Carbohydrate Polymers 89 (2012) 61–66

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Isolation, purification and antioxidant activities of polysaccharides from *Grifola frondosa*

Gui-tang Chen[a,*], Xue-mei Ma[a], Sheng-tao Liu[a], Yan-li Liao[a], Guo-qiang Zhao[b]

[a] *Department of Food Quality and Safety, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China*
[b] *Chestnut Research and Development Center of Qianxi County, Qianxi, Tang Shan 064300, China*

ARTICLE INFO

Article history:
Received 7 January 2012
Received in revised form 6 February 2012
Accepted 18 February 2012
Available online 25 February 2012

Keywords:
Grifola frondosa polysaccharides
Isolation
Purification
Antioxidant activity

ABSTRACT

The crude polysaccharides (GFP) were isolated from the fruiting bodies of *Grifola frondosa* and purified by DEAE cellulose-52 chromatography and Sephadex G-100 size-exclusion chromatography in that order. Three main fractions, GFP-1, GFP-2 and GFP-3, were obtained through the isolation and purification steps. Then the antioxidant activities of these three fractions were investigated *in vitro*. The results showed that GFP-1, GFP-2 and GFP-3 possessed significant inhibitory effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, hydroxyl radical and superoxide radical; their reducing power, ferrous ions chelating effect and the inhibition ability of the rat liver lipid oxidation where also strong. These results suggest that *G. frondosa* polysaccharides could be a suitable natural antioxidant and may be the functional foods for humans.
© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Polysaccharides are biological macromolecules, and they have immunomodulatory, antitumor, anti-inflammatory, and anti-fatigue effects, which are related to the antioxidant properties of polysaccharides. In general, many plant polysaccharides have exhibited strong antioxidant properties and can be explored as novel potential antioxidants. In addition, polysaccharides extracted from mushrooms have also shown antioxidant properties as shown by their free radical scavenging ability (Cheung, Cheung, & Ooi, 2003; Fan, Zhang, Yu, & Ma, 2007; Fu, Chen, Dong, Zhang, & Zhang, 2010; Mau, Lin, & Song, 2002; Tseng, Yang, & Mauc, 2008).

Grifola frondosa, which belongs to the family of Aphyllophorales and Polyporaceae, has been marketed in China, Japan, and other Asian countries as a medicinal and edible fungus. Recently, various bioactive properties of *G. frondosa* have been explored, which has attracted considerable attention around the world. Due to different biologically active compounds obtained from the fruiting body and liquid-cultured mycelium of *G. frondosa*, many interesting biological activities have been demonstrated. Various active substances isolated from fungi have been recorded in the literature (Lee et al., 2003; Yang, Gu, & Song, 2007), and polysaccharides were one of the important substances (Cao et al., 2010; Mori, Kobayashi, Tomita, Inatomi, & Ikeda, 2008). However, little information of antioxidant activities was available regarding the water-insoluble polysaccharides from *G. frondosa*. The present paper reports the antioxidant activities of three main polysaccharides fractions prepared from *G. frondosa* fruiting body. The scavenging effects on DPPH radical, hydroxyl radical and superoxide radical, the reducing power, the ferrous ions chelating effect, and the ability to inhibit the rat liver lipid oxidation were used to evaluate the antioxidant activities.

2. Materials and methods

2.1. Materials and reagents

Fruiting bodies of *G. frondosa* were provided by Chestnut Research and Development Center of Qianxi County (Qianxi, China).

Ethylene diamine tetra-acetic acid (EDTA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbate acid, butylated hydroxyanisole (BHA), thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). DEAE-52 and Sephadex G-100 were purchased from Whatman Co. (Maidstone, Kent, UK) and Pharmacia Co. (Sweden). All other chemicals and solvents were of analytical grade from China.

2.2. Preparation of G. frondosa polysaccharides

The fruiting bodies of *G. frondosa* were dried at 50 °C, pulverize into powder (60 mesh) and extracted with 95% ethanol for 24 h to remove impurities and small lipophilic molecules. The degreased powders (20 g) were dried, and extracted with distilled water (500 ml) by ultrasonic waves with a JY98-cell-breaking

* Corresponding author. Tel.: +86 25 83271414; fax: +86 25 83310606.
E-mail address: cpucgt@yahoo.com.cn (G.-t. Chen).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2012.02.045

apparatus prior (Scientz Biotechnology Co., Ningbo, China) for 15 min. The aqueous extract was centrifuged at 4500 rpm for 20 min, then the supernatant was concentrated by rotary vacuum evaporator (BC-R203, Shanghai Biochemical Equipment Co., Shanghai, China) at 65 °C, and precipitated by addition of a 4-fold volume of 95% ethanol and then incubated at 4 °C for 24 h. After centrifugation, the precipitate was washed with anhydrous ethanol, acetone and ether in turn, and then dried to yield the crude polysaccharides (GFP).

The GFP was re-dissolved in 100 ml distilled water, filtered through 0.45 μm filters and applied to a DEAE-52 cellulose column (2.6 cm × 30 cm) equilibrated with distilled water. The GFP was fractionated and eluted with distilled water and different concentrations of stepwise NaCl solution (0.1, 0.3, 0.5 and 1.0 M NaCl) at a flow rate of 2.0 ml/min. The elutes were concentrated to obtain the main fractions, which were then fractionated by size-exclusion chromatography on a Sephadex G-100 column (2.6 cm × 60 cm) eluted with 0.05 M NaCl at a flow rate of 1.0 ml/min. The fractions obtained were combined according to the total carbohydrate content quantified by the phenol–sulfuric acid method using glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The relevant fractions were collected, concentrated, dialyzed and lyophilized for further study, respectively.

2.3. Measurement of scavenging effect on DPPH radical

The scavenging effect of the polysaccharides on DPPH radical was measured by the method of Suda (2000) with some modifications. Sample of 2.0 ml (at different concentrations) was added to 2.0 ml of phosphate buffer (0.02 M, pH 6.0) and 2.0 ml of 0.2 mM DPPH in 95% ethanol. The mixture was shaken and left for 30 min at room temperature, and the absorbance of the resulting solution was measured at 517 nm by an uv-762 UV/VIS spectrophotometer (Shanghai Precision & Scientific Instrument Co., Shanghai, China). Ascorbic acid was used as positive control. The scavenging percentage was calculated according to the following equation:

$$\text{Scavenging percentage}\,(\%) = \left[\frac{Abs_{\text{blank}} - Abs_{\text{sample}}}{Abs_{\text{blank}}}\right] \times 100$$

where Abs_{blank} was the absorbance at 517 nm of 0.2 mM DPPH and Abs_{sample} was the absorbance at 517 nm of 0.2 mM DPPH with sample at different concentrations.

2.4. Measurement of hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was investigated by the method of Wang, He, and Liu (2008) with a minor modification. Briefly, the polysaccharides sample was dissolved in 10 ml of distilled water at the concentration of 0.2, 0.5, 1, 2 3, 4 and 5 mg/ml, respectively. The sample solution (0.25 ml) was mixed with 2.0 ml of 0.2 M phosphate buffer (pH 7.4), 1.75 mM deoxyribose, 0.1 mM ferrous ammonium sulfate and 0.1 mM EDTA, then 0.25 ml of 1.0 mM ascorbic acid and 0.25 ml of 10 mM H_2O_2 were added to the reaction solution. The reaction solution was incubated for 10 min at 37 °C and then 1.0 ml of 1% TBA and 2.0 ml of 2.8% TCA were added to the mixture. The mixture was boiled for 15 min and cooled on ice. Deionized water and ascorbic acid served as blank and positive control, respectively. The absorbance of the mixture was measured at 532 nm. The capability of scavenging hydroxyl radical was calculated according to the following equation:

$$\text{Scavenging percentage}\,(\%) = \left[\frac{Abs_{\text{blank}} - Abs_{\text{sample}}}{Abs_{\text{blank}}}\right] \times 100$$

where Abs_{blank} was the absorbance at 532 nm of deionized water and Abs_{sample} was the absorbance at 532 nm of the test sample mixed with reaction solution.

2.5. Measurement of superoxide radical scavenging activity

The superoxide radical scavenging activity was performed by the method of Liu, Ooi, and Chang (1997) with some modifications. The superoxide radical was generated in 3.0 ml of 0.1 M sodium phosphate buffer (pH 7.4) containing 0.156 mM β-nicotinamide adenine dinucleotide (NADH), 0.052 mM nitroblue tetrazolium (NBT) and 0.02 mM phenazine methosulfate (PMS). After addition of 1.0 ml sample (0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 mg/ml), the mixture was incubated at 25 °C for 5 min. The absorbance of the mixture was measured at 560 nm. Deionized water and ascorbic acid were used as the blank control and positive control, respectively. The scavenging activity of superoxide radicals (%) was calculated according to the following equation:

$$\text{Scavenging percentage}\,(\%) = \left[\frac{Abs_{\text{control}} - Abs_{\text{sample}}}{Abs_{\text{control}}}\right] \times 100$$

where Abs_{blank} is the absorbance of the control (deionized water, instead of sample), and Abs_{sample} was the absorbance of the test sample mixed with reaction solution.

2.6. Measurement of reducing power

The reducing power of the polysaccharides was measured according to the method of Oyaizu (1986). Each sample in 2.0 ml of distilled water was added to 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Then 2.5 ml of 10% (w/v) TCA was added to the reaction mixture, which was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) ferric chloride in test tube. After a 10 min reaction, the absorbance of the resulting solution was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used to compare the reducing power.

2.7. Measurement of ferrous ions chelating effects

The chelating activity of sample on ferrous ions was measured as reported (Dinis, Madeira, & Almeida, 1994) by measuring the formation of ferrous iron–ferrozine complex. Sample in different concentration (0.2, 0.5, 1, 2, 3, 4 and 5 mg/ml) was mixed with 3.7 ml of deionized water, and then reacted with 0.1 ml $FeSO_4$ (2.0 mM). After 0.2 ml of 5.0 mM ferrozine was added, the solution was mixed, left to stand for 10 min at room temperature, and then the absorbance of the mixture was determined at 562 nm. EDTA was co-assayed as a positive control. The chelating activity on ferrous ions was calculated according to the following equation:

$$\text{Chelating ability}\,(\%) = \left[\frac{Abs_{\text{blank}} - Abs_{\text{sample}}}{Abs_{\text{blank}}}\right] \times 100$$

where Abs_{blank} was the absorbance of the control (deionized water, instead of sample), and Abs_{sample} was the absorbance of the test sample mixed with reaction solution.

2.8. Measurement of lipid peroxidation inhibition

The assay was performed by using the method described by Mee, Han, and Ha (2001) with some modifications. Five healthy male SD rats were obtained from Experimental Animal Center of

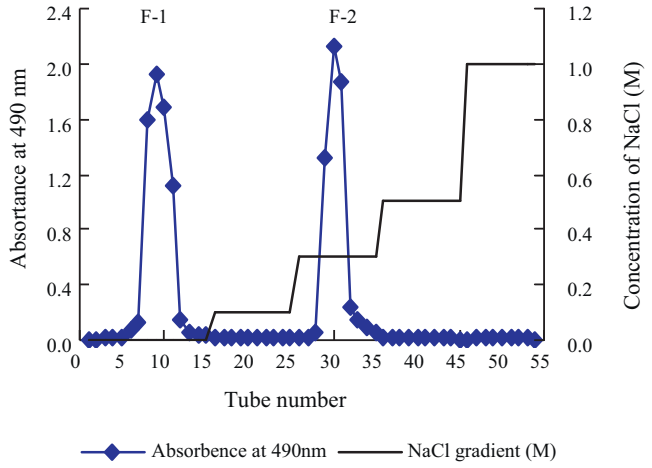

Fig. 1. The profile of GFP isolated from the fruiting bodies of *G. frondosa* on a DEAE-cellulose-52 chromatography eluted with distilled water and stepwise gradient of NaCl aqueous solutions (0.1, 0.3, 0.5 and 1.0 M) at a flow rate of 2.0 ml/min.

Tongji Medical College (Wuhan, China). After 12 h fasting, the rats were sacrificed by dislocation of cervical vertebra, and the livers were excised rapidly and made into 10% (w/v) homogenate with precooled phosphate buffer (50 mM, pH 7.4) using a pestle in a mortar. Each 2.0 ml of this homogenate was added to 1.0 ml of various concentrations of samples, then the mixed solution was incubated at 37 °C for 1.5 h. After the incubation period, 2.0 ml of 15% (w/v) TCA and 2.0 ml of 0.67% (w/v) TBA solution were added to the mixture. This mixture was then placed in a water bath at 100 °C for 10 min. After cooling, it was centrifuged at 3000 rpm for 15 min and the absorbance of the colored supernatant was then measured at 532 nm. BHA was used as positive control. The inhibition percentage was calculated according to the following equation:

$$\text{Inhibition percentage}\,(\%) = \left[\frac{Abs_{\text{blank}} - Abs_{\text{sample}}}{Abs_{\text{blank}}}\right] \times 100$$

where Abs_{blank} was 1.0 ml of sample substituted by an equal volume of distilled water, and Abs_{sample} was the absorbance of the test sample mixed with reaction solution.

2.9. Statistical analysis

All the data were subjected to analysis of variance and Duncan's multiple range tests, using a SPSS system (SPSS Inc., Chicago, USA). Significant levels were defined as probabilities of 0.05 or less.

3. Results and discussion

3.1. Isolation and purification of water-soluble polysaccharide

In the present study, GFP was isolated from *G. frondosa* and the yield was about 3.13%. Furthermore, the GFP solution was firstly separated through an anion-exchange chromatography of DEAE-52, affording two independent elution peaks (F-1 and F-2) as detected by the phenol–sulfuric acid assay (Fig. 1). The two fractions were collected, concentrated and purified by gel filtration chromatography of Sephadex G-100, respectively. As a result, F-1, generated two peaks, named as GFP-1 and GFP-2, respectively (Fig. 2A). And F-2, generated one single elution peak, named as GFP-3 (Fig. 2B). The recovery rates of GFP-1, GFP-2 and GFP-3 based on the amount of GFP were 33.3%, 21.5% and 30.8%, respectively.

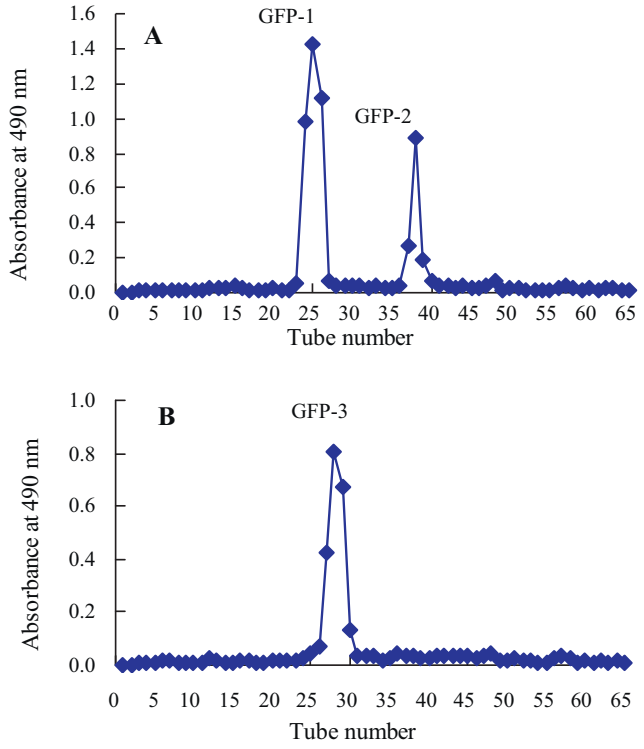

Fig. 2. The profile of GFP fractions (F-1 and F-2) eluted with 0.05 M NaCl aqueous solutions at a flow rate of 1.0 ml/min.

3.2. DPPH radical scavenging activity

Free radical scavenging is one of the generally accepted mechanisms against lipid oxidation, and DPPH as a stable free radical compound, which shows maximum absorbance at 517 nm that can readily undergo scavenging by an antioxidant, so it has been widely used to test the free radical-scavenging ability of various samples (Benvenuti, Pellati, Melegari, & Bertelli, 2004; Shimoji et al., 2002). The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. When DPPH encounters a hydrogen-donating substance, the radical would be scavenged and the absorbance is reduced (Shimada, Fujikawa, Yahara, & Nakamura, 1992). On the basis of this principle, the scavenging effects of purified polysaccharides on the DPPH radical were measured and the results are shown in Fig. 3. The scavenging activity of polysaccharides and ascorbic acid on inhibition of the DPPH radical was related to the concentration of the samples. Furthermore, the scavenging activities of ascorbic acid significantly increased with the increasing concentrations and were stronger than that of polysaccharides at every concentration point. Among all the samples, GFP-2 possessed higher DPPH scavenging activity than GFP-1 and GFP-3 in a concentration-dependent manner. At the concentration of 3.0 mg/ml, the scavenging effects of GFP-1, GFP-2 and GFP-3 on the DPPH radical were 48.6, 79.6 and 65.9%, respectively. The results mentioned above implied that the purified fractions of *G. frondosa* polysaccharides possibly contained substances that are hydrogen donors and could react with free radicals to convert them to more stable products and terminate the radical chain reaction. It indicates that *G. frondosa* polysaccharide is also an excellent DPPH scavenger.

3.3. Hydroxyl radical scavenging activities

The hydroxyl radical, known to be generated through the Fenton reaction in this system, was scavenged by polysaccharide samples.

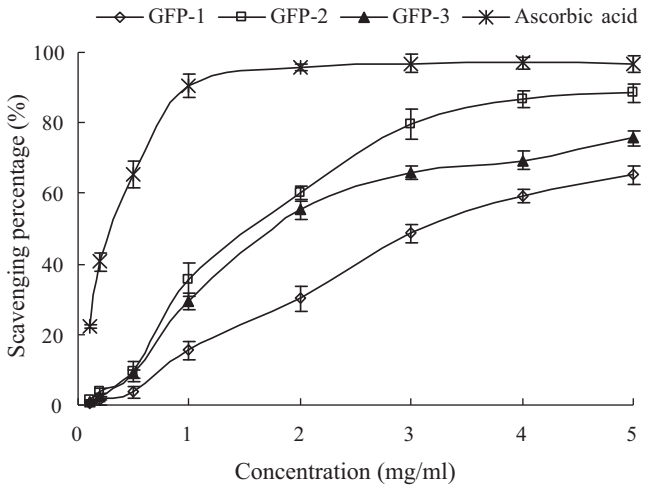

Fig. 3. Scavenging effects of different concentration of GFP-1, GFP-2, GFP-3 and ascorbic acid on DPPH radicals. The error bars indicate the standard deviation of 3 replicates.

The results of hydroxyl radical scavenging activities of GFP-1, GFP-2, GFP-3 and ascorbic acid are given in Fig. 4. Results showed that ascorbic acid has a high level of hydroxyl radical scavenging effect. For all the test samples, the effects of scavenging hydroxyl radicals were in a concentration-dependent manner, and increased in the order of GFP-1, GFP-3 and GFP-2. At the concentration of 3.0 mg/ml, the hydroxyl radical scavenging activity of GFP-1, GFP-3 and GFP-2 were 48.3, 52.6 and 91.6%, respectively. It showed that the three purified fractions of *G. frondosa* polysaccharides, especially GFP-2, which had no significant difference with ascorbic acid above the concentration of 3.0 mg/ml, can be the effective scavenger of hydroxyl radical.

3.4. Superoxide radical scavenging activity

The scavenging effects of different concentration of GFP-1, GFP-2, GFP-3 and ascorbic acid on superoxide radical were tested and significantly exhibited in a concentration-dependent manner (Fig. 5). For each polysaccharide fractions, the peak values were all observed at the polysaccharide concentration of 4.0 mg/ml.

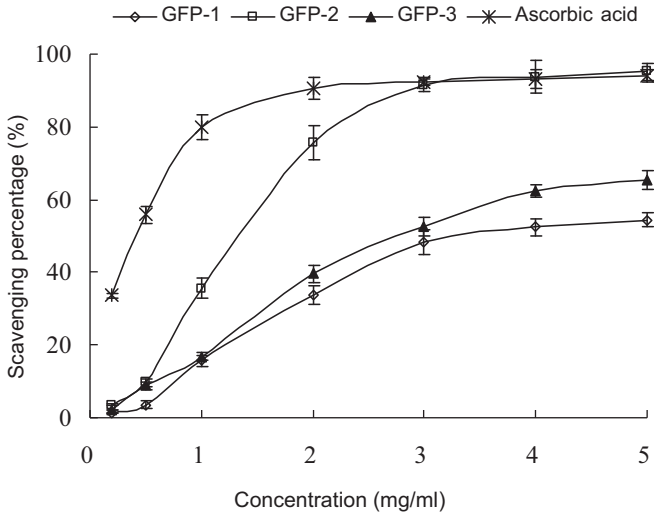

Fig. 4. Scavenging effects of different concentration of GFP-1, GFP-2, GFP-3 and ascorbic acid on hydroxyl radical. The error bars indicate the standard deviation of 3 replicates.

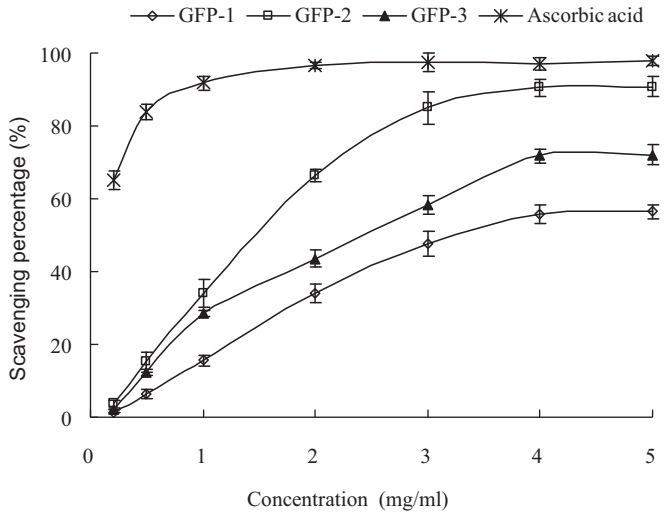

Fig. 5. Scavenging effects of different concentration of GFP-1, GFP-2, GFP-3 and ascorbic acid on superoxide radical. The error bars indicate the standard deviation of 3 replicates.

Among all samples, the scavenging ability on superoxide radical increased in the following order: GFP-1 < GFP-3 < GFP-2. In addition, the highest scavenging ability of 90.5% was obtained by GFP-2 at the concentration of 4.0 mg/ml, which was close to that of ascorbic acid (91.8%) at the concentration of 1.0 mg/ml. Although superoxide radical is a relatively weak oxidant in most organisms, it could produce hydrogen peroxide and hydroxyl radical through dismutation and other types of reaction and is the source of free radicals formed *in vivo*. Moreover, superoxide radical and its derivatives are cell-damaging through causing damage to DNA and membrane of cell. These results clearly indicated that the antioxidant activities of all samples were related to the abilities of scavenging superoxide radical.

3.5. Reducing power

It has been previously reported that there was a direct correlation between antioxidant activities and reducing power of certain plant extracts. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity (Meir, Kanner, Akiri, & Hadas, 1995). Measurement of reducing power may directly reflect the production condition of electron donor. The reducing power of GFP-1, GFP-2, GFP-3 and ascorbic acid determined at 700 nm is depicted in Fig. 6. As shown in the figure, the reducing power of ascorbic acid, which is a well recognized reducing agent, increased quickly at the concentration from 0.2 mg/ml to 1.0 mg/ml, and it showed the highest reducing power at the concentration of 1.0 mg/ml. The reducing power of all the samples increased gradually with the increasing concentration. Among the three fractions of polysaccharides, GFP-2 showed obviously higher ability than that of GFP-1 and GFP-3, but less than that of ascorbic acid. It suggested that reductone-associated and hydroxide groups of *G. frondosa* polysaccharides can act as electron donors and can react with free radicals to convert them to more stable products.

3.6. Ferrous ions chelating effect

Some research report some transition metals, such as Fe^{2+}, Cu^{+}, Pb^{2+}, Co^{2+} and so on, could trigger process of free radical reaction to magnify the cellular damage. Fe^{2+} is known as the most powerful pro-oxidant among various species of metal ions due to its high reactivity, which accelerates lipid oxidation by breaking

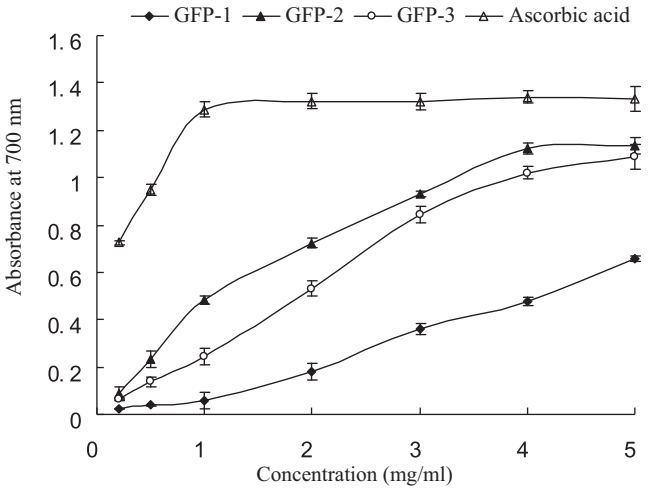

Fig. 6. Reducing power of different concentration of GFP-1, GFP-2, GFP-3 and ascorbic acid. The error bars indicate the standard deviation of 3 replicates.

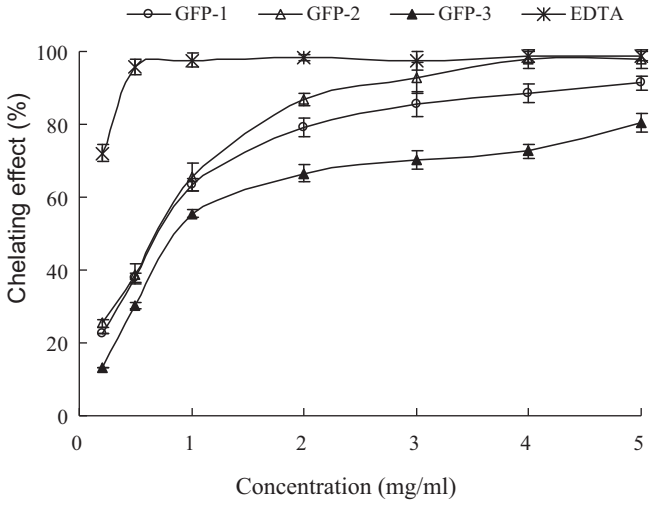

Fig. 8. Inhibition effect of different concentration of GFP-1, GFP-2, GFP-3 and BHA on lipid peroxidation of rat liver homogenates. The error bars indicate the standard deviation of 3 replicates.

down hydrogen and lipid peroxidase to reactive free radicals *via* the Fenton reaction (Sun & Kennedy, 2010). Metal chelating activity is claimed as one of antioxidant mechanisms, since it reduces the concentration of the catalyzing transition metal in lipid peroxidation (Qiao et al., 2009). The chelating abilities of GFP-1, GFP-2, GFP-3 and EDTA on Fe^{2+} are shown in Fig. 7. As shown in the figure, all the test samples and EDTA were found to have more potent chelating ability on Fe^{2+} in a concentration dependent manner from 0.2 to 5.0 mg/ml. Among all samples, the peak metal chelating ability of 97.8% was obtained by GFP-2 at the concentration of 4.0 mg/ml, which was close to that of EDTA (97.6%) at the concentration of 1.0 mg/ml. And the highest value of 91.3% and 80.4% was obtained by GFP-1 and GFP-3 at the concentration of 5.0 mg/ml, respectively. As described by Zha et al. (2009), since ferrous ions are the most effective prooxidants in the food system, and the chelating effect of polysaccharides on ferrous ions might affect the other activities of scavenging free radicals to protect organism against oxidative damage, the high ferrous ion chelating abilities of polysaccharides from *G. frondosa* would be somewhat beneficial.

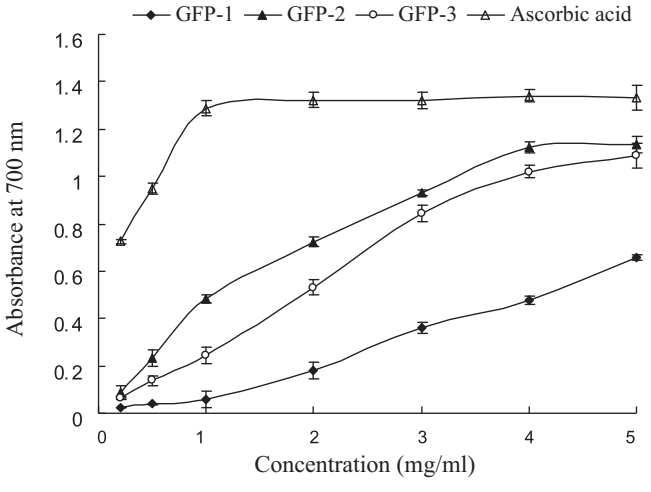

Fig. 7. Chelating effect of different concentration of GFP-1, GFP-2, GFP-3 and EDTA on ferrous ions. The error bars indicate the standard deviation of 3 replicates.

3.7. Lipid peroxidation inhibition

Unsaturated lipids in liver tissue are very susceptible to peroxidation when they are exposed to reactive oxygen species (ROS) or prooxidative metal ions such as ferrous ion. In the present investigation we have incubated the rat liver homogenate, and examined the effects of GFP-1, GFP-2, GFP-3 and BHA on homogenate by measuring the absorbance at 532 nm. The results showed that the generation of autoxidation in rat liver homogenate was obviously delayed by both purified polysaccharide fractions and BHA. As shown in Fig. 8, the inhibition capacity of all the GFP-1, GFP-2 and GFP-3 is week at low concentration, with only 5.5, 8.6 and 6.6% at the concentration of 0.2 mg/ml, respectively, but increased quickly with higher concentration. The inhibition capacity of GFP-2 and GFP-3 is even stronger than that of the positive control BHA at a dose more than 2.0 mg/ml and 3.0 mg/ml, respectively. In addition, the inhibition capacity of GFP-1 had no significant difference with that of BHA at the dose 4.0 mg/ml and 5.0 mg/ml. These results revealed that the three fractions of *G. frondosa* polysaccharides demonstrated great capacity for inhibiting effects on lipid peroxidation. Therefore, these polysaccharides from *G. frondosa* might serve as possible protective agents in human diets to help reduce oxidative damage.

4. Conclusion

The results from different *in vitro* assay systems, including the scavenging effects on DPPH radical, hydroxyl radical and superoxide radical, the reducing power, the ferrous ions chelating effect, and the ability to inhibit the rat liver lipid oxidation—demonstrated that the purified fractions of *G. frondosa* polysaccharides, especially GFP-2, have effective antioxidant activities. Hence, the *G. frondosa* polysaccharides can be used as an easily accessible source of natural antioxidants, as a food supplement, or in the pharmaceutical and medical industries.

Acknowledgements

This work was supported by the National Found for Fostering Talents of Basic Science (No. J0630858) and partly supported by the National Natural Science Foundation of China (No. 31101255).

References

Benvenuti, S., Pellati, F., Melegari, M., & Bertelli, D. (2004). Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of Rubus, Ribes, and Aronia. *Journal of Food Science*, *69*, 164–169.

Cao, X. H., Yang, Q. W., Lu, M. F., Hou, L. H., Jin, Y. Y., Yuan, J., et al. (2010). Preparation and anticoagulation activity of a chemically sulfated polysaccharide (S-GFB) obtained from *Grifola frondosa*. *Journal of Food Biochemistry*, *34*, 1049–1060.

Cheung, L. M., Cheung, P. C. K., & Ooi, V. E. C. (2003). Antioxidant activity and total phenolic of edible mushroom extracts. *Food Chemistry*, *81*, 249–255.

Dinis, T. C. P., Madeira, V. M. C., & Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Archives of Biochemistry and Biophysics*, *351*, 161–169.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Fan, L., Zhang, S., Yu, L., & Ma, L. (2007). Evaluation of antioxidant property and quality of breads containing *Auricularia auricula* polysaccharide flour. *Food Chemistry*, *101*, 1158–1163.

Fu, L., Chen, H., Dong, P., Zhang, X., & Zhang, M. (2010). Effects of ultrasonic treatment on the physicochemical properties and DPPH radical scavenging activity of polysaccharides from mushroom *Inonotus obliquus*. *Journal of Food Science*, *75*, 322–327.

Lee, B. C., Bae, J. T., Pyo, H. B., Choe, T. B., Kim, S. W., & Wang, H. J. (2003). Biological activities of the polysaccharides produced from submerged culture of the edible Basidiomycete *Grifola frondosa*. *Enzyme and Microbial Technology*, *32*, 574–581.

Liu, F., Ooi, V. E. C., & Chang, S. T. (1997). Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sciences*, *60*, 763–771.

Mau, J., Lin, H., & Song, S. (2002). Antioxidant properties of several specialty mushrooms. *Food Research International*, *35*, 519–526.

Mee, H. K., Han, J. H., & Ha, C. S. (2001). Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllostachys edulis*). *Journal of Agricultural and Food Chemistry*, *49*, 4646–4655.

Meir, S., Kanner, J., Akiri, B., & Hadas, S. P. (1995). Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *Journal of Agricultural Food and Chemistry*, *43*, 1813–1819.

Mori, K., Kobayashi, C., Tomita, T., Inatomi, S., & Ikeda, M. (2008). Antiatherosclerotic effect of the edible mushrooms *Pleurotuseryngii* (Eringi), *Grifola frondosa* (Maitake), and *Hypsizygus marmoreus* (Bunashimeji) in apolipoprotein E-deficient mice. *Nutritional Research*, *28*, 335–342.

Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidant activities of products browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, *44*, 307.

Qiao, D. L., Ke, C. L., Hu, B., Luo, J. G., Ye, H., Sun, Y., et al. (2009). Antioxidant activities of polysaccharides from Hyriopsis cumingii. *Carbohydrate Polymers*, *78*, 199–204.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidant properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural Food and Chemistry*, *40*, 945–948.

Shimoji, Y., Tamura, Y., Nakamura, Y., Nanda, K., Nishidai, S., Nishikawa, Y., et al. (2002). Isolation and identification of DPPH radical scavenging compounds in kurosu (Japanese unpolished rice vinegar). *Journal of Agricultural Food and Chemistry*, *50*, 6501–6503.

Suda, I. (2000). Antioxidative activity. In K. Shinohara, T. Suzuki, & S. Kaminogaw (Eds.), *The methods of food functions analysis* (pp. 218–220). Japan: Korin.

Sun, Y. X., & Kennedy, J. F. (2010). Antioxidant activities of different polysaccharide conjugates (CRPs) isolated from the fruiting bodies of *Chroogomphis rutilus* (Schaeff.: Fr.) O.K. Miller. *Carbohydrate Polymers*, *82*, 510–514.

Tseng, Y. H., Yang, J. H., & Mauc, J. L. (2008). Antioxidant properties of polysaccharides from *Ganoderma tsugae*. *Food Chemistry*, *107*, 732–738.

Wang, X., He, L., & Liu, B. (2008). Study on extraction, purification and scavenging activity to hydroxyl radicals of polysaccharides from leaves of *Ilex kudincha* C.J. Tseng. *Food Science*, *29*(6), 37–40.

Yang, B. K., Gu, Y. A., & Song, C. H. (2007). Chemical characteristics and immunomodulating activities of exo-biopolymers produced by *Grifola frondosa* during submerged fermentation process. *International Journal of biological macromolecules*, *41*, 227–233.

Zha, X. Q., Wang, J. H., Yang, X. F., Liang, H., Zhao, L. L., Bao, S. H., et al. (2009). Antioxidant properties of polysaccharide fractions with different molecular mass extracted with hot-water from rice bran. *Carbohydrate Polymers*, *78*, 570–575.