Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 364–370
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

In vitro free radical scavenging activities and effect of synthetic oligosaccharides on antioxidant enzymes and lipid peroxidation in aged mice

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

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

Received 8 January 2006; received in revised form 16 June 2006; accepted 20 June 2006
Available online 31 July 2006

Abstract

In this study we synthesized oligosaccharides using glucose as reactant via a route assisted by microwave irradiation and evaluated their antioxidant activity in vivo and in vitro. The results show that the oligosaccharides exhibited antioxidant activity in vitro as compared to standard antioxidants such as butylated hydroxytoluene (BHT), and α-tocopherol. This antioxidant activity depended on concentration and increased with increasing dose of sample. In addition, increased endogenous lipid peroxidation and decreased total antioxidant capacity (TAOC) were observed in aged mice. Thirty-day intraperitoneal administration of the oligosaccharides significantly decreased the lipid peroxidation in a dose-dependent manner. Oligosaccharides treatment increased TAOC and the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in all organs tested in aged mice. The present study suggests that the synthetic oligosaccharides possess promising future for their strong free radical scavenging activity. Therefore they can be employed in compensating the decline in TAOC and the activities of antioxidant enzymes and in reducing the risks of lipid peroxidation.

Keywords: Oligosaccharides synthesis; Antioxidant; MDA; In vivo; Superoxide anions

1. Introduction

Aging is a complex biological process that leads to gradual loss of ability of an individual to maintain homeostasis. During aging antioxidant functions decline in almost all mammals [1]. Reactive oxygen species (ROS), such as hydrogen peroxide and superoxide anions, which are produced as by-products in aerobic organisms, have been implicated in cellular processes such as mutagenesis, carcinogenesis and aging. Protection against oxidative stress in cells may be mediated by the antioxidant effects of compounds such as carotenoid [2,3]. To protect cells against oxidative damage by oxidants produced during the oxygen metabolism, an antioxidant system has presumably evolved in aerobic organisms. Antioxidant enzymes including superoxide dismutases (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) etc, constitute an important defense system to clear up the detrimental ROS in vivo. Although almost all organisms possess antioxidant defence and repair systems that have evolved to protect them against oxidative damage, these systems are insufficient to entirely prevent the damage. Against this background, the evaluation of the antioxidant properties of specific chemical scavengers is of particular value for their potential use in preventing or limiting the damage induced by free radicals.

Accordingly, various naturally occurring substances as well as biotechnological products are receiving continuous attention from the viewpoint of antioxidation. Many phenolic compounds have been found to be effective antioxidants in biological systems [4]. Another promising group of antioxidative compound is thought to be polysaccharides (including oligosaccharides) such as those extracted from plants [5], animals [6] and fungi [7,8]. Some of them have been accepted to be one of the important candidates for the development of effective and non-toxic medicines with stronger free radical scavenging and antioxidant actions. In recent years, reports on the antioxidant activity of oligosaccharides and their derivatives can be frequently

* Corresponding author. Tel.: +86 510 85869236; fax: +86 510 85869236.
E-mail address: lgw@sytu.edu.cn (G.-W. Le).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.06.041

observed in existing literature around the world. For example, marine polysaccharides including sulfated polysaccharides have been demonstrated to have antioxidant activity [9,10]. Polysaccharide extracted from mushrooms may prove to be one of the more useful candidates as the effective and non-toxic materials with free radical scavenging activity and metabolic regulatory activity [11–14]. Yuan et al. [15] reported that the feruloylated oligosaccharides significantly inhibited hemolysis of erythrocytes in a dose-dependent manner. However, few reports investigating antioxidant activity of synthetic oligosaccharides, which also carry polyhydroxyl groups, can be found to date.

In the present study, we prepared oligosaccharides using glucose as reactant via a route assisted by microwave irradiation and focused on investigation of their antioxidant activity in vivo and in vitro on the basis of our previous study [16].

2. Materials and methods

2.1. Chemicals and apparatus

Glucose ($C_6H_{12}O_6$) was purchased from Sinopharm Chemical Reagent Co., Ltd. in Shanghai (ShangHai, China); Maltose ($C_{12}H_{22}O_{11}\cdot H_2O$) was purchased from Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences (Shanghai, China); Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were obtained from ShangHai Dongfeng Institute of Biochemistry (ShangHai, China); Vitamin C injection was kindly provided by Tianjin Amino Acid Co., Ltd. (TianJin, China). All other chemicals used were of analytical grade.

Panasonic NN-S563JF frequency conversion microwave oven was purchased from Panasonic Company (Tokyo, Japan); UV-2102PC spectrophotometer was purchased from Unico Instruments Co., Ltd. (Shanghai, China); High pressure liquid chromatogram (HPLC) was performed on a Mlton Roy apparatus equipped with UV detector (Waters Ass., USA) (254 nm); High Speed Tabletop Centrifuge was obtained from Shanghai Anting Scientific Instrument Co., Ltd. (Shanghai, China).

2.2. Preparation of oligosaccharides

Reactant (glucose) was added into an open glass container, followed by the addition of water to initiate and catalyze. Then both were mixed in the open 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 result solution was then filtrated. The filtrated solution was allowed to dry at room temperature, then crushed up to obtain desired synthetic product.

2.3. High performance liquid chromatography (HPLC) conditions

The analytical column was a Sugarpack-1 (6.5 mm × 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.

2.4. Determination of in vitro free radical scavenging activity of synthetic oligosaccharides

2.4.1. Determination of superoxide anion radical scavenging activity of synthetic oligosaccharides

Superoxide anion radical generated by the xanthine/xanthine oxidase system was spectrophotometrically determined by monitoring the product of nitroblue tetrazolium (NBT) [17]. A reaction mixture containing 1.0 ml of 0.05 M phosphate buffer (pH 7.4), 0.04 ml of 3 mM xanthine, 0.04 ml of 3 mM EDTA, 0.04 ml of 0.15% bovine serum albumin, 0.04 ml of 15.0 mM NBT and varying amount of samples (oligosaccharides, BHT, α-tocopherol) was incubated at 25 °C for 10 min, and the reaction was then started by adding 0.04 ml of 1.5 U/ml xanthine oxidase and carried out at 25 °C for 20 min. The absorbance of the reaction mixture was measured at 560 nm. Decreased absorbance of the reaction mixture indicates increased superoxide anion radical scavenging activity.

$$\text{superoxide anion radical scavenging activity (\%)} = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of oligosaccharides and standards.

2.4.2. Determination of DPPH• scavenging activity of synthetic oligosaccharides

The free radical scavenging activity of oligosaccharides was determined by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Shimada et al. [18]. One millilitre of the reaction solution containing 0.1 mmol/l DPPH in ethanol was added to varying amount of oligosaccharides sample (50–250 μg) in 3 ml water. After 30 min, absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. BHT, α-tocopherol as standards, were measured in identical way described above. The DPPH• concentration in the reaction medium was calculated as the following equation ($R^2 = 0.9545$):

$$\text{absorbance} = 0.0036 \times [\text{DPPH}^\bullet].$$

2.4.3. Determination of hydrogen peroxide scavenging activity of synthetic oligosaccharides

The ability of oligosaccharides to scavenge hydrogen peroxide was evaluated according to the method of Ruch et al. [19]. A solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (pH 7.5). Hydrogen peroxide concentration was spectrophotometrically determined from absorption at 230 nm with molar absorbtivity 81 mol/l^{-1}/cm. Oligosaccharides samples (50–250 μg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 15 min against a blank solution containing in phosphate buffer without hydrogen peroxide. BHT, α-tocopherol as standards were measured in identical way described above. The scavenging activity of synthetic oligosac-

charides on hydroxyl radicals was expressed as:

$$\text{scavenged } H_2O_2\,(\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100;$$

A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of oligosaccharides and standards.

2.5. Determination of in vivo antioxidant activity of synthetic oligosaccharides

2.5.1. Animals grouping and treating

Aged Kunming mice (21 months old, 37–50 g) and young Kunming mice (3 months old, 19–26 g) were provided by Laboratory Animal breeding Center associated to our institute. The animals were maintained on a 12-h-dark/12-h-light cycle at about 22 °C. They had free access to standard laboratory pellet diet and water during the experiments.

Aged Kunming mice were randomly distributed into six groups comprising of 10 animals in each group: the aged control; Group I; Group II; Group III; Group IV; Group V. Group I were treated by intraperitoneal injection with oligosaccharides (450 mg/kg body weight; BW) dissolved in physiological saline once daily for 30 consecutive days. Single varying dose of sample mixture comprising of oligosaccharides plus Vitamin C (150 mg + 150 mg/kg BW; 300 mg + 300 mg/kg BW; 450 mg + 450 mg/kg BW) dissolved in physiological saline were respectively administered by intraperitoneal injection to Group II–IV once daily for 30 consecutive days. Group V serving as positive control received Vitamin C (450 mg/kg BW) in identical way. Young Kunming mice served as the normal control. Aged control and normal control received the same volume of physiological saline without medicine intraperitoneally. Twenty-four hours after the last drug administration, the animals were sacrificed by decapitation. Blood samples were harvested, kept at −20 °C until analyzed. Blood samples were centrifuged at 4000 rpm for 3 min at 4 °C and the serum was separated and the serum MDA level was measured. The organs (including liver, heart, brain, and lung) were removed, weighed and homogenized immediately with DY89-II homogenizer (NingBo Scientz Biotechnology Co., Ltd.) fitted with Teflon plunger, in ice chilled 10% KCl solution (10 ml/g of tissue). The suspension was centrifuged at 671 × *g* at 4 °C for 10 min and clear supernatant was used for the following estimations of activities of SOD, GSH-Px, TAOC, and level of MDA by spectrophotometric methods.

The activity of superoxide dismutase (SOD) was determined in tissue homogenates by using xanthine and xanthine oxidase system for production of superoxide radical and subsequent measurement of oligosaccharides as a scavenger of the radicals [20]. The SOD activity was expressed as NU/mg proteins.

Glutathione peroxidase (GSH-Px) was measured at 37 °C in tissue homogenates with cumene hydroperoxide as a substrate [21]. The GSH-Px coupled the reduction of cumene hydroperoxide to the oxidation of NADPH by glutathione reductase and concomitant oxidation is monitored in a spectrophotometer with the decrease in absorbance at 340 nm. The GSH-Px activity was expressed as U/mg proteins.

In this method, MDA levels, reflecting the lipid peroxidation in tissue homogenates, were determined spectrophotometrically by thiobarbituric acid. 1,1,3,3-Tetrae-thoxypropane was used as standard and the results were expressed as nmol MDA/mg protein[22].

The TAOC was measured by the method of Opara [23].

3. Results and discussion

3.1. Analysis of synthetic oligosaccharides by HPLC

The synthetic oligosaccharides were analysed by HPLC using a Sugarpack-1 column at 80 °C. The percent yield of synthetic oligosaccharides using glucose as reagent may maximally reach 82.3% according to peak area. As be shown in Fig. 1, main

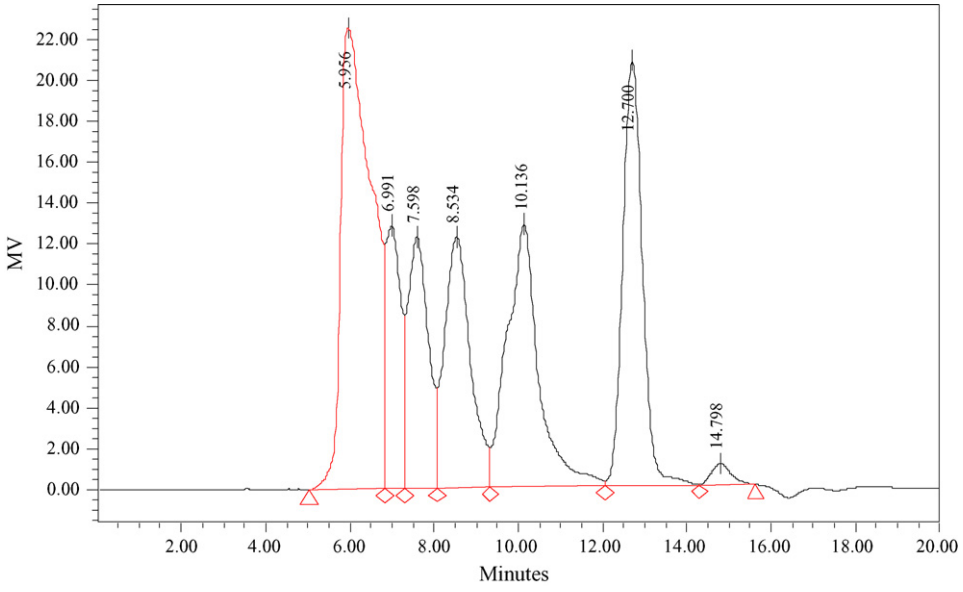

Fig. 1. The HPLC of the product (Sugarpack-1 column).

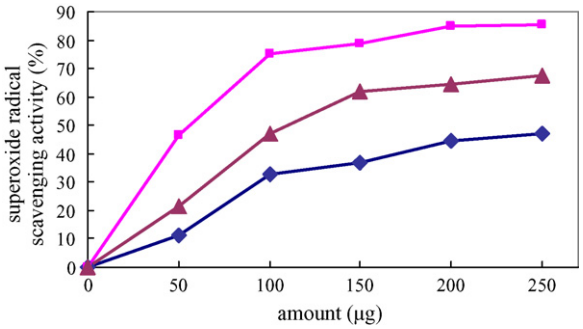

Fig. 2. Superoxide radical scavenging activity of oligosaccharides, BHT, and α-tocopherol. Results were mean ± S.D. of five parallel measurements. Differences were considered to be statistically significant if $P < 0.05$ when compared to control (standards). Oligosaccharides (◆); BHT (■); α-tocopherol (▲).

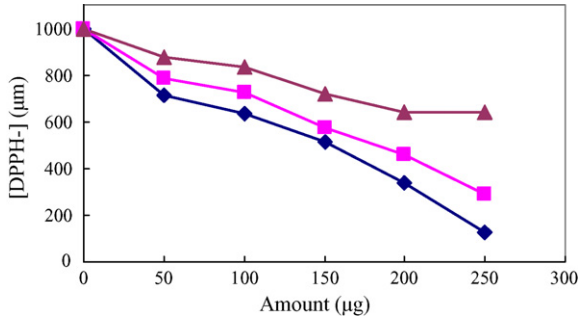

Fig. 3. Free radical scavenging activity of oligosaccharides, BHT, and α-tocopherol by 1,1-diphenyl-2-picrylhydrazyl radicals. Results were mean ± S.D. of five parallel measurements. Differences were considered to be statistically significant if $P < 0.05$ when compared to control (standards). Oligosaccharides (◆); BHT (■); α-tocopherol (▲).

constituents of the synthetic oligosaccharides could be detected and measured as following: in turn 17.73% monosaccharides (retention time: 12.700), 17.68% disaccharides (retention time: 10.136), 13.93% trisaccharides (retention time: 8.534), 11.00% tetrasaccharide (retention time: 7.598), 8.30% pentasaccharides (retention time: 6.991), 30.48% oligosaccharides of higher molecule weight (retention time: 5.956) according to peak area.

3.2. Superoxide anion radical scavenging activity

Superoxide anion radicals scavenging activity of varying amount of oligosaccharides was determined by the xanthine–xanthine oxidase system. Fig. 2 shows the percent inhibition of superoxide radical generation of 50, 100, 150, 200 and 250 μg of oligosaccharides and comparison with same amount of BHT, and α-tocopherol. Within given entire dosage ranges, oligosaccharides always exhibited weaker superoxide radical scavenging activity than BHT and α-tocopherol. The results were found statistically significant ($P < 0.05$). The percentage inhibition of superoxide generation by 250 μg of oligosaccharides, BHT, α-tocopherol was in turn found as 46.9%, 85.3% and 67.5%. The obtained data indicate that superoxide radical scavenging activity of those samples followed the order: BHT > α-tocopherol > oligosaccharides.

3.3. DPPH• radical scavenging activity

DPPH• radical is a stable lipophilic free radical which has been generally used for estimating antioxidant activity of food and medicine materials [24]. A significant decrease of the concentration of DPPH radical ($P < 0.05$) due to the scavenging ability of soluble solids in oligosaccharides and standards was observed in Fig. 3. Both BHT and α-tocopherol were used as standards. The scavenging effect of oligosaccharides and standards on the DPPH radical increased in an amount-dependent manner and followed the order: oligosaccharides (87.2%) > BHT (71.2%) > α-tocopherol (35.9%) at the dose of 250 μg, respectively, suggesting that oligosaccharides have a noticeable effect on scavenging DPPH• radical.

3.4. Hydrogen peroxide scavenging activity

The ability of oligosaccharides to scavenge hydrogen peroxide was evaluated according to the method of Ruch et al. [19]. Fig. 4. showed the scavenging effect of oligosaccharides on hydrogen peroxide and the comparison with BHT and α-tocopherol as standards in an amount-dependent manner. As be shown in Fig. 4, oligosaccharides, BHT, and α-tocopherol exhibited 68.5%, 83.3%, and 56.3% scavenging activity on hydrogen peroxide at 250 μg, respectively. In other words, hydrogen peroxide scavenging activity of oligosaccharides was higher than α-tocopherol but lower than BHT, suggesting that oligosaccharides had stronger hydrogen peroxide scavenging activity. There was statistically significant correlation between those values and control (standards) ($P < 0.05$). Hydrogen peroxide is a normal metabolite in living cells [25]. However it is important for cells to remove hydrogen peroxide because excess hydrogen peroxide may oxidize cellular components [26]. Thus, the removing of H_2O_2 is very important for antioxidant defence in cell or food systems.

3.5. In vivo antioxidant activity of oligosaccharides

SOD protects against oxygen free radicals by catalyzing the removal of superoxide radical ($O_2^{\bullet -}$), which damages the mem-

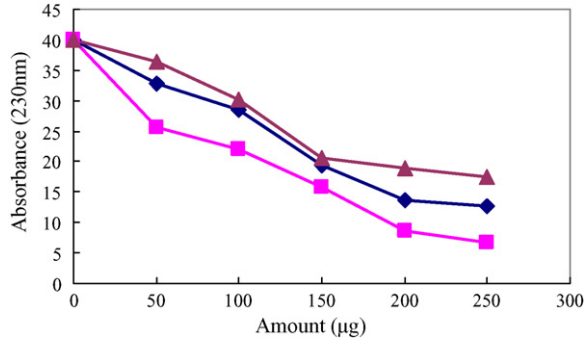

Fig. 4. Hydrogen peroxide scavenging activity of oligosaccharides, BHT, and α-tocopherol. Results were mean ± S.D. of five parallel measurements. Differences were considered to be statistically significant if $P < 0.05$ when compared to control (standards). Oligosaccharides (◆); BHT (■); α-tocopherol (▲).

Table 1
Effect of oligosaccharides on SOD activity in different organs in aged mice (NU/mg protein)

Groups	Lung	Liver	Heart	Brain
Normal control	10.71 ± 1.73	8.69 ± 0.88	15.58 ± 1.22	19.09 ± 1.06
Aged control	8.73 ± 2.44 c	7.61 ± 0.71 d	14.47 ± 1.19	18.8 ± 0.6
Group I	15.38 ± 2.37 b	22.49 ± 1.04 b	27.44 ± 1.45 b	30.73 ± 1.36 b
Group II	14.12 ± 2.06 b	19.6 ± 1.2 b	16.60 ± 2.31	20.07 ± 2.02
Group III	15.93 ± 1.01 b	22.88 ± 1.06 b	29.16 ± 0.91 b	24.11 ± 1.61 b
Group IV	18.44 ± 2.05 b	24.17 ± 0.94 b	30.37 ± 0.69 b	33.31 ± 0.12 b
Group V	10.83 ± 1.98 a	18.08 ± 0.97 b	25.97 ± 0.78 b	27.42 ± 0.86 b

a: $P<0.05$; b: $P<0.01$, compared with aged control group. c: $P<0.05$, d: $P<0.01$, compared with normal control group. The data were presented as means ± S.D. of 10 parallel measures and evaluated by one-way ANOVA followed by the Student's *t*-test to detect inter-group differences. Differences were considered to be statistically significant if $P<0.05$.

brane and biological structures[27]. As shown in Table 1, there was no significant difference in SOD activity observed in brain and heart between the aged and young mice ($P>0.05$) but could be found in liver and lung ($P<0.01$ and $P<0.05$). Declined SOD activity in aged tissues was significantly increased with oligosaccharides and Vitamin C administration. The antioxidant effect of oligosaccharides was higher than that of Vitamin C at 450 mg kg^{-1} BW. The age-related decrease in the activity of SOD documented in our study is corroborated by earlier investigations [28,29]. The possible reason can be the decreased synthesis of this enzyme or enhanced lipid peroxidation.

GSH-Px catalyzes the reduction of H_2O_2 to H_2O and O_2 at the expense of GSH. GSH peroxidase activity in our study is in agreement with the reports of earlier investigators [30]. As was shown in Table 2, significant decreased GSH-Px activity was observed in the heart, brain and liver in aged mice ($P<0.01$ and $P<0.05$). The activity of GSH-Px in lung slightly decreased with age ($P>0.05$). Both oligosaccharides and Vitamin C administration had been shown to have remarkable effects on increasing the activity of GSH-Px in different organs in aged mice. The inhibition of oligosaccharides administration against decreased GSH-Px activity induced by age was higher than that of Vitamin C at 450 mg kg^{-1} BW. In these organs tested (liver, heart, lung, brain), significant increase in GSH-Px activity for oligosaccharides treatment is in agreement with the trends of SOD described above.

Lipid peroxidation is a free radical induced process leading to oxidative deterioration of polyunsaturated lipids. Under normal physiologic conditions, low concentrations of lipid peroxides are found in tissues. Free radicals react with lipids and cause peroxidative changes that result in enhanced lipid peroxidation [31,32]. These free radical species formed are capable of oxidizing sulfhydryl moieties of proteins, thus leading to protein fragmentation and loss of cell viability. In our present study, a marked increase in MDA production, a main index of lipid peroxidation, was observed in all organs tested of aged mice (Table 3) ($P<0.01$ and $P<0.05$). Lipids act as vital substrates for lipid peroxidation, and the increase in lipid composition during aging [33] may be the cause for increased lipid peroxidation. In addition, higher levels of free radical production with increasing age have been reported [34]. Hence, it can serve as a potential marker of susceptibility or of early and reversible tissue damage and of decrease in antioxidant defense. Effects of oligosaccharides on lipid peroxidation were measured by the formation of free MDA in lung, heart, liver, brain and serum, and the data were shown in Table 3. Oligosaccharides treatment significantly inhibited the formation of MDA in all organs tested. Likewise, oligosaccharides exhibited stronger antioxidant effects than Vitamin C at 450 mg kg^{-1} BW. The obtained data suggest that oligosaccharides administration strongly decreased the MDA levels in comparison with age control and restored these alterations to near-normal levels in oligosaccharides-treated mice.

Oxidative stress occurs when there is an excessive production of reactive oxygen species or when total antioxidant capacity decreases [35]. Oxidative stress induced by reactive oxygen species has been implicated in many intrinsic and extrinsic physiological degeneration-associated problems. Total antioxidant capacity (TAOC) reflects the capacity of nonenzymatic antioxidant defense system. Therefore, measure of serum and tissue TAOC may give a more precise indication of the relationship

Table 2
Effect of oligosaccharides on GSH-Px activity in different organs in aged mice (U/mg protein)

Groups	Lung	Liver	Heart	Brain
Normal control	2.47 ± 0.33	9.23 ± 0.92	9.36 ± 0.93	3.91 ± 0.42
Aged control	2.32 ± 0.18	8.3 ± 0.45 c	8.43 ± 0.81 c	2.57 ± 0.56 d
Group I	6.73 ± 1.11 b	13.44 ± 0.97 b	14.62 ± 1.13 b	7.93 ± 0.97 b
Group II	4.33 ± 0.63 b	10.79 ± 1.18 b	9.38 ± 1.21	3.59 ± 0.31 b
Group III	5.34 ± 0.7 b	14.01 ± 1.04 b	10.97 ± 2.32 a	6.69 ± 0.8 b
Group IV	7.2 ± 0.75 b	19.65 ± 1.47 b	14.93 ± 1.08 b	8.82 ± 1.40 b
Group V	5.23 ± 0.94 b	10.4 ± 2.52 a	9.05 ± 0.60	7.60 ± 0.33 b

a: $P<0.05$, b: $P<0.01$, compared with aged control group. c: $P<0.05$, d: $P<0.01$, compared with normal control group. Statistical analysis was implemented in identical way described above.

Table 3
Effect of oligosaccharides on the MDA level in different organs in aged mice (nmol/mg protein)

Groups	Lung	Liver	Heart	Brain	Serum
Normal control	1.91 ± 0.18	12.02 ± 1.14	3.28 ± 0.22	9.61 ± 1.68	12.06 ± 0.26
Aged control	2.35 ± 0.14 d	16.38 ± 1.28 d	5.25 ± 0.17 d	11.04 ± 1.04 c	18.74 ± 0.51 d
Group I	1.97 ± 0.20 b	4.18 ± 0.45 b	3.43 ± 0.29 b	5.55 ± 0.63 b	11.40 ± 0.90 b
Group II	2.20 ± 0.27	9.27 ± 1.22 b	4.93 ± 0.65	10.07 ± 1.23	16.77 ± 1.60 a
Group III	1.81 ± 0.24 b	4.24 ± 0.30 b	4.14 ± 0.74 b	7.31 ± 1.85 b	13.60 ± 0.70 b
Group IV	1.54 ± 0.21 b	3.60 ± 0.39 b	2.37 ± 0.09 b	4.19 ± 0.46 b	10.13 ± 0.45 b
Group V	2.09 ± 0.47	4.80 ± 0.18 b	4.23 ± 1.16 a	7.45 ± 0.63 b	13.45 ± 0.63 b

a: $P<0.05$, b: $P<0.01$, compared with aged control group. c: $P<0.05$, d: $P<0.01$, compared with normal control group. Statistical analysis was implemented in identical way described above.

Table 4
Effects of oligosaccharides on total antioxidant capacity (TAOC) in different organs in aged mice (U/mg protein)

Groups	Lung	Liver	Heart	Brain
Normal control	0.96 ± 0.08	1.41 ± 0.09	0.73 ± 0.08	0.89 ± 0.11
Aged control	0.66 ± 0.06 d	0.79 ± 0.11 d	0.63 ± 0.15	0.55 ± 0.11 d
Group I	1.72 ± 0.14 b	1.98 ± 0.14 b	1.57 ± 0.17 b	2.75 ± 0.23 b
Group II	0.98 ± 0.05 b	1.03 ± 0.22 a	0.71 ± 0.12	0.75 ± 0.15 a
Group III	1.68 ± 0.13 b	1.77 ± 0.20 b	1.45 ± 0.11 b	2.19 ± 0.11 b
Group IV	1.85 ± 0.16 b	2.18 ± 0.30 b	1.95 ± 0.08 b	2.94 ± 0.11 b
Group V	1.45 ± 0.08 b	1.54 ± 0.17 b	1.46 ± 0.15 b	2.38 ± 0.06 b

a: $P<0.05$, b: $P<0.01$, compared with aged control group. d: $P<0.01$, compared with normal control group. Statistical analysis was implemented in identical way described above.

between antioxidants and the aging process. Data on TAOC are summarized in Table 4, except for a slight changes occurring in heart, decreased TAOC with age markedly happened to all tested organs. Administration of oligosaccharides and Vitamin C greatly elevated the TAOC in all tested organs.

Moreover, addition of Vitamin C to oligosaccharides can significantly and dose-dependently raise antioxidant activity of oligosaccharides, which is well reflected in the elevation of SOD activity, GSH-Px activity, TAOC and the reduction of MDA level (Tables 1–4).

In conclusion, our test indicates that synthetic oligosaccharides exhibit stronger free radical scavenging activity and that administration of synthetic oligosaccharides can prevent the formation of lipid peroxidation by overall enhancement of tissue enzymatic and nonenzymatic antioxidant defenses in aged mice. Therefore, the inhibitory effect of oligosaccharides on lipid peroxidation might be, at least in part, attributed to their influence on the antioxidant enzymes and non-enzymatic system by scavenging free radical.

Acknowledgements

The authors express their gratitude to Prof. Guowei Le and Prof. YongHui Shi for critical discussion and suggestions.

References

[1] D. Harman, Mutat. Res. 275 (1992) 257–266.
[2] N.C. Cook, S. Samman, J. Nutr. Biochem. 7 (1996) 66–76.
[3] O.A. Zaporozhets, O.A. Krushynska, N.A. Linkovska, V.N. Barvinchenko, J. Agric. Food Chem. 52 (2004) 21–25.
[4] Z.Q. Liu, K. Han, Y.J. Lin, X.Y. Luo, Biochim. Biophys. Acta 1570 (2002) 97–103.
[5] Q. Luo, Y.Z. Cai, J. Yan, M. Sun, H. Corke, Life Sci. 76 (2004) 137–149.
[6] C. Qin, K. Huang, H. Xu, J. Nutr. Biochem. 13 (2002) 592–597.
[7] C. Sun, J.W. Wang, L. Fang, X.D. Gao, R.X. Tan, Life Sci. 75 (2004) 1063–1073.
[8] C. Sun, C.Y. Shan, X.D. Gao, R.X. Tan, J. Biotechnol. 115 (2005) 137–144.
[9] M. Witvrouw, J. Desmyter, E. De Clercq, Antiviral Chem. Chemother. 5 (1994) 345–359.
[10] I.W. Sutherland, in: H.J. Rehm, G. Reed (Eds.), Biotechnology, Verlag Chemie, Weinhem, 1996, pp. 611–657.
[11] R.B. Herberman, M.E. Nunn, D.H. Lavrin, Int. J. Cancer 16 (1975) 216–229.
[12] G. Chihara, J. Hamuro, Y. Maeda, Y. Araki, F. Fukuoka, Cancer Res. 30 (1970) 2776–3781.
[13] K. Shigesue, N. Kodama, H. Nanba, Jpn. J. Pharmacol. 54 (2000) 293–300.
[14] N. Kodama, T. Kakuno, H. Nanba, Mycoscience 44 (2003) 257–261.
[15] X.P. Yuan, J. Wang, H.Y. Yao, Food Chem. 90 (2005) 759–764.
[16] X.-M. Li, G.-W. Le, Y.-H. Shi, Carbohydr. Polym. 64 (2006) 274–281.
[17] C.-H. Jung, H.-M. Seog, I.-W. Choi, M.-W. Park, H.-Y. Cho, LWT—Food Sci. Technol. 39 (2006) 266–274.
[18] K. Shimada, K. Fujikawa, K. Yahara, T. Nakamura, J. Agric. Food Chem. 40 (1992) 945–948.
[19] R.J. Ruch, S.J. Cheng, J.E. Klaunig, Carcinogenesis 10 (1989) 1003–1008.
[20] I. Fridovich, Advances in Enzymology 41 (1974) 35–97.
[21] A.L. Tappel, in: S. Fleischer, L. Packer (Eds.), Methods in Enzymology, vol. 52, Academic Press, New York, 1978, pp. 506–513.
[22] H. Ohkawa, N. Ohiski, K. Yogi, Anal. Biochem. 95 (1979) 351–358.
[23] E.C. Opara, E. Abdel-Rahman, S. Soliman, W.A. Kamel, S. Souka, J.E. Lowe, S. Abdel-Aleem, Metabolism 48 (1999) 1414–1417.
[24] C. Chen, H.R. Tang, L.H. Sutcliff, P.S. Belton, J. Agric. Food Chem. 48 (2000) 5710–5714.
[25] B. Chance, H. Sies, A. Boveris, Physiol. Rev. 59 (1979) 527–605.
[26] N. Masuoka, M. Wakimoto, T. Ubuka, T. Nakano, Clin. Chim. Acta 254 (1996) 101–111.
[27] B. Halliwell, J.M. Gutteridge, C.E. Cross, J. Lab. Clin. Med. 119 (1992) 598–620.

[28] J.M.C. Gutteridge, Clin. Chem. (Washington, DC, USA) 41 (1995) 1819–1828.
[29] J.Q. Mo, D.G. Hom, J.K. Andersen, Mech. Ageing Dev. 81 (1995) 73–82.
[30] P. Arivazhagan, T. Thilakavathy, C. Panneerselvam, J. Nutr. Biochem. 11 (2000) 122–127.
[31] B.N. Ames, M.K. Shigenaga, T.M. Hagen, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 7915–7922.
[32] A.W. Girotti, J. Free Rad. Biol. Med. 1 (1985) 87–95.
[33] Y. Lion, E. Grandin, A. Van De Vorst, Photochem. Photobiol. 31 (1980) 305–309.
[34] R.S. Sohal, H.H. Ku, S. Agarwal, M.J. Forster, H. Lal, Mech. Ageing Dev. 74 (1994) 121–133.
[35] I. Nakashima, W. Liu, A.A. Akhand, K. Takeda, Y. Kawamoto, M. Kato, H. Suzuki, Mol. Aspects Med. 24 (2003) 231–238.