Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 64 (2006) 274–281
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Microwave-assisted solid-phase oligosaccharides synthesis reaction and scavenging activity of synthetic product to free radical

Xin-Ming Li [a,b], Guo-Wei Le [a,b,*], Yong-Hui Shi [a,b]

[a] School of Food Science and Technology, Southern Yangtze University, Wuxi, Jiangsu 214036, People's Republic of China
[b] The Key Laboratory of Industrial Biotechnology, Ministry of Education, Southern Yangtze University, Wuxi, Jiangsu 214036, People's Republic of China

Received 11 November 2005; accepted 23 November 2005
Available online 20 January 2006

Abstract

Using phosphoric acid as the catalyst and glucose as substrate, microwave-assisted solid-phase bioactive oligosaccharide synthesis was studied. The optimum reaction conditions were that microwave power output of 800 W, microwave irradiation time 10 min, adding 30% water to initiate reaction, adding 10% heteropoly acid A as the catalyst. The yield ratio of product was 76.56%. The product was characterized with HPLC. The component and proportion of synthetic oligosaccharides included glucose 49.25%, maltose 10.10%, isomaltose 21.78%, maltotriose 2.15%, panose 10.08%, isomaltotriose 5.16%, tetrasaccharide and pentasaccharides 1.61%.

Free radical scavenging test demonstrated that synthetic oligosaccharides possessed property of scavenging oxygen and hydroxyl free radical.

Keywords: Microwave irradiation; Oligosaccharides synthesis reaction; Free radical; Vitamin C; Functional oligosaccharides

1. Introduction

The biological importance of oligosaccharides is increasing very rapidly. Their key role in most of the specific recognition phenomena is more and more underlined. Basing on lots of studies, the beneficial actions of oligosaccharides are assumed as following: inhibit growth of pathogenic and/or harmful bacteria, stimulate the immune functions, reduce level of cholesterol and risk of cancer, improve digestion and/or absorption of food ingredients/minerals and vitamins (Crittenden & Playne, 1996). In fact, their highly complex structure allows very specific interactions. But this complex structure is also a limitation to the possibility of developing efficient synthesis methods. The large scale application of such oligosaccharides in various fields (agrochemistry, cosmetics, food and feed) and particularly health applications (drug target, inflammatory phenomena, immuno-stimulation, etc.,)will need whole-newly synthesis methods (Monsan & Paul, 1995). Oligosaccharides are usually synthesized by enzymes, it takes a long time and has low yield. The current chemical synthesis of oligosaccharides usually have high cost and bothering purification steps (Flitsch, 2000).

In the electromagnetic spectrum, the microwave radiation region is located between infrared radiation and radio waves. Microwaves have wavelengths of 1 mm ± 1 m, corresponding to frequencies between 0.3 and 300 GHz. In inorganic chemistry, microwave technology has been used since the late 1970s, while it has only been implemented in organic chemistry since the mid-1980s. Since the mid-1990s, however, the number of publications has increased significantly. The demand for diverse compound libraries for screening in drug discovery and materials science promotes the development of new technologies for rapid parallel and combinatorial synthesis. One of those high-speed techniques is microwave-assisted organic synthesis, which has attracted a substantial amount of attention in the past few years (Lidstrom, Tierney, Wathey, & Westman, 2001). The main benefits of performing reactions under microwave (MW) irradiation conditions are the significant rate-enhancements, cleaner reaction profiles, inexpensive reagents and simple experimental/product isolation procedures and the higher product yields that can frequently be observed. Not surprisingly, these features have recently also attracted interest from the drug discovery and medicinal

* Corresponding author. Address: School of Food Science and Technology, Southern Yangtze University, Wuxi, Jiangsu 214036, People's Republic of China. Tel./fax: +86 510 5869236.
E-mail address: lgw@sytu.edu.cn (G.-W. Le).

0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.11.043

chemistry communities, for which reaction speed is of great importance (Larhed & Hallberg, 2001; Yadav & Reddy, 2002). The combination of microwave heating technology and combinatorial chemistry applications therefore seems a logical consequence of the increased speed and effectiveness offered by using microwave irradiation instead of conventional methods. Regardless of the origin/existence of a special microwave effect, it is extremely efficient and applicable to a broad range of practical syntheses (Larhed & Hallberg, 2001; Lidstrom et al., 2001). The first true example of microwave-assisted combinatorial synthesis was reported by Khmelnitsky and co-workers (Cotterill et al., 1998) in 1998 and described the rapid parallel synthesis of combinatorial libraries of functionalized pyridines in 96-well plates by microwave irradiation in a domestic microwave oven. After that, there are lots of pertinent reports to come from around the world. In a recent study by Stadler and Kappe (2001), using dedicated multimode microwave reactors for chemical synthesis, it was demonstrated that microwave irradiation can be effectively employed to attach aromatic carboxylic acids to chloromethylated polysaccharides resins via the cesium carbonate method. In the reaction significant rate-accelerations and higher loadings were also observed when the microwave-assisted protocol was compared with the conventional thermal method. Reaction times were reduced from 12 to 48 h with conventional heating at 80 °C to 5–15 min with microwave flash heating at 200 °C in 1-methyl-2-pyrrolidone, employing open glass vessels. Importantly, no degradation of the polysaccharides resins even under prolonged exposure to microwave irradiation at 200 °C was observed. Today, lots of facts demonstrate that microwave-assisted solid-phase organic synthesis technology is practical and high-efficient in organic combination industry.

The key informative role of oligosaccharides in biological systems is becoming more obvious every day (Sharon & Lis, 1993). This role has been completely underestimated for a while. This is mainly due to the difficulty to elucidate the structure of carbohydrate units and to synthesize standard derivatives efficiently. Rapid advance of oligosaccharides synthesis technology spurs people to focus more interest on its physiology function (Etzioni et al., 1992). In vitro studies have shown that soluble forms of sialyl Lewisx-, Lewisx- or Lewisa-containing oligosaccharides can inhibit selectin-mediated PMN-endothelial interaction (Cummings & Smith, 1992; Foxall et al., 1992). Yuan, Wang, Yao, and Chen (2005) reports that feruloylated oligosaccharides from wheat bran can effectively inhibit radical-induced hemolysis of erythrocytes in vitro. Ying Fan et al. (2005) report that acidic oligosaccharide sugar chain can induce cognitive improvement via its antioxidant activity. Przybyło, Litynska, Pochec (2005) support the view that oligosaccharides in the form of glycoprotein are involved in several steps of the metastatic process. There is also evidence emerging that oligosaccharides may have effects in the small intestine, particularly in enhancing calcium absorption (Cummings, Edmond, & Magee, 2004).

To the best of our knowledge, there is little literature on the microwave-assisted solid-phase oligosaccharides synthesis around the world so far. Our present study implemented oligosaccharides synthesis reaction under microwave-assisted solid-phase condition using glucose as substrate. The study mainly focused on the investigation of effect of four crucial synthesis factors—microwave power output, microwave irradiation time, catalyst adding quantity and initiator adding quantity, on the yield ratio of synthetic oligosaccharides. It has long been known that oxygen is toxic to aerobic organisms when they are exposed to it in concentrations greater than that of normal air, and the main cause of this toxicity is the intracellular reduction of O_2 into highly reactive chemical species or free radicals (Frank, 1985). To effectively scavenge free radical becomes increasingly important to maintain health. So, at last, scavenging activities of synthetic oligosaccharides to oxygen free radical and hydroxyl free radical were evaluated.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Glucose ($C_6H_{12}O_6$) (AR class) from Sinopharm Chemical Reagent Co., Ltd in Shanghai; Maltose ($C_{12}H_{22}O_{11}\cdot H_2O$) (BR class) from Shanghai Institutes for Biologocal Sciences, Chinese Academy of Sciences; Heteropoly acid A; Compound enzyme (glucose oxidase and peroxidase) from Institute of Microbiology, Chinese Academy of Sciences; superoxide dismutase (SOD) (AR class) and safremine (AR class) from ShangHai DongFen Biochemistry Institute. All other chemicals used were of analytical grade.

2.1.2. Apparatus

Panasonic NN-S563JF frequency conversion microwave oven from PANASONIC company; 722 spectrometry from Shanghai No. 3 instrument company; high pressure liquid chromatogram (HPLC), Waters400E America; High Speed Tabletop Centrifuge from Shanghai Anting Scientific Instrument Co., LTD; GDJF - 004 grating spectrophotometer from Shanghai No. 3 instrument company.

2.2. Preparation of synthetical oligosaccharides

Reaction substrate (glucose) was added into a closed glass container, and then water was also added into it to initiate and catalyze. Then both were mixed in the closed glass container and subjected to microwave irradiation for a specific time with stirring. After reaction finished, reaction mixture was cooled and dissolved into deionized water. The solution was then filtrated. The filtrated solution was allowed to dry at room temperature, then crushed up to afford synthetical product. The yield ratio of oligosaccharides may be calculated by the

following formulas:

the yield ratio of oligosaccharides (%)

= (the amount of initial glucose

− the amount of residual glucose)/the amount of initial glucose

× 100%.

2.3. Determining content of glucose

Total glucose concentration were measured by phenol–vitriol method with some slight modification. For the preparation of sample solution, 5 g phenol was dissolved in a little distilled water, shaken up and distilled water was then supplied to the volume of 100 ml with a final concentration of 5%. Then 0.2 ml sample was accurately taken and filled into a 10 ml cuvette. Then 0.4 ml, 5% phenol was added into the cuvette and shaken up. Then 2 ml vitriol was filled into the mixture in cuvette, shaken up and stand for 30 min at room temperature. At last, absorbance values were recorded by 722 spectrophotometer at the wavelength of 490 nm. At the same time, the wash solution was measured as blank control according to identical way. Elution curve may be protracted by absorbance values and elution solution volume.

2.4. HPLC conditions

The analytical column was a Spherisorb NH_2 (4.6× 250 mm. i.d.). The mobile phase was acetonitrile/water (70/30 v/v) with 1 mL min^{-1} flow rate. The column oven was kept at 30 °C. The volume of the injection was 10 μL.

The analytical column was a Sugarpark1 (6.5× 300 mm. i.d.). The mobile phase was water with 0.4 mL min^{-1} flow rate. The column oven was kept at 80 °C. The volume of the injection was 10 μL.

2.5. Free radical scavenging activity of synthetic oligosaccharides

2.5.1. Determining of oxygen free radical scavenging activity of synthetic oligosaccharides

Tris–HCl buffer solution of 4.5 ml, 0.05 mol L^{-1}, pH 8.2 was accurately taken, placed in cuvettes and pre-incubated at 25 °C for 20 min. Then three groups of different concentration of oligosaccharides solution or vitamin C solution were accurately taken 0.1 ml, respectively, and added into the cuvettes. Then 0.4 ml, 2.5 mmol L^{-1}, pyrogallol solution were also added into the cuvettes and mixed. After all cuvettes were incubated at 25 °C for 4 min, two drops of 8 mol L^{-1} HCL were immediately added into reaction solution for terminating the reaction. Absorptivity was recorded at 299 nm (distilled water was used to adjust to zero). Samples measured were replaced by 0.1 ml distilled water in blank group and at the same time, a reagent blank tube was set up. At last, scavenging rates were calculated.

2.5.2. Determining of Hydroxyl free radical scavenging activity of synthetic oligosaccharides

Hydroxide free radicals were generated by ferrous ion catalyzing H_2O_2. Hydroxide free radical could make safremine decolour. In the experiment, mannitol which is special scavenger to hydroxide free radicals was taken as positive control. A mixture of 1 ml, 0.15 mmol L^{-1}, pH 7.4 phosphor buffer solution, 1.00 ml, 40 g ml^{-1} safremine, 0.51 ml samples measured, 1.00 ml, 1.3% H_2O_2 (prepared refreshly), 1.00 ml, 0.954 mmol L^{-1} EDTA–FeII (prepared freshly) was incubating at 37 °C for 30 min, and absorptivity was then recorded at 520 nm. Samples measured were replaced by 0.50 ml distilled water in blank group. EDTA–FeII and Samples measured were replaced by 1.50 ml distilled water in control group. At the same time, a reagent blank tube was set up. At last, scavenging rates were calculated.

2.6. Statistics analysis

Statistics were performed utilizing SPSS for Windows version 10.0 (SPSS Inc., Chicago, IL). The data in this study were expressed as the mean ± SD ($n = 10$). Statistical comparisons between groups were performed by t-test. $P < 0.05$ was considered to be significant difference.

3. Results and discussions

3.1. Protracting of the standard curve of glucose

Twenty milligram glucose were accurately taken and added into a 500 ml capacity flask. Water was supplied to scale. 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 ml glucose solution prepared were, respectively, taken and in turn added into 20 cuvettes of 2 ml cubage, and then supplied water to 2.0 ml. Then 1.0 ml, 6% phenol and 5.0 ml H_2SO_4 were in turn added into cuvettes, shaken up and stood for 20 min at room temperature. At last, absorbance values were recorded by 722 spectrophotometer at the wavelength of 490 nm. At the same time, 2.0 ml wash solution was measured as blank control according to identical way. Standard curve of glucose may be protracted. Abscissa stand for glucose concentration (μg ml^{-1}). Vertical coordinates stand for OD value.

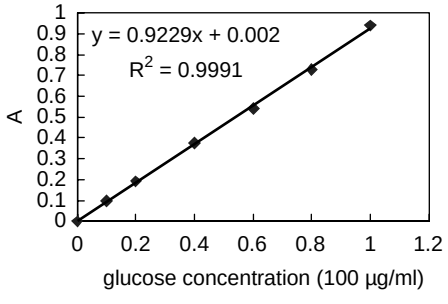

Fig. 1. The standard curve of glucose.

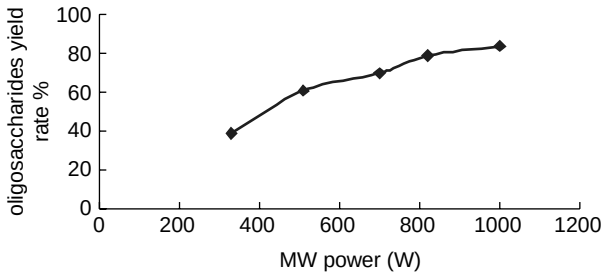

Fig. 2. Effect of microwave power on the yield of product.

Standard curve of glucose was shown in Fig. 1. Regression equation:

$$Y = 0.9229X + 0.002.$$

3.2. The conditions for polymerization

3.2.1. The effect of microwave power on the polymerization

This work had studied the effect of microwave power on glucose polymerization when different microwave power (200, 400, 600 and 800 W) was set under the reaction conditions as follows: 30% initiator, 10% catalyst, 10 min microwave irradiation. The results are demonstrated in Fig. 2.

Microwave possesses the character of penetrating materials and generating efficient internal heat-transfer, resulting in even heating throughout material irradiated (Strauss & Trainor, 1995). The mechanism of microwave generating heat may be primarily contributed to two major factors: dipolar polarization and conduction. (Gabriel et al., 1998; Giguere, 1986; Langa, 1997; Loupy, 1998; Mingos & Baghurst, 1991; Strauss & Trainor, 1995). When microwaves penetrates materials, the energy of microwaves can be absorbed by materials, which can further be translated into heat energy. The ability of a solvent to convert microwave energy into heat energy will be dependent not only the frequency, but also on the temperature (Baghurst & Mingos, 1992). When microwaves come into materials, the electromagnetic intensity and power will continuously weaken, but enhancing the power of microwaves can usually increase the capacity of penetration and the speed of the reaction. Singha, Sethia, Tewaria, Srivastavaa, and Sanghib (2003) observes that with MW power rising, yield of synthesical product will increase, which is in agreement with result of our experiment (Fig. 2)—during the microwave irradiation the yield ratio of synthesical oligosaccharides increased with elevating MW power. When the microwave power was over 400 W, the yield ratio of oligosaccharides could exceed 40%. In the experiment, we adopted 800 W MW power for economically accomplishing the synthesis.

3.2.2. The effect of microwave irradiation time on the polymerization

This experiment in turn adopted 2, 4, 6, 8, 10, 12, 14 min microwave irradiation to investigate the effect of microwave irradiation time on polymerization. Other experimental conditions were as follows: microwave power output of 800 W; 30% initiator; 10% catalyst.

Microwave heat involves a direct interaction with certain classes of absorbing molecules. This direct absorption can lead to localized introduction of energy to a region from the remote microwave source and raises the solution temperature (Galema , 1997). In chemical reactions, microwave irradiation time which may decide degree of rising temperature is an very important factor for the yield ratio of products. Generally speaking, the yield ratio of products rises as microwave irradiation time is extended. However, due to the rapid reactive speed in microwave irradiation, excessive irradiation may result in the products' coking or decomposability. It could be seen in Fig. 3 that the yield ratio of polymerization gradually went up with the microwave irradiation time's lengthening. It reached the peak value at 10 min. But after that, the yield ratio of oligosaccharides started to go down, and the colour of products evidently became darker. The reason for this may be assumed that products and glucose are broken up in such conditions as the overmuch irradiation time and higher temperature in the reactive system.

3.2.3. The effect of the adding quantities of initiators on the polymerization

Glucose in solid state system could not effectively absorb microwave, so it is necessary to add some initiators to help reactants to absorb microwave more effectively. For rapid microwave heating, solvent selection is important. Solvents must have a dipole to absorb the microwave energy and provide rapid super heating; therefore, polar solvents such as water, alcohols and acetic acid are optimal solvents whereas nonpolar solvents such as benzene and toluene are poor solvents (Houmes & zur Loye, 1997; Kappe, 2002, 2003; Larhed & Hallberg, 2001; Ley & Baxendale, 2002; Lidstrom et al., 2001; Loupy & Perreux, 2001; Wilson, Gilroy, Dolan, & Snyder, 2004), and may make reactions get on more successfully. In theory, the quantity of the initiator mostly affects initial speed of the reaction but does not affect the ongoing speed of the reaction (Wathey, Tierney, Lidstrom, & Westman, 2002). Once the solid state reaction is initiated, water which is one of the products in the reaction and behaves as a substitute for organic solvent at elevated temperature (Lidstrom et al., 2001) can easily absorb microwave to make the reaction continue. Some quantities of water behaving as initiator can also avoid the products' cooking or decomposability to help the reaction get on smoothly.

This experiment adopted 5, 10, 15, 20, 25, 30, 35, 40% water as initiators to study the effect of the quantities of

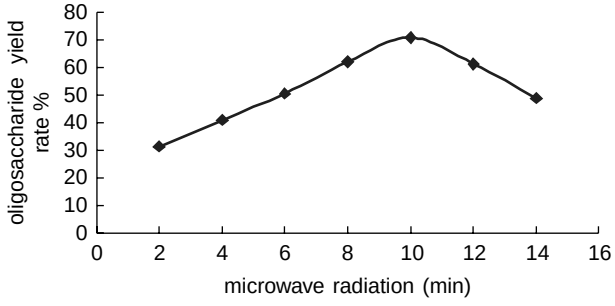

Fig. 3. Effect of microwave irradiation time on the yield of product.

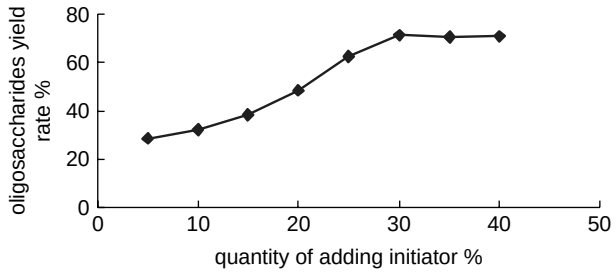

Fig. 4. Effect of initiator on the yield of product.

different initiators on polymerization. Other experimental conditions were as follows: 10% catalyst; 10 min microwave irradiation; microwave power output of 800 W.

Fig. 4 shows that the ratio of products rose as the quantity of initiators increased. It reached the peak value at adding 30% initiator. The polymerization of oligosaccharides is catalyzed by the acid. And it often happens in the polar-state (water-state) parts of the reactive mixture. When less initiator is added, the initiator cannot effectively absorb microwave energy. The reason for this is that microwave irradiation can accelerate the volatilization of water. Besides this, heteropoly acid used as catalyst in the experiment makes it effective to absorb on the surface of oligosaccharides which results in the polymerization of oligosaccharides. If the initiator is much less, acid and oligosaccharides could not mix together equably. Moreover, if the concentration of acid separates unequably, the dense parts easily results in coking under microwave irradiation, so that acid cannot play an effective part as catalyst.

Microwave irradiation also can promote the polymerization towards products. In nature, the polymerization of oligosaccharides is dehydration. Microwave irradiation generates energy from the inside of molecule and then water vapour rapidly generating from the inside of materials forms a kind of enormous impetus which drive water to move onto the surface with the state of water vapour and even sometimes produce very large grads of total pressures. The grads makes some water range on the surface of materials before it is boiled away. So water volatilizes very quickly. Rapid water volatilization is good for the polymerization towards products which raises the yield ratio of products. It is the merit that conventional heating method cannot be comparable to.

3.2.4. The effect of the quantity of catalyst on the polymerization

This experiment adopted heteropoly acid A as the catalyst. The results suggested that the different quantities of acid as the catalyst had different effect on the polymerization. We used different quantities of acid as the catalyst for the investigation of its effect on the polymerization. Other experimental conditions were as follows: 30% initiator; 10 min microwave irradiation; microwave power output of 800 W.

It could be shown from Fig. 5 that the yield ratio of oligosaccharides rose as the quantity of acid increased. It approached the peak value at adding 10% initiator. When adding more than 10% acid, coking happened to the products which led to the lower yield ratio. Generally speaking, in the solvent with higher concentration of oligosaccharides, acid can easily replace water in polar solvent or extend the distances between polyanions to enter liquid state. In some sense, acid absorbing a lot of polar molecule is similar to a kind of thick solvent whose state stands between solid and liquid. It is often called 'will-be liquid'. In the reactive system, owing to the shorter chains of glucose, it can sufficiently come up to and mix with acid, so that most of acid becomes 'will-be liquid' and only a little gets very dispersive solid particles. This kind of multiply-state system makes the osculant areas between catalyst and glucose increase to large extent so that it accelerates the polymerization of oligosaccharides (Seddon, 1996).

3.3. Analysis of synthetic product by HPLC

The reaction mixture was analysed by HPLC to detect the different sugars present. The samples were analysed on HPLC using a Sugarpark1 column at 80 °C (Fig. 6). Main constituents of synthetic oligosaccharides could be detected and measured as following: in turn monose, disaccharides, trisaccharides, tetrasaccharide, pentasaccharides and higher oligosaccharides according to peak order from right to left.

Basing on HPLC chromatogram (Fig. 6), the yield ratio of microwave-assisted solid-phase oligosaccharides synthesis using glucose as substrate may maximally reach 76.56%. The component percentages of synthetic product were in turn as following: disaccharides 20.81%, trisaccharides15.50%, tetrasaccharide10.84%, pentasaccharides 7.79%, higher oligosaccharides 21.63%. Fig. 7 was HPLC chromatogram ($SpherisorbNH_2$ column, 30 °C) of monose, disaccharides and trisaccharides. It could be observed that main constituents of synthetic oligosaccharides were in turn glucose (49.25%), unknown component (1.48%), maltose (10.10%), isomaltose 21.78%, maltotriose 2.15%, panose 10.08%, isomaltotriose 5.16% according to peak order. Because of poor analysis ability of $SpherisorbNH_2$ column to higher oligosaccharides than trisaccharides, concrete constituent and proportion of these higher oligosaccharides were unclear. The results suggested that synthetic oligosaccharides by microwave-assisted solid-phase organic synthesis technology were chiefly composed of disaccharides and trisaccharides.

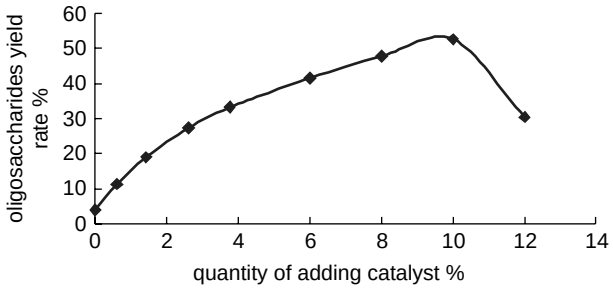

Fig. 5. Effect of catalyst on the yield of product.

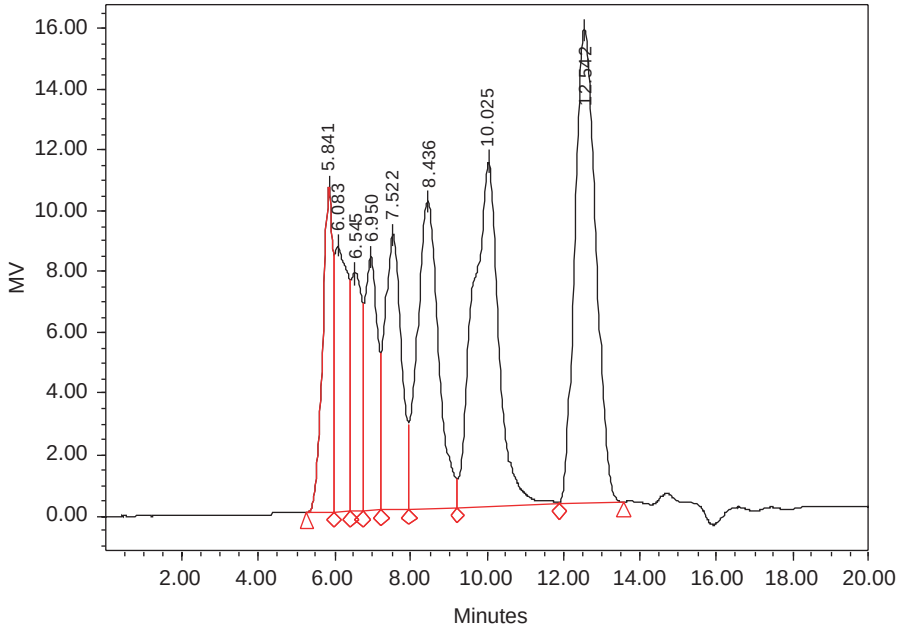

Fig. 6. The HPLC of the product (Sugarpark1 column).

3.4. Free radical scavenging activity of synthetic oligosaccharides

3.4.1. Oxygen free radical scavenging activity of synthetic oligosaccharides

Scavenging activities of synthetic oligosaccharides to oxygen free radical were demonstrated in Table 1. All data had been revised by reagent blank tube.

Results (Table 1) showed that scavenging activities of synthetic oligosaccharides for oxygen free radical generated in pyrogallol self-oxidation system were statistically significant ($P<0.01$) and there existed a dose-dependent pattern, $r=0.92186$, correlation coefficient was significant ($P<0.05$), but scavenging activity of synthetic oligosaccharides was smaller than that of vitamin C of same dose.

3.4.2. Hydroxide free radical scavenging activity of synthetic oligosaccharides

Scavenging activities of synthetic oligosaccharides to hydroxide free radical generated by hydroxide free radical production system ($FeSO_4+H_2O_2$) were demonstrated in Table 2. All data had been revised by reagent blank tube.

Results (Table 2) showed that scavenging activity of synthetic oligosaccharides for hydroxyl free radical generated

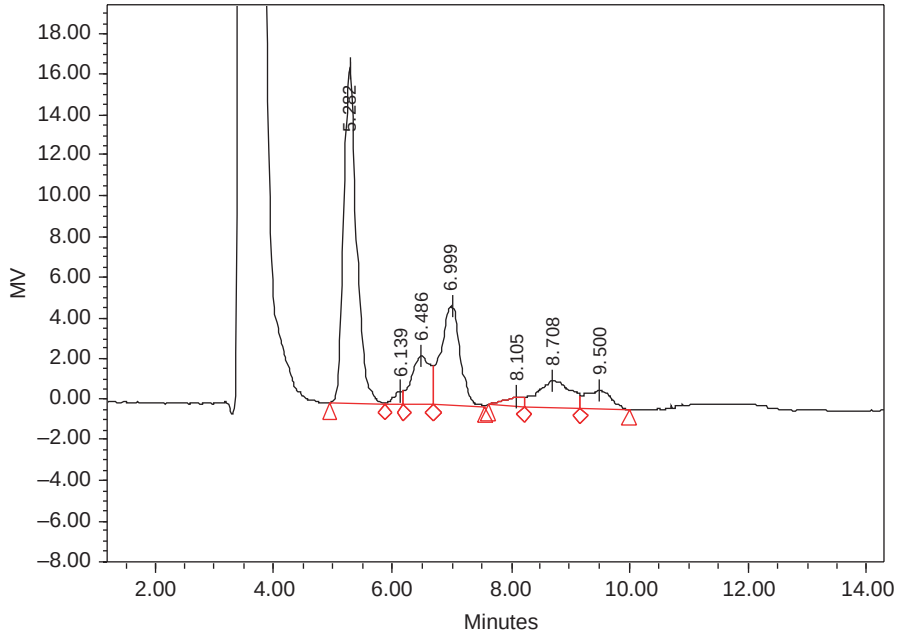

Fig. 7. The HPLC of monose, disaccharides and trisaccharides (SpherisorbNH_2 column).

Table 1
Oxygen free radicals cavenging activity of oligosaccharides

Group	Number	Oligo saccharides concentration	A_{299} ray absorptivity	Scavenging rate (%)
Contrast	10	0	0.4116±0.0040	0
Oligosaccharides	10	2.82	0.2309±0.0028*	44.28
Vitamin C	10	2.82	0.1084±0.0031*	70.92
Oligosaccharides	10	1.88	0.2506±0.0029*	41.16
Vitamin C	10	1.88	0.1266±0.0030*	66.88
Oligosaccharides	10	0.94	0.3001±0.0028*	29.84
Vitamin C	10	0.94	0.2010±0.0031*	51.26

*$P<0.01$, in comparison with contrast.

was statistically significant ($P<0.01$) in comparison with blank group and control one. There existed a dose-dependent pattern between oligosaccharides concentration and scavenging activity, $r=0.94058$, correlation coefficient was significant ($P<0.01$). Moreover, scavenging activity of synthetic oligosaccharides for hydroxyl free radical was significantly higher than that of mannitol of same dose ($P<0.01$).

So far, mechanism of oligosaccharides scavenging free radical is still a matter of hot debate. A possible explaination is that some special groups contained in oligosaccharides, like feruloyl mzoiety (Xiaoping Yuan et al., 2005), α-tocopheryl moiety (Martina Lahmann & Joachim Thiem, 1997), etc. are well responsible for the scavenging activity. The second opinion is that it was chelation and absorption function of oligosaccharides to scavenge free radical. Carbohydrates have long been known to form stable complexes with metal ions (Gyurcsik & Nagy, 2000). Metal ions are one of factors of stimulating lipid and protein oxidation. Oxidative damage may be inhibited both through the direct radical scavenging and the copper-chelation mechanism (Tamba & Torreggiani, 2003). The third theory assumes that oligosaccharides scavenging free radical is determined by its space structure (Karin Kollarova, Maria Henselova, & Desana Liškova, 2005). Monosaccharides without antioxidation potential interact each other and form polysaccharides of complex structure, which start to possess antioxidation potential. A possible explanation is that change of space structure result in free energy declining of compound in itself. In our experiments, the scavenging activities of synthetic oligosaccharides to free radical were significantly observed. On one hand, the results confirm the antioxidant activities of synthetic oligosaccharides, on the other hand, indicate the involvement of inhibition against free radical can occur in the absence of special function groups. Namely, oligosaccharides in itself possess the property of scavenging free radical.

Table 2
Hydroxyl free radical scavenging activity of oligosaccharides

Group	Number	Oligo saccharides concentration ($g\,L^{-1}$)	A_{299} ray absorptivity	Scavenging rate (%)
Blank	10	0	0.1042±0.0039	0
Contrast	10	0	0.0699±0.0021	0
Oligosaccharides	10	3.67	0.1109±0.0024*	76.89
Mannitol	10	3.67	0.1207±0.0023**	52.78
Oligosaccharides	10	2.82	0.1177±0.0025*	60.85
Mannitol	10	2.82	0.1278±0.0030**	36.88
Oligosaccharides	10	1.88	0.1200±0.0035*	51.00
Mannitol	10	1.88	0.1341±0.0030**	21.06

*$P<0.01$, in comparison with contrast and mannitol of the same dose. ** $P<0.01$, in comparison with contrast.

4. Conclusions

In the reaction of microwave-assisted solid-phase oligosaccharides synthesis, the optimum reaction conditions were that microwave power output of 800 W, microwave irradiation time 10 min, adding 30% water to initiate reaction, adding 10% heteropoly acid A as the catalyst. The product was characterized with HPLC and included glucose 49.2%, maltose 10.1%, isomaltose 21.78%, maltotriose 2.15%, panose 10.08%, isomaltotriose 5.16%, tetrasaccharide and pentasaccharides 1.61%. Results indicated that the microwave-assisted solid-phase oligosaccharides synthesis technology had some advantages of higher speed, cleaner reaction profiles, and the higher product yields.

Free radical scavenging test demonstrated that synthetic oligosaccharides possessed properity of scavenging oxygen and hydroxyl free radical.

Acknowledgements

The authors thank for the financial assistance of the Nutrition and Safety institute of the Department of Food Science and Technology in Southern Yangtze University. The authors express their gratitude to Professor Guowei Le, Professor YongHui Shi for critical discussion and suggestions.

References

Baghurst, D. R., & Mingos, D. M. P. (1992). *Journal of the Chemical Society, Chemical Communications* , 674–677.

Cotterill, I. C., Usyatinsky, I. Y., Arnold, J. M., Clark, D. S., Dordick, J. S., Michels, P. C., et al. (1998). Microwave assisted combinatorial chemistry. Synthesis of substituted pyridines. *Tetrahedron Letters*, *39*, 1117–1120.

Crittenden, R. G., & Playne, M. J. (1996). Production, properties and application of food-grade oligosaccharides [J]. *Trends in Food Science and Technology*, *7*, 353–361.

Cummings, John. H. , Edmond, Laurie M., & Magee, Elizabeth A. (2004). Dietary carbohydrates and health: Do we still need the fibre conception. *Clinical Nutrition Supplements*, *1*, 5–17.

Cummings, R. D., & Smith, D. F. (1992). The selectin family of carbohydrate-binding proteins: Structure and importance of carbohydrate ligands for cell adhesion. *BioEssays*, *14*, 849–856.

Etzioni, A., Frydman, M., Pollack, S., Avidor, I., Phillips, M. L., Paulson, J. C., et al. (1992). Recurrent severe infections caused by a novel leukocyte adhesion deficiency. *The New England Journal of Medicine*, *327*, 1789–1792.

Fan, Ying, Hu, Jinfeng, Li, Jing, Yang, Zhao, Xin, Xianliang, Wang, Jian, et al. (2005). Effect of acidic oligosaccharide sugar chain on scopolamine-induced memory impairment in rats and its related mechanisms. *Neuroscience Letters*, *374*, 222–226.

Flitsch, Sabine L. (2000). Chemical and enzymatic synthesis of glycopolymers. *Biopolymers*, *6*, 619–625.

Foxall, C., Watson, S. R., Dowbenko, D., Fennie, C., Lasky, L. A., Kiso, M., et al. (1992). The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis(x) oligosaccharide. *The Journal of Cell Biology*, *117*, 895–902.

Frank, L. (1985). Oxygen toxicity in eukaryotes. In L. W. Oberly (Vol. Ed.), *Superoxide dismutase, pathological states: Vol. 3* (pp. 1–43). Boca Raton: CRC Press.

Gabriel, C., Gabriel, S., Grant, E. H., Halstead, B. S. J., & Mingos, D. M. P. (1998). Dielectric parameters relevant to microwave dielectric heating. *Chemical Society Reviews*, *27*, 213–223.

Galema, S. A. (1997). *Chemical Society Reviews*, *26*, 233–238.

Giguere, R. J. (1986). Application of commercial microwave ovens to organic synthesis. *Tetrahedron Letters*, *27*, 4945–4948.

Gyurcsik, B., & Nagy, L. (2000). *Coordination Chemistry Reviews*, *203*, 81–149.

Houmes, Joel D., & zur Loye, Hans-Conrad (1997). Microwave synthesis of ternary nitride materials. *Journal of Solid State Chemistry*, *130*, 266–271.

Kappe, C. O. (2002). High-speed combinatorial synthesis utilizing microwave irradiation. *Current Opinion in Chemical Biology*, *6*, 314–320.

Kappe, C. O. (2003). Scalability of microwave-assisted organic synthesis: From single-mode to multimode parallel batch reactors. *Organic Process Research and Development*, *7*, 707–716.

Kollarova, Karin, Henselova, Maria, & Liškova, Desana (2005). Effect of auxins and plant oligosaccharides on root formation and elongation growth of mung bean hypocotyls. *Plant Growth Regulation*, *46*, 1–9.

Lahmann, Martina, & Thiem, Joachim (1997). Synthesis of α-tocopheryl oligosaccharides. *Carbohydrate Research*, *299*, 23–31.

Langa, F. (1997). Microwave irradiation: More than just a method for accelerating reactions. *Contemporary Organic Synthesis*, *4*, 373–386.

Larhed, M., & Hallberg, A. (2001). Microwave-assisted high-speed chemistry: A new technique in drug discovery. *Drug Discovery Today*, *6*, 406–416.

Ley, S. V., & Baxendale, I. R. (2002). New tools and concepts for modern organic synthesis. *Nature Reviews Drug Discovery*, *1*, 573–586.

Lidstrom, P., Tierney, J., Wathey, B., & Westman, J. (2001). Microwave assisted organic synthesis—A review. *Tetrahedron*, *57*, 9225–9283.

Loupy, A. (1998). New solvent-free organic synthesis using focused microwaves. *Synthesis* , 1213–1234.

Loupy, A., & Perreux, L. (2001). A tentative rationalization of microwave effects in organic synthesis according to the reaction medium and mechanistic considerations. *Tetrahedron*, *57*, 9199–9223.

Mingos, D. M. P., & Baghurst, D. R. (1991). Application of microwave dielectric heating effects to synthetic problems in chemistry. *Chemical Society Reviews*, *20*, 1–47.

Monsan, Pierre, & Paul, Francois (1995). Enzymatic synthesis of oligosaccharides. *FEMS Microbiology Reviews*, *16*, 187–192.

Przybyło, M., Litynska, A., & Pochec, E. (2005). Different adhesion and migration properties of human HCV29 non-malignant urothelial and T24 bladder cancer cells: Role of glycosylation. *Biochimie*, *87*, 133–142.

Seddon, K. R. (1996). *Kinetika i Kataliz*, *37*, 743–748.

Sharon, N., & Lis, H. (1993). Carbohydrates in cell recognition. *Scientific American*, *1*, 74–81.

Singha, Vandana, Sethi, Rupali, Tewari, Ashutosh, Srivastava, Vasundhara, & Sanghi, Rashmi (2003). Hydrolysis of plant seed gums by microwave irradiation. *Carbohydrate Polymers*, *54*, 523–525.

Stadler, A., & Kappe, C. O. (2001). High-speed couplings and cleavages in microwave-heated, solid-phase reactions at high temperatures. *European Journal of Organic Chemistry* , 919–925.

Strauss, C. R., & Trainor, R. W. (1995). Development in microwave-assisted organic chemistry. *Australian Journal of Chemistry*, *48*, 1665–1692.

Tamba, Maurizio, & Torreggiani, Armida (2003). Allopurinol and its interactions with Cu^{2+} ions: Radical reactions and complex structure. *Research on Chemical Intermediates*, *6*, 533–552.

Wathey, Bernard, Tierney, Jason, Lidström, Pelle, & Westman, Jacob (2002). The impact of microwave-assisted organic chemistry on drug discovery. *DDT*, *7*, 373–380.

Wilson, N. S., Gilroy, J., Dolan, J. W., & Snyder, L. R. (2004). Development and applications of a practical continuous flow microwave cell. *Organic Process Research and Development*, *8*, 535–538.

Yadav, J. S., & Reddy, B. V. S. (2002). Microwave-assisted rapid synthesis of the cytotoxic alkaloid. *Tetrahedron Letters*, *43*, 1905–1907.

Yuan, Xiaoping, Wang, Jing, Yao, Huiyuan, & Chen, Feng (2005). Free radical-scavenging capacity and inhibitory activity on rat erythrocyte hemolysis of feruloyl oligosaccharides from wheat bran insoluble dietary fiber. *LWT*, *38*, 877–883.