The Preparation and Bioactivity Research of Agaro-Oligosaccharides

Hai-Min Chen¹, Li Zheng² and Xiao-Jun Yan¹*

¹Key Laboratory of Marine Biotechnology, Post Box 71, Ningbo University, Ningbo 315211, P. R. China
²Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, P. R. China

Received: June 4, 2004
Revised version: September 16, 2004
Accepted: November 22, 2004

Summary

Agaro-oligosaccharides were hydrolytically obtained from agar using hydrochloric acid, citric acid, and cationic exchange resin (solid acid). The FT-IR and NMR data showed that the hydrolysate has the structure of agaro-oligomers. Orthogonal matrix method was applied to optimize the preparation conditions based on α-naphthylamine end-labeled HPLC analysis method. The optimal way for oligosaccharides with different degree of polymerization (DP) was achieved by using solid acid degradation, which could give high yield and avoid solution neutralization process. Agaro-oligosaccharides with high purity were consequently obtained by activated carbon column isolation. Furthermore, the antioxidant and α-glucosidase inhibitory activity of three fractions were also investigated. The result indicated that 8 % ethanol-eluted fraction showed highest activity against α-glucosidase with IC₅₀ of 8.84 mg/mL, while 25 % ethanol-eluted fraction possessed excellent antioxidant ability.

Key words: agaro-oligosaccharides, orthogonal matrix, HPLC, acid, degradation, antioxidant, α-glucosidase

Introduction

Agar extracted from red seaweed has been used as food substance in China and Japan for a long time. From the structural point of view, agar is a linear sugar chain consisting of alternative galactose (3-O-linked β-D-galactopyranose) and anhydrogalactose (4-O-linked 3,6-anhydro-α-L-galactopyranose). Two forms of oligosaccharides can be formed depending on the moiety of end sugar, namely, agaro-oligosaccharides and neogaro-oligosaccharides (1). In recent years, versatile biological activities of agaro-oligosaccharides have been observed, and the structure and bioactivity relationship research also revealed that the functionality is directly correlated to the degree of polymerization (DP). The agaro-oligosaccharides with DP 2–4 possess the ability to suppress the production of the pro-inflammatory cytokine TNF-α and the expression of inducible nitric oxide synthase (iNOS), an enzyme associated with the production of NO (2). The oligosaccharides with DP 6–8 can elicit physiological response in algae (3). Therefore, these oligosaccharides have a potential to be developed into a highly safe functional ingredient in food, drink or pharmaceutical composition.

Some methods have been used to hydrolyze agar, such as enzymatic (4), physical (5), and chemical degradation (6,7). Enzymatic hydrolysis can be performed by two types of agarases, including β- and α-agarase (8). Most of the agarases isolated in recent years have been identified to be β-type, while the α-agarase has scarcely
been described. Therefore, this method is used primarily to get neoagaro-oligosaccharides. Moreover, enzyme system has the problems of low activity, low stability and productivity, which limit their wide application in industry. However, chemical degradation, especially acid hydrolysis, is available for industrial preparation because of its simplicity, rapidity, low cost and high yield.

Several acids have been used to hydrolyze agar (9), each of which focused primarily on the structural characterization of the polymer chain (10). However, quantitative study on the optimization of hydrolysis method is surprisingly scarce, possibly owing to the lack of quantitative measurement method. In the course of our studying program for bioactivities of agaro-oligosaccharides, it was found that an ordinary α-naphthylamine end-labeled HPLC analysis method enables us to tackle this hydrolysis optimization problem, which is the cornerstone for large scale of agaro-oligosaccharide preparation technology. Therefore, we established a precolumn labeling HPLC method for quantitative detection of agaro-oligosaccharides in our previous study (11).

In the present work, we selected three types of acids including hydrochloric acid, citric acid and solid acid to hydrolyze agar, and their products were also investigated based on this HPLC method. Orthogonal matrix method was employed to optimize the preparation conditions. The bioactivities including antioxidant activity and α-glucosidase inhibition activity for different agaro-oligosaccharide fractions were further evaluated.

Materials and Methods

Materials

1,1-diphenyl-2-picryl-hydrazyl (DPPH), Dowex 50 W × 2 cation exchange resin and α-glucosidase were purchased from Sigma (St. Louis, MO, USA). Other reagents were all of analytical grade and commercially available. HPLC analysis was performed on a Waters Millennium HPLC system with two Waters 515 pumps and a Waters 2996 photodiode array detector. Prior to HPLC, all solvents were degassed.

Experimental design

Hydrochloric acid, citric acid, and cationic exchange resin (solid acid) were used to hydrolyze agar. In order to investigate the relationships among variables of acid content, agar content, temperature, hydrolysis time, and the yield of agaro-oligosaccharides, as well as the ratio of oligosaccharides, the orthogonal matrix L9 (3^4) method was adopted (Table 1). After hydrolysis, the solutions were neutralized with 1 M NaOH, and lyophilized for further analysis.

Fractionation of hydrolysate

The hydrolysis solution (50 mL) was loaded onto an activated carbon (50 mesh) column (1.6 × 50 mm). The column was washed with 500 mL of water to remove salts and monosaccharides, then eluted sequentially with 5, 8, 15, 20 and 25 % aqueous ethanol. Each fraction was concentrated under reduced pressure and lyophilized.

Structure determination

The structure elucidation of agaro-oligosaccharide was performed by FTIR and NMR. The Fourier-transformed infrared spectra of KBr pellets of the oligosaccharides were recorded on the FTIR-8900 spectrophotometer. Scans were carried out over the range of 4000–450 cm\(^{-1}\).

The nuclear magnetic resonance (NMR) spectra were acquired on a Bruker DRX-300 NMR spectrometer. Samples were dissolved in D\(_2\)O and \(^1^3\)C NMR spectra of 4 % (\(m/V\)) solutions were recorded at 80 °C under 100.69 MHz. Proton decoupled \(^1^3\)C NMR chemical shifts were measured in parts per million. For \(^1^H\)-NMR, samples (7–10 mg) were dissolved in D\(_2\)O (0.5 mL), and spectra were recorded at room temperature using a spectral width of 5.7 kHz, 90° pulse and an acquisition time of 4.4 s for 144 scans.

Analysis of agaro-oligosaccharides

The quantitative analysis of agaro-oligosaccharides was carried out using α-naphthylamine end-labeled HPLC method (11). After the derivation agaro-oligosaccharides were analyzed in isocratic mode on a Waters symmetry C\(_8\) RP column (150×3.9 mm, 5 μm) at a flow rate of 0.5 mL/min with the mobile phase of 40 % MeOH in distilled water. The peak area of each oligosaccharide was calculated automatically when baseline correction was appropriate, and the contents of these oligosaccharides were calculated according to the content and peak area correlation of each individual oligosaccharide, i.e. agaro-biose, agaro-triose, agaro-hexose, agaro-octaose, agaro-decaose, which have been purified from gel chromatography.

Table 1. Experimental design of hydrolysis modes with three different acids

<table>
<thead>
<tr>
<th>Acid</th>
<th>w(acid)/%</th>
<th>T/K</th>
<th>t/h</th>
<th>w(agar)/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl degradation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.18</td>
<td>310</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0.37</td>
<td>323</td>
<td>3.5</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>0.55</td>
<td>353</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Citric acid degradation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.2</td>
<td>323</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0.35</td>
<td>343</td>
<td>3.5</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>363</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Solid acid degradation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>333</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>343</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>363</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

Determination of antioxidant activity of agaro-oligosaccharides

The level of antioxidant ability was determined by examining the DPPH radical scavenging activity (12). Three fractions of agaro-oligosaccharides eluted from activated carbon column were dissolved into 50 % ethanol. The amount of 0.15 mM of DPPH in ethanol containing 10 % of water was used as the colorimetric reagent. The volume of 1 μL of oligosaccharide solution was added into 100 μL of DPPH, and incubated at 25 °C for 30 min in darkness. The absorbance was measured at λ=492 nm using a microplate reader. Radical scavenging activity was calculated by the following equation:
where: \( A_{\text{blank}} \) is the absorption of blank sample \((t=0 \text{ min})\) and \( A_{\text{test}} \) is the absorption of test sample \((t=30 \text{ min})\).

**α-Glucosidase assay**

Enzyme activity was assayed according to the method by Kwon (I3). Briefly, yeast α-glucosidase \((50 \mu\text{g/mL})\) was dissolved in the \(0.1 \text{ M phosphate buffer (pH=7.2)}\). The amount of \(10 \text{ mM nitrophenyl-α-D-glucopyranoside} \) in the same buffer was used as a substrate solution. The reaction mixture contained \(40 \mu\text{L of phosphate buffer, 20 \muL of enzyme solution and 20 \muL of sample containing different concentration of oligosaccharides. The mixture was incubated for 5 min at 37 °C, then the reaction was started by adding 20 \muL of substrate solution. After incubation for another 30 min at 37 °C, the amount of 10 mM of nitrophenyl-D-glucopyranoside was dissolved in the 0.1 M glycine buffer (pH=10.4) were added to end the reaction. The absorbance at 410 nm was measured. The concentration showing 50% inhibition was determined from a plot of percent inhibition vs the concentration.

**Results and Discussion**

**The structure elucidation of acid hydrolyzed products**

To validate the HPLC method, the structure and degree of polymerization of agar-oligosaccharides should be identified firstly. Therefore, FTIR and NMR were used to elucidate the structure information of agar hydrolysate. The FTIR spectra showed a well-defined peak at about 930 cm\(^{-1}\) corresponding to 3,6-anhydrogalactose. There were no absorption bands around 1225 or 817 cm\(^{-1}\), which indicated that no sulfate group existed in the hydrolysate (Fig. 1).

The composition and structural information of solid acid hydrolyzed fragments were further investigated by \(^1\text{H-NMR and }^{13}\text{C-NMR spectroscopy. The typical de-}\)

shielded \(^1\text{H-NMR and }^{13}\text{C-NMR signals corresponding to the anomeric hydrogens and carbons were obtained and presented in Fig. 2. Assignments were based on the close similarity with literature values, and the interpretation of these signals was indicated in Table 2. The \(^{13}\text{C-NMR spectrum of an oligosaccharide is very consistent with those previously published for agarose series (10). It gives out two distinctive major anemic carbon signals (G and A), which were expected for the major disaccharide repeat unit, and the signals (G’nr and A’nr) that belong to the residues towards the nonreducing end of the agaro-oligosaccharides. The presence of these signals indicates the presence of floridean starch in this fraction, because all of the signals illustrate the galactose ring structures present in seaweed galactans (14,15). In addition, this }^{13}\text{C-NMR spectrum presents a major difference compared with the neoagaro-oligosaccharides. Any signals for 3,6-anhydrogalactose at the nonreducing ending were not observed (16). As for the neoagaro series, the chemical shifts of carbons of unit G-1’α and G-1’β were identical (92.4 and 96.4 ppm, respectively, with intensities in the ratio of 1:2). From these results, it is seen that the reaction product was a mixture of typical agaro-oligomers with galactose units at their reducing ends.**

**Optimization of acid hydrolysis conditions**

In order to investigate the effect of various parameters, such as the concentration of acid, temperature, hydrolysis time, and the concentration of agar on the yield and ratio of oligosaccharides, a three-level four-factor design including 9 experimental runs for each acid was conducted to optimize these conditions. After the statistical analysis, the order of effect of all factors on the yield of oligosaccharides and agarobiose could be determined by the magnitude order of R (Mix Dif). The results indicated that the temperature was the most significant factor. High temperature could promote the speed of hydrolysis, as well as increase the yield of oligosaccharides, while a further improvement of temperature would result in the oxidation of saccharides, and the amount of monosaccharide was also enhanced. In the same way, high concentration of acid accelerated the hydrolysis process, but also the degree of oxidation. Agar concentration is another important factor that affects the yield of agaro-oligosaccharides. High yield of oligosaccharides, especially of the high-DP-oligosaccharides,
such as octaose and decaose, could be obtained with 2 % agar. Lower concentration of agar (1 %) reduced the yield of oligosaccharides, but the percentage of agarobiose was higher.

To optimize each factor, the intuitive analysis based on statistical calculation was carried out. Table 3 summarizes the optimal conditions of the three acid hydrolysis forms, as well as the results under these conditions. The maximum yield of agaro-oligosaccharides (70.6 %) was obtained under the optimal conditions of 70 °C, 10 % of solid acid, 2 % of agar for 8 h. In its products, the high-DP-oligosaccharides were the main saccharides (containing 43.35 % of octaose and decaose), while the content of agarobiose was low (22.66 %) (Fig. 3). Comparing the three degradation forms, HCl hydrolysis was the best way to obtain oligosaccharides with DP lower than 6. Under the conditions of 80 °C, 0.37 M HCl and 1 % of agar for 5 h, the maximum ratio of agarobiose was achieved at 65.43 %. To obtain the optimization levels for agarotetraose and agarohexaose, the optimum conditions were 50 °C, 0.55 M HCl and 1.5 % of agar for 2 h. Citric acid was another acid used to hydrolyze agar. In our assay, a very small amount of oligosaccharides was detected by using this acid. Even under the optimal conditions, the yield of oligosaccharides was only 18.23 %. However, the highest ratio of agarooctaose and agarodecaose (80.25 %) was observed when citric acid was used.

After the hydrolysis, it is remarkable to observe a large quantity of salt in the products of HCl and citric acid degradation. Therefore, the use of these two acids will inevitably cause the problem of desalting, which is a disadvantage in a large scale preparation and in the subsequent bioactivity research. On the other hand, by using the solid acid degradation, the free proton linked to the cationic exchange resin is scarcely released into the solution during the reaction, so it avoids the procedure of neutralization and desalting. From the data in
Table 3, it is indicated that under the conditions of 90 °C, 20 % of solid acid and 1 % of agar for 8 h, the yield of oligosaccharides and the ratio of agarobiose were all relatively high. In addition, under certain conditions, high yield and high purity of agarotetraose and agarohexaose could also be achieved. When these factors are combined, solid acid degradation was the optimal form for the preparation of agaro-oligosaccharides.

**Fractionation of agaro-oligosaccharides by activated carbon column**

Although with the optimal conditions the maximum yield and highest content of agaro-oligosaccharides could be obtained, the compositions in the hydrolysate were still complex. In order to improve the purity of oligosaccharides with different range of DPs, the hydrolysate needs to be fractionated further.

Activated carbon column has been used to isolate sugars since 1940s, the activated carbon column permits loading a large volume and high concentration of the sample without the disturbance of salt or other factors, which allows easy concentration and further fractionation. Therefore, it is suitable for the preparation of a large quantity of oligosaccharides with high purity. In our experiment, 5, 8, 10, 15, 20 and 25 % aqueous ethanol were used to elute the column, and the fractions were detected using HPLC method. As a result, agarobiose with high purity was present in the fractions eluted with 5 and 8 % ethanol (85.47 and 87.68 %); high
percentage of agarotetraose and agarohexaose (59.87 and 49.71 %) present in 10 and 15 % ethanol-eluted fraction; 56.67 % of agarooctaose and agarodecaose present in 25 % ethanol-eluted fraction (Table 4). The result illustrated that oligosaccharides with different DPs could be fractionated effectively by activated carbon. Here, we used the fractions eluted by 8, 15 and 25 % ethanol for the following bioactivity assays.

**Antioxidant activity**

Antioxidant compound is proved to be prospective and useful to prevent and treat the diseases with oxidative stress involvement such as liver injury, atherosclerosis, diabetes, and aging process (17,18). Agaro-oligosaccharides have been reported to display antioxidant effects, such as inhibiting the production of lipid peroxide radicals and inhibiting the production of NO. In this assay, we investigated the antioxidant activity of agaro-oligosaccharides with different ranges of DPs by DPPH method. DPPH scavenging activity is easy-use in vitro assay to assess the activity of various antioxidants through proton donation mechanism (19). It is indicated that 15 % ethanol-eluted fraction, which contains high percentage of agarotetraose and agarohexaose (49.71 %) exhibited highest DPPH scavenging activity (IC50=2.85 mg/mL), followed by 8 % fraction whose IC50 was 3.32 mg/mL, in which agarobiose was the primary component (87.68 %). The fraction containing 56.67 % of agarooctaose and agarodecaose showed lowest scavenging activity, and its IC50 was 4.93 mg/mL (Fig. 4).

**α-Glucosidase inhibitory activity**

Agar has been used as functional dietary fiber for diabetics in the health food market because of its non-nutritive nature. Nevertheless, its α-glucosidase inhibitory ability should also be considered for retarding the carbohydrate digestion. Some inhibitors with pseudosaccharide structure including acarbose and 1-deoxynojirimycin have been used very successfully in controlling diseases such as diabetes, obesity or hyperlipemia (20). In the course of our research program for bioactivities of agaro-oligosaccharides, we also investigated the α-glucosidase inhibitory ability through the use of a crude α-glucosidase preparation from human small intestine (21). The results are illustrated in Table 3 that the agaro-oligosaccharides from the hydrolysis of agar in different conditions have high inhibitory activity. Table 4 shows three different elutions in terms of ethanol concentration; 10, 15, and 25 %, which also gave high inhibitory activity. It can be seen that ethanol elution is a powerful mechanism for fractionation.

**Table 3. Optimal hydrolysis conditions of three different acids and corresponding results**

<table>
<thead>
<tr>
<th>Acid</th>
<th>Optimal conditions</th>
<th>w(agarooligosaccharide)/%</th>
<th>w(agarobiose)/%</th>
<th>w(agarotetraose+agarohexaose)/%</th>
<th>w(agarooctaose+agarodecaose)/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>80 °C, HCl 0.37 M, agar 2 % for 5 h</td>
<td>30.90</td>
<td>63.54</td>
<td>18.01</td>
<td>18.34</td>
</tr>
<tr>
<td></td>
<td>80 °C, HCl 0.37 M, agar 1 % for 5 h</td>
<td>29.48</td>
<td>65.43</td>
<td>11.40</td>
<td>23.17</td>
</tr>
<tr>
<td></td>
<td>50 °C, HCl 0.55 M, agar 1.5 % for 2 h</td>
<td>24.48</td>
<td>25.30</td>
<td>59.36</td>
<td>15.34</td>
</tr>
<tr>
<td></td>
<td>37 °C, HCl 0.18 M, agar 1 % for 3.5 h</td>
<td>12.15</td>
<td>5.27</td>
<td>22.40</td>
<td>72.33</td>
</tr>
<tr>
<td>Citric acid</td>
<td>90 °C, citric acid 0.5 M, agar 2 % for 3.5 h</td>
<td>18.23</td>
<td>40.66</td>
<td>29.67</td>
<td>28.66</td>
</tr>
<tr>
<td></td>
<td>90 °C, citric acid 0.35 M, agar 1.5 % for 2 h</td>
<td>12.11</td>
<td>41.96</td>
<td>53.01</td>
<td>5.02</td>
</tr>
<tr>
<td></td>
<td>70 °C, citric acid 0.35 M, agar 2 % for 5 h</td>
<td>10.12</td>
<td>5.42</td>
<td>44.98</td>
<td>49.60</td>
</tr>
<tr>
<td></td>
<td>50 °C, citric acid 0.5 M, agar 1 % for 3.5 h</td>
<td>4.55</td>
<td>3.52</td>
<td>16.73</td>
<td>80.25</td>
</tr>
<tr>
<td>Solid acid</td>
<td>70 °C, solid acid 10 %, agar 2 % for 8 h</td>
<td>70.6</td>
<td>22.66</td>
<td>33.99</td>
<td>43.35</td>
</tr>
<tr>
<td></td>
<td>90 °C, solid acid 20 %, agar 1 % for 8 h</td>
<td>33.2</td>
<td>57.80</td>
<td>34.87</td>
<td>7.33</td>
</tr>
<tr>
<td></td>
<td>90 °C, solid acid 10 %, agar 1 % for 6 h</td>
<td>36.58</td>
<td>27.26</td>
<td>42.53</td>
<td>30.21</td>
</tr>
<tr>
<td></td>
<td>60 °C, solid acid 10 %, agar 2 % for 6 h</td>
<td>15.3</td>
<td>3.21</td>
<td>22.78</td>
<td>74.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>φ(ethanol in elution)/%</th>
<th>w(agarobiose)/%</th>
<th>w(agarotetraose and agarohexaose)/%</th>
<th>w(agarooctaose and agarodecaose)/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>85.47</td>
<td>8.11</td>
<td>6.42</td>
</tr>
<tr>
<td>8</td>
<td>87.68</td>
<td>1.9</td>
<td>10.42</td>
</tr>
<tr>
<td>10</td>
<td>20.83</td>
<td>59.87</td>
<td>19.29</td>
</tr>
<tr>
<td>15</td>
<td>39.82</td>
<td>49.71</td>
<td>10.46</td>
</tr>
<tr>
<td>20</td>
<td>14.30</td>
<td>43.28</td>
<td>42.42</td>
</tr>
<tr>
<td>25</td>
<td>10.19</td>
<td>33.14</td>
<td>56.67</td>
</tr>
</tbody>
</table>

Table 4. Effect of elution conditions on the composition of agaro-oligosaccharides
cosidase inhibition activity of the three fractions. As a result, the 8 % ethanol-eluted fraction showed the highest activity in the three samples (IC$_{50}$=8.84 mg/mL). The 15 % ethanol-eluted fraction also had inhibitory ability (IC$_{50}$=14.05 mg/mL), while low activity was observed for the fraction containing 56.67 % of high-DP-oligosaccharides, and its IC$_{50}$ was greater than 40 mg/mL (Fig. 5).

Fig. 4. Effect of three fractions eluted from activated carbon column on the DPPH scavenging activity

Theoretically, a potential α-glucosidase inhibitor will always be a substrate analog which can mimic the transition state formed during the catalysis (21). In our assay, agarobiose showed high inhibitory activity. It may be possible that this disaccharide is a maltose structural analog. However, with the increase of DPs, it is much more difficult for oligosaccharides to bind with the α-glucosidase.

Conclusion

Since the discovery of multiple bioactivities for agar-oligosaccharides, an optimal condition for the preparation of the oligosaccharides has been necessary. In this work, an orthogonal design was used to optimize the hydrolysis conditions. The result indicated that solid acid hydrolysis was the optimal form to obtain agarooligosaccharides. The crude hydrolysate could further be purified by activated carbon column. The antioxidant and α-glucosidase inhibition assay indicated that the bioactivities were relative to the DP of oligosaccharides. Agarobiose possessed good α-glucosidase inhibitory activity, while agarotetraose and agarohexaose could scavenge the DPPH effectively.

Acknowledgements

This work was supported by grants from China Key Medicine Project (2003AA2Z3511), China Key Basic Research Project (G19992004), and Zhejiang Provincial Science and Technology Project (2003C29030).

References


Priprava i iskorištenje bioaktivnosti agaro-oligosaharida

Sažetak

Agaro-oligosaharidi dobiveni su hidrolizom agara pomoću solne i limunkske kiseline te kationske izmjenjivačke smole. Podaci dobiveni Fourier-transformiranim infracrvenim spektrom i nuklearnom magnetskom rezonancijom pokazali su da su hidrolizati agaro-oligomeri. Ortoqonalna matrica primijenjena je da bi se utvrdili optimalni uvjeti priprave hidrolizata koristeći postupak HPLC analize uzoraka koji su na svojim krajevima bili označeni α-naftilaminom. Primjenom kationske izmjenjivačke smole postignuto je optimalno iskorištenje oligosaharida različitog stupnja polimerizacije te izbjegnuta neutralizacija reakcijske otopine. Agaro-oligosaharidi velike čistoće dobiveni su nakon toga prolaskom kroz kolut s aktivnim ugljenom. Nadalje, ispitana je antioksidativna te inhibitorna aktivnost prema α-glukozidazi triju frakcija. Frakcija eluirana 8 %-tnim etanolom s IC₅₀=8,84 mg/mL pokazivala je najveću inhibiciju α-glukozidaze, a frakcija eluirana s 25 %-tnim etanolom imala je izvrsna antioksidativna svojstva.