Carbohydrate Polymers 106 (2014) 22–24

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Preparation and antioxidant activity of the oligosaccharides derived from *Laminaria japonica*

Sheng-Jun Wu*

School of Marine Science and Technology, Huaihai Institute of Technology, 59 Cangwu Road, Xinpu 222005, China

ARTICLE INFO

Article history:
Received 29 December 2013
Received in revised form 13 January 2014
Accepted 28 January 2014
Available online 6 February 2014

Keywords:
Oligosaccharides
Laminaria japonica
Hydrogen peroxide
Antioxidant activity

ABSTRACT

Hydrogen peroxide (H_2O_2) can degrade polysaccharides and has bleaching effect. In this study, the oligosaccharides derived from *Laminaria japonica* were prepared by hydrolysis with H_2O_2 and their antioxidant activity was investigated. The optimal hydrolysis conditions were determined as follows: reaction time 24 h, reaction temperature 75 °C, and H_2O_2 concentration 4%. Under the optimum conditions, the maximum yield of the oligosaccharides reached 17.65%, which was higher than that of aqueous extraction, and at the same time, the maximal decoloration rate reached 79.85%. The oligosaccharides sample contained 94.82% sugar, of which the average degree was approximately 8, and showed light green. The oligosaccharides derived from *L. japonica* showed high hydroxyl radical scavenging activity (91.31%) at the concentration of 100 μg/mL.

1. Introduction

For thousands of years, seaweeds have been valued as an edible and health-enhancing resource in oriental countries, e.g. China, Japan, and Korea. Seaweeds have high essential minerals, vitamins, dietary fibers, and polysaccharides contents (Christine, Rainer, & Gerhard, 2007).

The brown seaweed, *Laminaria japonica*, is one of common seafoods in China and many other countries, and documented as a drug in traditional Chinese medicine for over a thousand years (Wang, Zhang, Zhang, & Li, 2008). Recent studies demonstrated that the polysaccharides isolated from *L. japonica* have anti-tumor, anti-apoptosis, anti-virus, anti-coagulant, anti-oxidant, anti-fatigue, and anti-radiation activities (Kim, Kim, Kim, Lee, & Lee, 2006; Luo et al., 2011; Tomohiro, Jynji, Takashi, Noriyuki, & Makoto, 2006; Wang et al., 2008; Zhao, Xue, & Li, 2008). However, the oligosaccharides derived from *L. japonica* have not been reported frequently (Kim et al., 2006).

In this study, oligosaccharides were prepared from *L. japonica* by hydrolysis with hydrogen peroxide (H_2O_2). The extraction conditions were optimized, and the antioxidant activity of *L. japonica*-derived oligosaccharides (LOs) was determined.

2. Materials and methods

2.1. Materials

L. japonica was purchased from a local supermarket (Xinpu, China). H_2O_2 (30%, v/v) was purchased from the Laiyang Kant Chemical Co., Ltd. (Laiyang, China). All other chemicals were reagent-grade.

2.2. Preparation of LOs

L. japonica was washed with tap water, shredded, dried in a hot air oven (JK-OOI-240A, China) at 70 °C for 6 h, pulverized, and sifted through a 60-mesh sieve.

The lipids in the dried powder were separated by the Soxhlet extraction method using light petroleum as the solvent. The resulting samples were suspended in tap water to yield a suspension at a concentration of 1% (w/v). H_2O_2 (2%, 2.5%, 3%, 3.5%, 4%, 4.5%, and 5%, respectively) were added to a reactor containing 200 mL of the suspension, and the reactor was incubated in a thermostatic water bath at different temperatures (45 °C, 50 °C, 55 °C, 60 °C, 65 °C, 70 °C, 75 °C, 80 °C, and 85 °C, respectively) for designated time periods (6 h, 12 h, 18 h, 24 h, 30 h, and 36 h, respectively). Aliquots of the suspension were withdrawn periodically and cooled below 15 °C to terminate the reaction.

The hydrolysates were filtered, concentrated (approximately 15%). proteins removed using the Sevag method, precipitated using

* Tel.: +86 518 85895427; fax: +86 518 85895428.
E-mail address: wushengjun008@sina.com

0144-8617/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2014.01.098

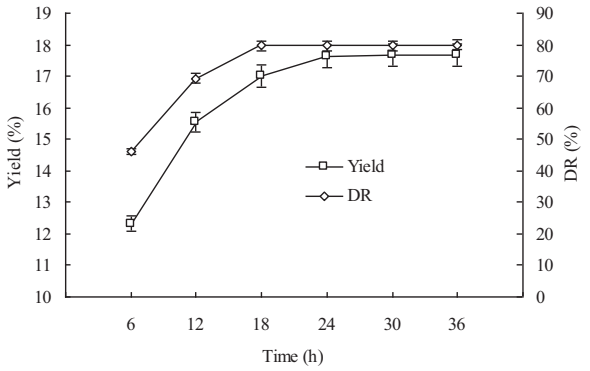

Fig. 1. Effect of time on the yield of LOs and DR. Data are shown as mean ± SD (n = 3).

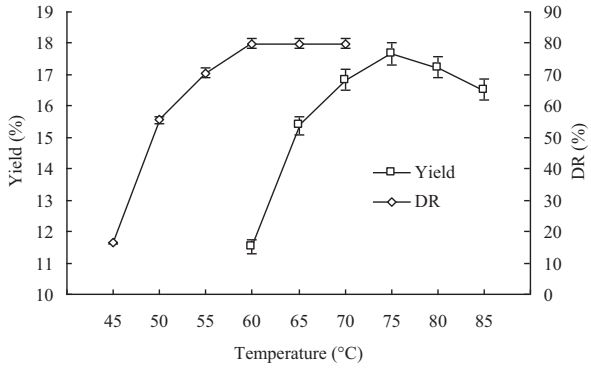

Fig. 2. Effect of temperature on the yield of DOs and DR. Data are shown as mean ± SD (n = 3).

5 volumes of absolute ethanol, filtered again, and freeze-dried. The percentage yield of LOs was calculated using Eq. (1).

$$\text{Yield} = 100\frac{W_2}{W_1} \quad (1)$$

where W_1 and W_2 represent the weights of the recovered LOs and the original *L. japonica* powder, respectively.

2.3. Analytical methods

Ash, moisture, protein, and total sugar contents of the samples were determined according to standard methods (Hou, 2004). The reducing sugars were estimated by the Somogyi method and expressed as a dextrose equivalent (DE) value (Nelson, 1944).

Decoloration rate (DR) was calculated as follows:

$$\text{DR}(\%) = \frac{OD_1 - OD_2}{OD_1} \times 100 \quad (2)$$

where OD_1 is the optical density value of the *L. japonica* suspension filtrate at 320 nm, OD_2 is the optical density value of the hydrolysates filtrate at 320 nm.

Hydroxyl radical scavenging activity (HRSA) of the hydrolysates was measured according to the method of Andrews (1986). LOs hydroxyl scavenging activity was calculated as follows:

$$\text{HRSA}(\%) = \frac{A_1 - A_2}{A_1 - A_0} \times 100 \quad (3)$$

where A_0 is the absorbance of the reagent blank absorbance, A_1 is the positive control absorbance, A_2 is the absorbance of the sample.

2.4. Statistical analysis

All data are presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used for comparing the groups. A P value of <0.05 was considered to be statistically significant.

3. Results and discussion

3.1. Effect of time on the yield of LOs and DR

Effect of reaction time on the yield and DR of LOs with H_2O_2 was determined over a period of 36 h (Fig. 1). The yield of LOs increased sharply within 18 h, gradually increased from 18 h to 24 h, and did not increase further after 24 h. Therefore, the optimal reaction time for the yield of LOs yield was 24 h. However, maximal DR of Los was obtained at 18 h, indicating that decoloration of the reaction broth needed shorter reaction time than that of getting maximum yield of LOs.

3.2. Effect of temperature on the yield of DOs and DR

The reaction temperature crucially affects *L. japonica* polysaccharides hydrolysis and the broth decoloration with H_2O_2. The temperatures studied were from 45 °C to 85 °C. The maximum LOs yield was obtained at 75 °C (Fig. 2). Increasing temperature further decreased LOs yield. This can be attributed to the excessive hydrolysis of *L. japonica* polysaccharides at high temperature, which produced oligosaccharides with an extremely low degree of polymerization that made precipitation with ethanol difficult. In contrast, the optimal temperatures for chitosan, curdlan, and *Lycium barbarum* polysaccharides hydrolysis using H_2O_2 are 40–60 °C (Tian, Liu, Hu, & Zhao, 2004), 60 °C (Wu, Cai, & Sun, 2012), and 70 °C (Jiang, 2014). The differences could be due to the discrepancies in polysaccharide type. However, maximum DR of the broth was obtained at 60 °C, indicating that decoloration of the reaction broth needed milder temperature condition than that of getting maximum yield of LOs.

3.3. Effect of H_2O_2 concentration on the yield of LOs and DR

Effect of the H_2O_2 concentration on the yield of LOs and DR was shown in Fig. 3. The maximum LOs yield and broth DR were achieved at H_2O_2 concentration of 4% and 3.5%, respectively. It is worth noting that increasing in H_2O_2 concentration above 4% decreased LOs yield, which could also be attributed to the excessive hydrolysis of H_2O_2 in the presence of excess H_2O_2. Under the optimum conditions, i.e. time 24 h, reaction temperature 75 °C, and H_2O_2 concentration 4%, the maximum yield of the oligosaccharides reached 17.65% and was higher than that of aqueous extraction (Yu, 2006).

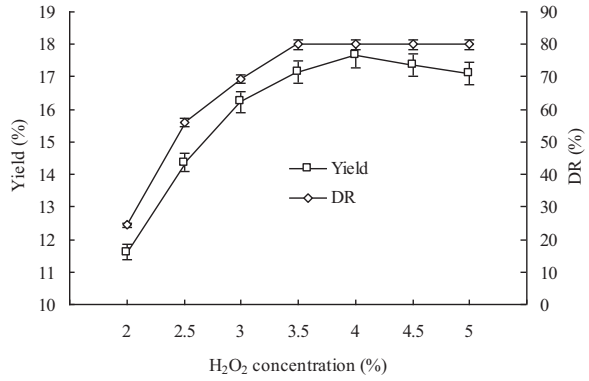

Fig. 3. Effect of H_2O_2 concentration on the yield of DOs and DR. Data are shown as mean ± SD (n = 3).

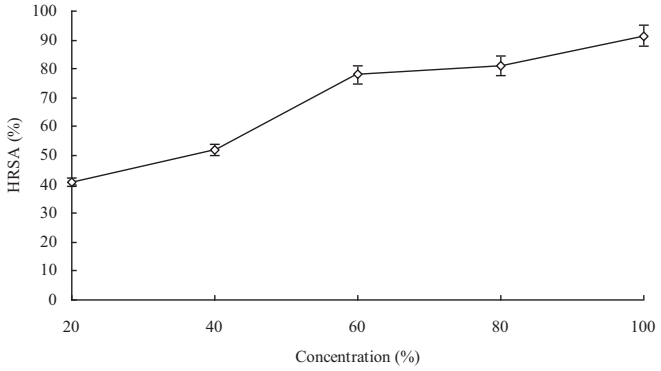

Fig. 4. HRSA of LOs. Data are shown as mean ± SD ($n = 3$).

3.4. Product characterization

Ash, moisture, and total sugar contents in the products were 3.21%, 1.92%, and 94.82%, respectively. The products did not contain any protein. DE of the resulting products was 12.27, indicating that the average degree of polymerization was approximately 9. The products were light green and water soluble powders.

3.5. HRSA of LOs

Hydroxyl radicals are the highest activity among all reactive oxygen species. They can induce severe damage to biomolecules such as polysaccharides, protein, and nucleic acid and thus have been widely accepted as a tool for estimating the free-radical scavenging activities of antioxidants (Jiang, 2014). The HRSA of the LOs reached 91.31% at a concentration of 100 mg/mL (Fig. 4).

4. Conclusions

In this study, we prepared water soluble LOs by hydrolysis with H_2O_2. The yield of DOs and the DR of the broth were affected by the hydrolysis conditions. LOs were partially characterized and their antioxidant activity were determined. The results of this study demonstrate that LOs have high HRSA at a concentration of 100 mg/mL.

Acknowledgment

This research was supported by A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

References

Andrews, A. T. (1986). *Electrophoresis: Theory, techniques and biochemical and clinical applications* (2nd ed., pp. 53–75). Oxford: Clarendon.
Christine, D., Rainer, S., & Gerhard, J. (2007). Amino acids, fatty acids and dietary fibre in edible seaweed products. *Food Chemistry*, *103*, 891–899.
Hou, M. L. (2004). *Food analysis*. Beijing, China: Chemical Industry Press (in Chinese).
Jiang, L. F. (2014). Preparation and antioxidant activity of *Lycium barbarum* oligosaccharides. *Carbohydrate Polymers*, *99*, 646–648.
Kim, K., Kim, Y., Kim, H., Lee, B., & Lee, D. (2006). Anti-apoptotic activity of laminarin polysaccharides and their enzymatically hydrolyzed oligosaccharides from *Laminaria japonica*. *Biotechnology Letters*, *28*, 439–446.
Luo, Q., Liu, J., Yan, J., Zhou, Y., Cui, X., & Yang, M. (2011). The effect of *Laminaria japonica* polysaccharides on the recovery of the male rat reproductive system and mating function damaged by multiple mini-doses of ionizing radiations. *Environmental Toxicology and Pharmacology*, *31*, 286–294.
Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry*, *153*, 375–380.
Tian, F., Liu, Y., Hu, K. A., & Zhao, B. Y. (2004). Study of the depolymerization behavior of chitosan by hydrogen peroxide. *Carbohydrate Polymers*, *57*, 31–37.
Tomohiro, O., Jynji, Y., Takashi, Y., Noriyuki, Y., & Makoto, N. (2006). Two fucoidans in the holdfast of cultivated *Laminaria japonica*. *Journal of Natural Medicines*, *60*, 236–239.
Wang, J., Zhang, Q., Zhang, Z., & Li, Z. (2008). Antioxidant activity of sulfated polysaccharide fractions extracted from *Laminaria japonica*. *International Journal of Biological Macromolecules*, *42*, 127–132.
Wu, S. J., Cai, R. Z., & Sun, Y. Y. (2012). Degradation of curdlan using hydrogen peroxide. *Food Chemistry*, *135*, 2436–2438.
Yu, H. (2006). Study on the extracting condition and deproteinization of *Laminaria japonica* aresch polysaccharide. *China Food Additives*, *3*, 39–43 (in Chinese).
Zhao, X., Xue, C. H., & Li, B. F. (2008). Study of antioxidant activities of sulfated polysaccharides from *Laminaria japonica*. *Journal of Applied Phycology*, *20*, 431–436.