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Chitin and chitosan biopolymer production from the Iranian medicinal fungus
Ganoderma lucidum: Optimization and characterization

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**ABSTRACT**

Chitin and chitosan with unique properties and numerous applications can be produced from fungus. The production of chitin and chitosan from the mycelia of an Iranian Ganoderma lucidum was studied to improve cell growth and chitin productivity. Inoculum size and initial pH as two effective variables on the growth of G. lucidum and chitin production were optimized using response surface method (RSM) by central composite design (CCD). The results verified the significant effect of these two variables on the cell growth and chitin production. In optimum conditions, including pH = 5.7 and inoculum size of 7.4%, the cell dry weight was 5.91 g/L and the amount of chitin production was 1.08 g/L with the productivity of 0.083 g/(L day). The produced chitin and chitosan were characterized using XRD and FTIR. Moreover, the antibacterial activity of the produced chitosan was investigated and compared with the commercial chitosan. The results showed that the produced chitin and chitosan had suitable quality and the Iranian G. lucidum would be a great source for safe and high-quality chitin and chitosan production.

**KEYWORDS**

Antibacterial properties; chitin; chitosan; Ganoderma lucidum; Medicinal fungus; optimization

**Introduction**

Chitin is a natural amino polysaccharide made from chains of N-acetyl glucosamine. It is the second most abundant natural biopolymer after cellulose.\[1\] Cellulose and chitin are polysaccharides that have protective roles in plants and animals. Chitin is found in crustaceans, Mollusca, marine diatoms, insects and cells walls of algae, fungi, yeasts, and exoskeleton of marine zooplankton species.\[2\] The annual estimate of chitin synthesis is more than ten giga tons. Also, 150,000 tons of this polymer is used for the commercial activities.\[3\] Chitin is insoluble in ordinary solvents. It can be converted into chitosan by enzymatic or chemical deacetylation methods. Chitosan is soluble in aqueous solutions of some acids.\[4,5\]

Biocompatibility, biodegradability, and antimicrobial and selective adsorption properties are factors that increase the widespread use of chitosan in various forms, such as beads, powder, membrane, gel, sponge, and fiber. Several inherent properties of chitosan, as well as its poly-cationic nature, lead to its widespread use in different fields, including medicine, pharmacy, food industries, textile, water purification, cosmetics, agriculture, paper, and so forth.\[6–9\] The degree of deacetylation and molecular weight (MW) are two important factors for this polysaccharide. They affect physical, chemical and biological properties of chitosan. For example, the antimicrobial activity of chitosan was found to be directly proportional to its DD, which in turn is related to the number of its protonated amine groups.\[10\]

Decreasing the MW of this polymer causes its antibacterial activity to increase on the gram-negative bacteria and reduce the activity on the gram-positive bacteria.\[6,11,12\] Also, reduction in chitosan MW increases its solubility in aqueous solution in natural pH, decreases its solution viscosity, and makes it more suitable for the biological application.\[13,14\]

The traditional source for production of chitin and its derivative-chitosan is shells of crabs, shrimps, and krills which are wastes left from the processing of marine products. However, crustacean shell wastes can be limited and subject to seasonal supply. In recent years, the researchers have directed their attention to the production of chitin from fungi. In various structures of fungi such as the cell wall of mycelium, stem, and spores of ascomycetes, zygomycetes, basidiomycetes, and phycomycetes, there exists chitin.\[15\]

Ganoderma lucidum is a famous medicinal fungus and is one of the traditional Asian medicines that have been used for centuries.\[16\] Therefore, metabolites produced by G. lucidum are safe and can be utilized in medicine and food industry. Over 300 species of Ganoderma have been reported in various places of the world.\[17\] In Iran, seven species of Ganoderma have been reported in north forests. One of these species of Ganoderma is G. lucidum.\[18–21\]
Fruiting body, mycelium, and spores of *G. lucidum* have about 400 bioactive compounds that can be used in medicine in order to improve the quality of life and prevent and treat the diseases.\[17,22\] According to results of the previous studies, fungal chitosan offers significant advantages, the most important of which are crustacean waste sources that are restricted by seasons and locations, but fungal mycelium can be produced by a fermentation process in all seasons. The cell wall material of fungus can be recovered by simple chemical procedures. The process of fungal chitosan extraction does not need demineralization treatment, because the fungal mycelia have a lower level of inorganic materials compared to crustacean wastes. Moreover, the fungal chitosan is free of heavy metal contents especially mercury which is high in marine organisms. The physico-chemical properties of fungal chitosan are relatively constant compared to crustacean wastes. Moreover, the fungal chitosan can be produced by a fermentation process in all seasons. Controlling the product quality is also difficult during its cultivation. Fungi mycelia can be cultivated by submerged or solid fermentation. These processes, especially submerged cultivation, are rapid. In addition, the produced mycelial in each batch of these processes is homogeneous.\[14,23,24\]

The published reports indicated that different factors such as the composition of the culture media, temperature, incubation time, pH, inoculum size, harvest time, ambient oxygen content, relative humidity, ambient water content, light intensity, and fungal species affect the amounts of the produced mycelium, chitin, and chitosan by fungus in fermentation process.\[25-30\] Statistical designs of experiments, such as response surface method (RSM) are a powerful and effective method for obtaining optimum conditions of processes.\[31,32\]

This study aimed at optimizing the culture medium for the chitin production from Iranian *G. lucidum*, which was isolated from forests in Nowshahr city in Northern Iran using the response surface method. The obtained chitin was converted into chitosan by deacetylation. Finally, the properties of the produced chitosan were compared to the commercial chitosan.

**Materials and methods**

**Preparation and maintenance of fungal samples**

The mushroom *G. lucidum*, native to Iran, was isolated from forests of Nowshahr in northern Iran. This fungus was identified in our previous study with GenBank accession number: KX765192.\[33\] The Chinese *G. lucidum* was also donated by the Ferdowsi University of Mashhad, a state university in Northeastern Iran. Furthermore, potato dextrose agar (PDA) was used for culture maintenance and stored at 4°C.

**Growth**

The inoculum was prepared in potato dextrose broth (PDB) at 25°C and 132 rpm for 7 days. After the adjustment of pH and inoculation, the growth and production medium, PDB, was incubated for 14 days at 25°C and 132 rpm. The mycelium was separated by the centrifugation of fermentation broth at 10,000 rpm for 10 min and washed twice with distilled water. The separated mycelia were dried using freeze drying. Furthermore, the dry weight of the produced mycelia was measured.

**Chitin extraction**

The powder of mycelia was added to NaOH 4 M with the ratio of 1:20 W/V (1 g of powder of mycelia was added to 20 mL NaOH 4 M) and was incubated at 90°C for 3 h. After centrifugation at 10,000 rpm for 20 min, sediment was washed twice with deionized water. This sediment was chitin, which was dried by freeze dryer. Additionally, the weight was measured.\[24\]

**Deacetylation of chitin and chitosan production**

Chitosan was produced by deacetylation of chitin. For this purpose, chitin was mixed with concentrated sodium hydroxide (45%) with a ratio of 1:15 W/V (1 g chitin was added to 15 mL NaOH 45%) and was incubated for 4 h at 90°C. After centrifugation at 10,000 rpm and after 15 min, the supernatant was thrown away. Moreover, the sediment, which was chitosan, was washed with distilled water until it became neutral. Then, it was dried by freeze dryer.

The degrees of deacetylation of chitin and chitosan that had hydroxyl as well as the first and second amide groups were determined using FTIR.\[34-36\] Deacetylation degree of the sample was determined using Eq. (1).\[36\]

\[
DD = 100 - \left[ \left( \frac{A_{1655}}{A_{3450}} \right) \times 115 \right]
\]  

where \(A_{1655}\) is adsorption pick of the first amide in 1655 cm\(^{-1}\) as the amount of N-acetyl group and \(A_{3450}\) is adsorption pick of hydroxyl (–OH) group in 3450 cm\(^{-1}\).

**Statistical optimization of chitin production**

Several environmental and processing variables such as the kind and concentration of media components, temperature, pH, aeration, and inoculum age and size affected the chitin production from *G. lucidum*. The published reports showed that two variables of the initial pH and inoculum size are more effective on the growth of *G. lucidum* and chitin production in a determined medium.\[25,29,30\] The RSM using central composite design (CCD) was employed to achieve an optimum condition. A \(2^2\)-factorial central composite experimental design, with four axial points, seven center points, and the total number of 13 experiments, was applied for the optimization of the variables.\[31\] The levels of the studied variables were determined using preliminary experiments and the results of the studies conducted by other researchers (Table 1).\[13,24,25,28\] Table 2 shows the CCD for two variables. The obtained results were analyzed using Design Expert 7.0 software.
Characterization

Microscopic morphological

The morphology of the produced samples was studied using field emission scanning electron microscope (FE-SEM). For this purpose, the samples were powdered. Some powders were utilized as thin layers attached on a special surface and were coated with thin layer of gold using DS-sputtering under relative vacuum condition and 24 mA. Then, the image was prepared under the rapid voltage of 30 kV.

XRD analysis

The produced chitosan by Iranian G. lucidum was characterized by X-ray diffraction (XRD) technique using an X-ray diffractometer (X’Pert PRO MPD, PANalytical Company, Almelo, The Netherlands) in the diffraction angle (2θ) range from 3.01 to 79.97, using CuKx radiation (k = 1.5406 Å) generated at 40 kV and 40 mA.

FTIR analysis

FTIR is a usual method for identification of unknown materials based on functional groups. The infrared spectroscopy can determine the degree of deacetylation of chitosan that has hydroxyl as well as the first and second amine groups. For this analysis, 15 mg of powder samples were mixed with KBr powder and was pressed under the pressure. Also, a tablet with 2 mm diameter was prepared. The spectra of the prepared sample were determined using FTIR (Brucker, EQUINOX55 model was made in Germany) in 400–4000 cm\(^{-1}\) range of wavenumber.\[35\]

Antibacterial properties

In disk diffusion method, the solution of chitosan with 1%, 2%, and 3% concentration was prepared using 1% V/V acetic acid acetic. The disks were put in chitosan solution for 24 h. They were extracted and dried in oven at 37°C for 1 h and put on cultured plate.\[37–39\]

For standard determination of the number of bacteria in laboratory, the standard solution of 0.5 McFarland was used. Muller–Hinton agar media were also used for microbial sensitivity test. A gram-positive bacterium, Staphylococcus aureus, and a gram negative bacterium, Escherichia coli were cultured as pour plate. After the maximum time of 15 min after culture, the disks must have been put on culture surface plates on the sterile condition. Then, the plates were incubated for 18–24 h at 37°C.

Results and discussion

Optimization of chitin production

For optimizing chitin production, two variables that were selected based on the preliminary experiments and other published reports were studied by RSM using CCD. The cell growth and chitin production were measured as responses. The obtained results are indicated in Table 2. The results were analyzed by Design Export software. These results are also shown in Tables 3 and 4 for cell growth and chitin production respectively. According to these tables, the first and second order main effects of two variables are significant with \(p < 0.05\) for two responses, cell growth and chitin production. However, the interaction between these two variables is not significant for both responses. ANOVA analysis showed that the predicted models are highly significant (\(p = 0.001\)) and these models can effectively predict the responses in different conditions of these two variables. The \(p\) value of lack of fit of models is not significant. This also verified that the predicted models fit with the experimental data. The goodness fit of the predicted models was confirmed using \(R^2\) of models. The value of determination of coefficients (\(R^2 = 0.972\) for growth and \(R^2 = 0.977\) for chitin production) demonstrated that more than 97% of changeability in the responses could be described by models.

Figure 1 indicates normal plots of residuals for cell growth (CDW) and chitin production (P). These plots also verify the accuracy of the predicted models because the residuals of the experimental responses are very small and close to the predicted responses.

The predicted models obtained for cell growth and chitin production are as follows:

\[
\text{CDW}(\text{g/L}) = -13.759 + 5.587A + 1.076B + 0.043AB - 0.518A^2 - 0.089B^2
\]

\[
P(\text{g/L}) = -2.582 + 1.044A + 0.214B + 0.007AB - 0.096A^2 - 0.017B^2
\]

In these models, CDW is cell dry weight (g/L), \(P\) is chitin production (g/L), \(A\) is pH, and \(B\) is inoculum size (V/V%). The coefficient of variables in the predicted models also

### Table 1. Selected levels of factors for the central composite design of growth and chitin production of G. lucidum.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coded value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-x)</td>
<td>(-1)</td>
</tr>
<tr>
<td>(0)</td>
<td>(+1)</td>
</tr>
<tr>
<td>(+x)</td>
<td></td>
</tr>
<tr>
<td>Initial pH</td>
<td>3.4</td>
</tr>
<tr>
<td>Inoculum size (%V/V)</td>
<td>2.06</td>
</tr>
</tbody>
</table>

### Table 2. The optimization of growth and chitin production of Iranian G. lucidum using central composite design.

<table>
<thead>
<tr>
<th>Trial</th>
<th>A: Initial pH</th>
<th>B: Inoculum size (%V/V)</th>
<th>CDW (g/L)</th>
<th>Chitin production (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>3.5</td>
<td>3.25</td>
<td>0.63</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>3.5</td>
<td>3.52</td>
<td>0.68</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>10.5</td>
<td>3.66</td>
<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>10.5</td>
<td>4.83</td>
<td>0.89</td>
</tr>
<tr>
<td>5</td>
<td>3.4</td>
<td>7</td>
<td>3.57</td>
<td>0.69</td>
</tr>
<tr>
<td>6</td>
<td>7.6</td>
<td>7</td>
<td>4.16</td>
<td>0.78</td>
</tr>
<tr>
<td>7</td>
<td>5.5</td>
<td>2.06</td>
<td>3.95</td>
<td>0.74</td>
</tr>
<tr>
<td>8</td>
<td>5.5</td>
<td>11.94</td>
<td>4.09</td>
<td>0.76</td>
</tr>
<tr>
<td>9</td>
<td>5.5</td>
<td>7</td>
<td>6.25</td>
<td>1.17</td>
</tr>
<tr>
<td>10</td>
<td>5.5</td>
<td>7</td>
<td>5.85</td>
<td>1.12</td>
</tr>
<tr>
<td>11</td>
<td>5.5</td>
<td>7</td>
<td>6.28</td>
<td>1.2</td>
</tr>
<tr>
<td>12</td>
<td>5.5</td>
<td>7</td>
<td>6.1</td>
<td>1.15</td>
</tr>
<tr>
<td>13</td>
<td>5.5</td>
<td>7</td>
<td>6.18</td>
<td>1.16</td>
</tr>
</tbody>
</table>
showed the significance of main and cubic effects of pH and inoculum size on CDW and $P$. The coefficient of $AB$ was very small in both models, which indicated that the interaction between pH and inoculum size was not significant in both models.

Figures 2 and 3 show the three dimensional plots that represent the cell growth and chitin production with respect to pH and inoculum size. According to these figures, each of these two responses reached its maximum level at about the mid of the studied range of variables. Significant effects of variables are clearly seen in these figures. In terms of pH and inoculum size, circle contour plots of CDW and $P$ show that interaction between these variables is not significant.[32]

The optimum conditions of variables were estimated using design expert software. The software estimated the optimum condition based on the predicted model. The maximum cell dry weight and chitin production were estimated in pH = 5.7 and inoculum size = 7.4. The accuracy of the estimated optimum condition was surveyed by applying the experiment in this condition. In the optimum condition, cell dry weight was 5.91 g/L and chitin production was 1.08 g/L that had 96% and 93% agreement with prediction optimum responses, respectively.

The optimum pH = 5.7 was similar to the results reported by Zhou et al.[29] with optimum pH = 5.5 for a Chinese $G$. lucidum and the findings reported by Zhong-hua et al.[30] with optimum pH = 6 for $G$. applanatum. These findings verified the optimum pH that was obtained for Iranian $G$. lucidum. According to the results of the present study as well as the reports of the existing literature, $G$. lucidum, like other fungi, prefers to grow in acidic conditions. The pH of the medium always influences the physiology of a

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### Table 3. Analysis of the central composite design results for the growth of Iranian $G$. lucidum.

<table>
<thead>
<tr>
<th>Term</th>
<th>Sum of square</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>$F$ value</th>
<th>$p$ value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>16.95</td>
<td>5</td>
<td>3.39</td>
<td>48.78</td>
<td>0.001</td>
<td>Significant</td>
</tr>
<tr>
<td>$A$</td>
<td>0.65</td>
<td>1</td>
<td>0.65</td>
<td>9.3</td>
<td>0.0186</td>
<td></td>
</tr>
<tr>
<td>$B$</td>
<td>0.46</td>
<td>1</td>
<td>0.46</td>
<td>6.61</td>
<td>0.0369</td>
<td></td>
</tr>
<tr>
<td>$AB$</td>
<td>0.2</td>
<td>1</td>
<td>0.2</td>
<td>2.91</td>
<td>0.1316</td>
<td></td>
</tr>
<tr>
<td>$A^2$</td>
<td>9.45</td>
<td>1</td>
<td>9.45</td>
<td>135.91</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>$B^2$</td>
<td>8.23</td>
<td>1</td>
<td>8.23</td>
<td>118.43</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Residual error</td>
<td>0.49</td>
<td>7</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack-of-fit</td>
<td>0.37</td>
<td>3</td>
<td>0.12</td>
<td>4.13</td>
<td>0.102</td>
<td>Not significant</td>
</tr>
<tr>
<td>Pure error</td>
<td>0.12</td>
<td>4</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. Analysis of the central composite design results for chitin production of Iranian $G$. lucidum.

<table>
<thead>
<tr>
<th>Term</th>
<th>Sum of square</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>$F$ value</th>
<th>$p$ value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>0.59</td>
<td>5</td>
<td>0.12</td>
<td>61.39</td>
<td>0.001</td>
<td>Significant</td>
</tr>
<tr>
<td>$A$</td>
<td>0.017</td>
<td>1</td>
<td>0.017</td>
<td>8.73</td>
<td>0.0213</td>
<td></td>
</tr>
<tr>
<td>$B$</td>
<td>0.012</td>
<td>1</td>
<td>0.012</td>
<td>6.15</td>
<td>0.0422</td>
<td></td>
</tr>
<tr>
<td>$AB$</td>
<td>0.0049</td>
<td>1</td>
<td>0.0049</td>
<td>2.54</td>
<td>0.1552</td>
<td></td>
</tr>
<tr>
<td>$A^2$</td>
<td>0.33</td>
<td>1</td>
<td>0.33</td>
<td>169.4</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>$B^2$</td>
<td>0.3</td>
<td>1</td>
<td>0.3</td>
<td>157.88</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Residual error</td>
<td>0.014</td>
<td>7</td>
<td>0.00193</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of fit</td>
<td>0.01</td>
<td>3</td>
<td>0.00374</td>
<td>3.97</td>
<td>0.1081</td>
<td>Not significant</td>
</tr>
<tr>
<td>Pure error</td>
<td>0.0034</td>
<td>4</td>
<td>0.00085</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Normal plots of residuals for (A) cell growth (CDW) and (B) chitin production ($P$).
microorganism by affecting nutrient desirability, enzyme activity, oxidative–reductive reactions, and most importantly cell membrane morphology.[13,40]

The optimum inoculum size, which was obtained in the current study, was (7.4%) approximately equal to the results of the study that was conducted by Zhong-hua et al.[30] on G. applanatum. In fact, the inoculum size influenced the fermentation profile. The lag phase of profile decreased with increasing of inoculum size. The final biomass density was also affected with regard to the inoculum size. Also, inoculum size affected pellet size distribution and the metabolite production.[25]

**Comparison of Chinese and Iranian G. lucidum**

Chinese and Iranian G. lucidum were cultured in optimum conditions of triplicate. The cell growth and chitin production for each sample were measured. The average responses
indicated that the CDW of Chinese *G. lucidum* was 5.67 g/L and the CDW of Iranian *G. lucidum* was 5.91 g/L. In fact, there was a significant increase in the CDW of Iranian *G. lucidum*. The chitin production of Chinese *G. lucidum* was 1.06 g/L that did not show any significant difference from chitin production of Iranian *G. lucidum*. The amount of metabolite production besides environmental and process conditions depended on the type of organism. The obtained results indicated that growth of Iranian *G. lucidum* was higher than the growth of Chinese *G. lucidum*; whereas the content of chitin in the mycelium of Iranian *G. lucidum* was lower than other polymers.

**Characterization of the produced chitin and chitosan**

**Microscopic morphology**

The morphology of two samples of the produced chitin and chitosan was studied by FE-SEM. The images of FE-SEM of chitin and chitosan with different magnifications are shown in Figure 4. According to these images, the chitin and chitosan samples are spheres with approximately smooth surface. When the samples were dried, the aggregation of particles was seen because of the attachment of particles. Also, this figure shows that the size of the produced fungal chitin particles is in the range from 30 to 50 nm and the size of the produced fungal chitosan particles is in the range from 20 to 40 nm. Also, the size of chitin particles is slightly greater than that of chitosan particles.

**XRD analysis**

The XRD pattern of chitosan that was obtained from Iranian *G. lucidum* illustrates two individual broad diffraction peaks at 2θ = 10 and 20, which are representative fingerprints of semicrystalline chitosan as shown in Figure 5. The peaks around 2θ = 10 and 2θ = 20 are related to crystal I and crystal II in chitosan structure and are characterized by a high degree of crystallinity to the produced chitosan.

**FTIR analysis**

The FTIR spectrum was mainly used for qualitative evaluation of chitin and chitosan characterization and determination of their main functional group. Also, FTIR could determine the degree of acetylation/deacetylation of chitin and chitosan. Figure 6 displays FTIR analysis and compares the produced fungal chitin and chitosan with the commercial chitosan (molecular weight 1.506 × 10⁵ kDa and degree of deacetylation 80%, Sigma, Japan). As illustrated in Figure 6, the FTIR spectrum of the fungal chitosan is similar to the FTIR spectrum of the commercial chitosan. The spectra reveal the presence of a broad band at 3,450 cm⁻¹, which corresponds to the vibrational stretching of the hydroxyl groups. Also, the C–H stretching band at 2,885 cm⁻¹ is seen in the spectra. The band at 1,655 cm⁻¹ corresponds to the amide I stretching of C=O, while the band at 1,558 cm⁻¹ corresponds to the stretching or N–H deformation of amide II. Moreover, the band at 894 cm⁻¹ corresponds to the symmetrical deformation of amide III. The sharp peak at 1,427 cm⁻¹ can be attributed to the symmetric deformation of –CH₃. In addition, the band at 1,072 cm⁻¹ presents the internal vibration of chitin cycle, which corresponds to C–O–C group.

In the deacetylation of chitin, the band at 1,655 cm⁻¹ decreased, while the band at 1,558 cm⁻¹ increased because of the presence of –NH₂ group. In fungal chitosan spectrum, the band at 1,558 was higher than absorbance compared to the band at 1,655 cm⁻¹. This finding indicated that the deacetylation of chitin has been effectively conducted. To determine the degree of deacetylation, the band of amid I group at 1,655 and the stretching band of hydroxyl group are important. According to FTIR spectra of fungal and commercial chitosan, and Eq. (1), the degrees of deacetylation of fungal and commercial chitosan are 79% and 70%, respectively. This finding showed that the produced fungal chitosan had suitable quality for medical application. These Deacetylation degrees also indicated that the ratio of acetyl glucosamine groups to amine groups was an
important parameter for specific application and solubility of chitosan. Deacetylation degree, as the most important chemical parameter, affected the efficiency of chitosan for different uses. Indeed, some researchers pointed out that the term “chitosan” can be used, when the degree of deacetylation is higher than 70%. However, most researchers use “chitosan”, when the deacetylation degree of chitin is higher than 50%.

Figure 5. XRD analysis of the chitosan produced from Iranian G. lucidum.

Figure 6. FTIR spectrum of chitin and chitosan produced from Iranian G. lucidum and the commercial chitosan.
The FTIR spectra of fungal chitin and chitosan also showed that different vibrations occurred after deacetylation. By decreasing C-O functional groups, at 1655 cm\(^{-1}\), the vibration (absorption) was reduced. Also, the absorption peak at 849 cm\(^{-1}\) increased due to functional groups of \(-\text{NH}_2\). The most important difference between chitin and chitosan FTIR spectra is seen at 3270–3450 cm\(^{-1}\). Probably, the reason is the existence of the higher number of \(-\text{NH}\) and \(-\text{OH}\) functional groups in chitosan compared to chitin. The peak value at 2885 cm\(^{-1}\) in chitin was higher than chitosan due to the higher C–H bonds in chitin.

The comparison between the fungal and the commercial chitosan FTIR spectra indicated that fungal chitosan had a higher deacetylation degree than the commercial chitosan. Bio properties of chitosan relied on its cationic properties and deacetylation degree determined this property. Deacetylation degree, crystallization, and molecular weight were three structural parameters in chitosan that affected its solubility, degradability, and mechanical resistance.

**Antimicrobial properties**

Antimicrobial properties of chitosan that obtained from Iranian *G. lucidum*, was examined by disk diffusion method using a gram-positive bacteria, *S. aureus*, and a gram-negative bacteria, *E. coli*. Figure 7 shows the results of antibacterial tests. Three concentrations of chitosan, including 1%, 2%, and 3% w/w, were studied. The results showed that the zone of growth inhibition for *E. coli* was only observed in disk with 3% concentration of chitosan, whereas disks with 2% and 3% chitosan concentrations showed the zone of growth inhibition for *S. aureus*. These results revealed that *E. coli* is more resistant to chitosan than *S. aureus*. In addition, gram-positive bacteria were significantly more sensitive to the antimicrobial activity of chitosan compared to gram-negative ones. This difference in sensitivity is mainly recognized to the different buildings of their cell covers. The term cell cover includes both the cell wall and the cytoplasmic membrane of a bacterial cell; it also contains the semi-permeable lipid bilayer of gram-negative bacteria. This layer is an additional diffusion obstacle for gram-negative bacteria.\(^{[10]}\)

None of these two bacteria showed sensitivity in 1% of chitosan concentration. Indeed, this result indicated that the low concentration of chitosan had not any antibacterial effect. This observation verifies the results of other reports.\(^{[12]}\)

**Conclusion**

The Mushroom pharmaceutical *G. lucidum* is an excellent source of polysaccharides, proteins, and secondary metabolites. Chitin forms about 50% of the cell wall of these fungi. In the current study, an Iranian *G. lucidum* was introduced for chitin production. The growth of Iranian *G. lucidum* and its chitin production were optimized using RSM. In the optimum condition (initial pH = 5.7 and inoculum size = 7.4%), the CDW and chitin production were 5.91 and 1.08 g/L, respectively. Characterization of the produced chitosan using FTIR, XRD analysis, and the antimicrobial test indicated that this polymer was very similar to the commercial chitosan. Furthermore, the deacetylation degree of the produced fungal chitosan (79%) was high. Therefore, it seems that it is appropriate for the numerous applications. Regarding the source of the produced chitosan, i.e. *G. lucidum*, which has been used in the traditional medicine for centuries; this biopolymer is safe, nontoxic, and suitable for medical applications.

### References


[13] Vaingankar, P.N.; Juvekar, A.R. Fermentative Production of Mycelial Chitosan from Zygomycetes: Media Optimization and

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**Figure 7.** Antibacterial effect of chitosan produced from Iranian *G. lucidum* at different concentrations (a: 1, b: 2, c: 3, and d: 0%) using disk diffusion method: (A) *E. coli*; (B) *S. aureus*. 


