SUPPLEMENTARY MATERIAL

A new method for fast chitin extraction from shells of crab, crayfish and shrimp

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Abstract
A new method for quick chitin isolation from the shells of crab, crayfish and shrimp is described. The main difference between the new method and the conventional method is two sodium hypochlorite (NaClO) treatments for 10 minutes each before the processes of demineralization and deproteinization. After the NaClO treatment, only 15 minutes is adequate for the demineralization and 20 minutes for the deproteinization processes. Newly extracted chitin from crab, crayfish and shrimp shells and commercial chitin were characterized using FTIR, TGA, X-ray diffractometry (XRD) and elemental analysis. From the results, it was observed that the chitins isolated with the new method and the commercial chitin had almost the same physicochemical properties. The advantage of the new method compared to traditional methods is the relatively rapid chitin extraction. When compared to the traditional chitin extraction method, the proposed method appears to be promising regarding its time and energy saving nature.

Keywords: new method, chitin, isolation, characterization, sodium hypochlorite

1. Experimental
1.1. Materials
The crab, crayfish and shrimp shells used in this study were obtained from the waste products of a restaurant located in Istanbul. The physicochemical characteristics of the chitins obtained using the new method was compared to commercial chitin, which was bought from SIGMA ALDRICH, Missouri, United States (shrimp shells, Pcode: 1001416772).

1.2. Chitin extraction process
The crab, crayfish and shrimp shells were dried at 60 °C for a week in an oven. The samples were ground to a fine powder using a mortar. The extraction method proposed here involved four chemical treatment steps, with each step followed by rinsing with distilled water until a neutral pH was reached. In the first step, the samples (10 g of powder per sample) were refluxed in 100 mL sodium hypochlorite (NaClO) solution (3%, v:v) at 100 °C for 10 min. After washing with distilled water, the second step of the procedure was to repeat the first step. The third step was demineralization of the sample in a mineral acid solution. The samples were refluxed in 50 mL of 1 M HCl at 75 °C for 15 min. In the last step, the samples were refluxed in 50 mL of 1 M NaOH (sodium hydroxide) solution at 100 °C for 20 min. to remove any protein residues. Finally, following washing, the extracts were filtered off and placed in an oven at 60 °C for a week. The dry mass of the each chitin sample was calculated and then the percent chitin content was determined. Three repeats of the new method was applied. The method used by Kaya et al. (2014) was followed for the FTIR, TGA, XRD and elemental analysis of the isolated chitins and the commercial chitin. A Perkin Elmer brand
FTIR Spectrometer was used to determine absorbance in the wave number between 4000–625 cm$^{-1}$. Thermogravimetric analysis was conducted by EXSTAR S11 7300 with 10 °C heating rate per minute from 25 to 650 °C. XRD measurements were done at 40 kV, 30 mA and 20 with the scan angle from 5 to 45°. A Flash 2000 brand of elemental analyzer was used to determine the C, H and N contents.

References

Table captions

Table S1: Elemental analysis results of chitins extracted with the new method and commercial chitin.

Table S2: XRD data and crystalline values of chitins extracted with the new method and commercial chitin.
Table S1

<table>
<thead>
<tr>
<th>Sample</th>
<th>N %</th>
<th>C %</th>
<th>H %</th>
<th>C/N (%)</th>
<th>DA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitin from crab shell</td>
<td>6.03</td>
<td>42.9</td>
<td>5.65</td>
<td>7.11</td>
<td>114</td>
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<td>Chitin from crayfish shell</td>
<td>6.09</td>
<td>42.88</td>
<td>6.02</td>
<td>7.04</td>
<td>110</td>
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<td>Chitin from shrimp shell</td>
<td>6.17</td>
<td>43.2</td>
<td>6.42</td>
<td>7</td>
<td>108</td>
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<td>Commercial chitin</td>
<td>6.13</td>
<td>43.28</td>
<td>6.04</td>
<td>7.06</td>
<td>112</td>
</tr>
</tbody>
</table>

Table S2

<table>
<thead>
<tr>
<th>Chitin sources</th>
<th>XRD peaks of chitins (°)</th>
<th>Crystalline index values (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crab shell chitin</td>
<td>9.56, 13.14, 19.56, 20.92, 23.34, 26.72</td>
<td>89.5</td>
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<tr>
<td>Crayfish shell chitin</td>
<td>9.38, 13.04, 19.54, 20.64, 23.26, 26.36</td>
<td>86.7</td>
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<td>Shrimp shell chitin</td>
<td>9.31, 13.01, 19.44, 21.06, 23.42, 26.76</td>
<td>91.4</td>
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<tr>
<td>Commercial chitin</td>
<td>9.51, 13.08, 19.36, 20.86, 23.62, 26.49</td>
<td>85.2</td>
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</table>
Figure captions

**Figure S1**: FTIR spectrum of the chitins extracted with the new method and commercial chitin (a. crab shell chitin, b. crayfish shell chitin, c. shrimp shell chitin and d. commercial chitin).

**Figure S2**: TGA diagrams of the chitins extracted with the new method and commercial chitin (a. crab shell chitin, b. crayfish shell chitin, c. shrimp shell chitin and d. commercial chitin).

**Figure S3**: XRD peaks of the chitins extracted with the new method and commercial chitin (a. crab shell chitin, b. crayfish shell chitin, c. shrimp shell chitin and d. commercial chitin).
Figure S1
Figure S2
Figure S3