>94.43±1.04</td>
</tr>
</tbody>
</table>

$^	ext{a, d}$ Means within the same column with different superscript differ significantly $(p<0.05)$.

CONCLUSIONS

The present study showed the successful conversion of waste species of Indian white shrimp to chitosan, and was confirmed with FTIR analysis. Our results showed no significant variation of treatment in moisture, ash and fat content, and the appropriate protein content and solubility of samples produced...
by more concentration of alkali solution (50%) used in deacetylation. The results demonstrated that significant correlations were observed between protein content and solubility, WBC and DDA, and FBC and Mw of chitosan. We focused on the improved extraction method by using mild conditions and the design of a microwave method by using the simple, clean, cheap and environmentally friendly method for the synthesis of chitosan with suitable physiochemical and functional properties which improved the quality of the final product. These results suggest that waste species of Indian white shrimp could possibly be used as basis for the industrial production of commercial chitosan.

REFERENCES


