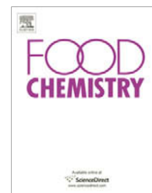
Food Chemistry 115 (2009) 939–944

Contents lists available at ScienceDirect

Food Chemistry

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



# The in vitro antioxidant activity of lotus germ oil from supercritical fluid carbon dioxide extraction

Jianlin Li, Min Zhang, Tiesong Zheng *

*Department of Food Science and Nutrition, Nanjing Normal University, 122 Jinghai Road, Nanjing 210093, People's Republic of China*

ARTICLE INFO

*Article history:*
Received 30 August 2008
Received in revised form 9 December 2008
Accepted 7 January 2009

*Keywords:*
Lotus germ oil
Antioxidant activities
Oxygen radicals
Lipid peroxidation

ABSTRACT

The in vitro antioxidant activity of lotus germ oil extracted by supercritical fluid extraction (SFE) has been investigated. The distinctly high total phenolic compounds content and tocopherol content in lotus germ oil composition were found to be 9.06 ± 0.11% and 485.1 ± 50 mg/100 g, respectively. The lotus germ oil exhibited a dose-dependent inhibitory effect on the hydroxyl free radical and superoxide anion free radical. However, the scavenging effects on the superoxide anion free radical were deceased when the extract concentration was greater than 70 mg/mL. Lotus germ oil showed substantial antioxidant activity in the mice liver and kidney tissues homogenates in a dose-dependent manner. The auto-haemolysis of mice red blood cells was also blocked by lotus germ oil in a dose-dependent manner. Lotus germ oil showed a higher antioxidant activity in the lard system. The high content of phenolic compounds and tocopherol in the lotus germ oil could partially account for the antioxidant activity. These results suggest the lotus germ oil can be used as healthcare oil to develop.

© 2009 Elsevier Ltd. All rights reserved.

## 1. Introduction

Reactive oxygen species (ROS) including hydrogen peroxide ($H_2O_2$), superoxide ($\cdot O_2^-$) and the hydroxyl radical ($\cdot OH$) are metabolic intermediates in human body. Generally, the generation and scavenging of ROS keep dynamic equilibrium in human normal metabolism (Pahlavani & Harris, 1998). When the generation of ROS induced by various stimulators in the organism exceeds the antioxidant capacity of the organism, it will result in cell death and tissue damage. ROS are increasingly recognised as the pathogenesis of certain human diseases, including cancer, ageing and chronic arterial disease (Moskovitz, Yim, & Chock, 2002). Antioxidants could increase the stability of foods and prevent oxidative damage in living systems by scavenging ROS. Therefore, there is obvious importance in the development of natural antioxidants and scavengers to protect biomolecules against the attack of ROS.

*Nelumbo nucifera* Gaertn also known as lotus is a large aquatic herb and grows widely in the eastern Asian countries. Almost all parts of the lotus plant are edible as vegetable and traditional herbal medicine. Several physiological active substances of *N. nucifera* Gaertn including alkaloids (Qian, 2002), flavonoids (Kashiwada et al., 2005), unsaturated fatty acids (Bi, Yang, Li, Zhang, & Guo, 2006), microelements (Lou, Yuan, & Ji, 1995) and plant sterols (Bi et al., 2006) have been identified. *N. nucifera* Gaertn is reported to be capable of antidiarrhoeal (Mukherjee et al., 1995), diuretic (Mukherjee, Das, Saha, Pal, & Saha, 1996), antipyretic (Mukherjee et al., 1996), antimicrobial (Mukherjee, 2002), hypoglycaemic (Mukherjee, Pal, Saha, & Saha, 1995), anti-inflammation (Liu et al., 2006), anti-obesity (Ono, Hattori, Fukaya, Imai, & Ohizumi, 2006) and antioxidant activity (Rai, Wahile, Mukherjee, Pada, & Mukherjee, 2006; Sohn et al., 2003; Yen, Duh, & Su, 2005; Yen, Duh, Su, Yeh, & Wu, 2006).

Many studies have confirmed the antioxidant activity of lotus seeds. The organic solvent extracts of lotus seeds were reported to have high levels of DPPH radical scavenging activity and inhibited both the production of serum enzymes and cytotoxicity (Sohn et al., 2003). The water extracts of lotus seeds exhibited stronger antioxidant activity and the large amounts of phenolic compounds were responsible for the antioxidant activity (Yen et al., 2005). The water and organic solvent extracts of lotus seeds could also inhibit nitric oxide accumulation and scavenge reactive nitrogen species (Yen et al., 2006). The extracts of lotus roots and leaves also inhibited NO production and scavenged small carbon-centred radicals (Borgi, Reciob, Ríos, & Chouchane, 2008; Hu & Skibsted, 2002). However, the previous studies are focused on the leaves, seeds and rhizomes of lotus and little information of lotus germ, especially regarding its antioxidant activity, is available.

Under the supercritical fluid conditions, supercritical fluid extraction (SFE) technology is suitable to decrease volatility and thermal degradation during compounds extraction. In comparison with conventional liquid extraction, supercritical fluids have a higher diffusivity and lower density, viscosity, and surface tension. Supercritical fluid is nontoxic, nonexplosive and easily removable

* Corresponding author. Tel.: +86 25 83598286; fax: +86 25 83707623.
*E-mail address:* tieszheng@sina.com (T. Zheng).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.01.008

from products. Furthermore, the previous studies have shown that the antioxidant activity of extracts by SFE was significantly higher than that of extracts by conventional extractions (Diaaz-reinoso, Moure, Domianguez, & Parajoa, 2006; Hu, Xu, Chen, & Yang, 2004). However, to our knowledge, the antioxidant activities of lotus germ oil extracts prepared by SFE have never been evaluated.

Here, the composition of lotus germ oil extracted by SFE was detected and identified. The antioxidant capacity of lotus germ oil was investigated by in vitro assay. The scavenging effect of lotus germ oil on oxygen radicals and its inhibitory effect on lipid peroxidation (LPO) were studied. In addition, inhibiting effect of lotus germ oil on the lard peroxidation was compared with that of α-tocopherol.

## 2. Materials and methods

### 2.1. Materials

Lotus germ was purchased from Xinsheng Medicine Corporation (Nanjing, China). Standard DL-α-tocopherol was obtained from the Supelco Corporation (Pennsylvania, USA). Sucrose ester of fatty acid was purchased from Jin He Food Corporation (Hangzhou, China). Tris base was from Promega Corporation (Wisconsin, USA). 2-Thiobarbituric acid, myristic acid, hexadecanoic acid, stearic acid, oleic acid, linoleic acid and linolenic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). 6,9,12,15-Docosatetraenoic acid and eicosapentaenoic acid were from Fluka Chemie AG (Buchs, Switzerland). Carbon dioxide with purity >99% was supplied by Nan Jing Gas Inc. (Nanjing, China). All other reagents from local sources were of analytical grade.

Adult mice with a mass from 23 to 27 g were supplied by the Experimental Animal Center of Nanjing Medical University (Nanjing, China).

### 2.2. Supercritical $CO_2$ extraction procedure

Lotus germ sample (200 g) with the moisture of 5.06 ± 0.20% were ground into powder with 25 μm mean particle size and weighed accurately before filled into the extractor vessel. The extraction conditions were as follows: 0.5 L/min $CO_2$ flow rate, 2 h time, 32 MPa extraction pressure, 50 °C extraction temperature, 9 MPa separate pressure and 45 °C separate temperature.

### 2.3. GC–MS identification

Qualitative and quantitative analysis of lotus germ oil was carried out using a CP3800-Saturn 2200 gas chromatography–mass spectrometry (GC–MS) system (Varian, USA). EI mode was 70 eV and injector temperature was 250 °C. The carrier gas was helium (flow rate of 1.0 mL/min), split ratio, 1:10, and the capillary columns were VF-5 ms (30 m × 0.25 mm × 0.25 μm) (Varian, USA). The temperature programs were 80–260 °C at a rate of 3 °C/min and held at 260 °C for 20 min. Ion source temperature was 200 °C and the scan range was 20–500 amu. The identification of individual compounds was based on comparison of their relative retention times with those of the authentic standards. The compounds without the authentic standards were tentatively identified by comparing their relative retention times with those of the Wiley, NIST and NBS mass spectral databases and the previous publication (Bi et al., 2006).

### 2.4. Tocopherol analysis

Tocopherol was assayed according to the method described by Guo, Li, and Meng (2003). About 1.012 mg/mL DL-α-tocopherol solution was prepared by dissolving 25.3 mg of standard DL-α-tocopherol in hexane. The standard curve of DL-α-tocopherol was obtained by the 960 fluorescence spectrophotometer (Shanghai, China) with an excitation wavelength of 295 nm and an emission wavelength of 325 nm. About 0.1 g lotus germ oil and 0.3 g ascorbic acid were dissolved in 12 mL of absolute ethanol. Then, 3 mL of 60% potassium hydroxide were added in the mixture and saponified for 15 min in the boiled water bath. About 30 mL of distilled water were added in the mixture and moved to a 250 mL separatory funnel. Then, 10 mL of hexane were added in the mixture and shaken for 8 min, and hexane was collected. Two drops of 60% concentrated sulphuric acid were added in the mixture. The mixture was washed with 10 mL of distilled water for three times. About 1 mL of hexane phase solution in the mixture was diluted to 10 mL for the detection. The fluorescence intensities of samples were determined by the fluorescence spectrophotometer with an excitation wavelength of 295 nm and an emission wavelength of 325 nm.

$$\text{Tocopherol content (mg/100 g)} = X \times 10/G$$

Here, $X$ (μg/mL) is the tocopherol content according to the standard curve, $G$ is the sample mass. Results are average value from eight independent samples ($N = 8$).

### 2.5. Hydroxyl free radical scavenging activities

The scavenging activities of hydroxyl free radical were assayed by a modified Fenton reaction protocol (Caillet et al., 2007). The assay system consisted of 1.4 mL of 0.02 mM crystal purple solution, 1.0 mL of 5 mM $Fe^{2+}$ solution, 1.0 mL of tris–HCl (pH 5.5), 1 mL of 2.5 mM $H_2O_2$ and distilled water. The final volume was 10 mL. The different volumes of lotus germ oil were dissolved into 0.1% sucrose ester of fatty acid for emulsification. The different concentrations of the test compounds were incubated at 37 °C for 1 h. The scavenging activities of hydroxyl free radical of positive (contains the lotus germ oil) and control samples were evaluated by the following formula:

$$\text{AA } (\%) = [(A_2 - A_1)/(A_0 - A_1)] \times 100\%$$

where AA is the scavenging activities of hydroxyl free radical, $A_2$ is the absorbance of sample with fenton reagents at 580 nm, $A_0$ is the absorbance of sample without fenton reagents at 580 nm, and $A_1$ is the absorbance of sample with fenton reagents and 0.5 mL of 0.2 M KF.

### 2.6. Superoxide anion free radical scavenging activities

The scavenging activities of superoxide free radical were determined by Marklund and Marklund method (1974). About 0.1 mL of lotus germ extracts (positive) or solvent (control) were added to 2.8 mL of 50 mM tris–HCl buffer for 10 min at 25 °C, then 0.1 mL of 60 mM pyrogallol (positive) or 10 mM HCl (control) were added in the assay system. After the mixture was rapidly shaken, its absorption value was determined at 420 nm in 0.5 min interval for 4 min (keeping the auto-oxidation rate of pyrogallol at 0.050–0.065 OD/min). The changing curve of the absorption value (OD) to time was obtained and the curve slope was defined as antioxidation activities of the sample or scavenging activities of lotus germ extracts on superoxide anion. The scavenging rate was calculated by the following formula:

$$R\ (\%) = \frac{\Delta A'_{420}/\Delta T - \Delta A_{420}/\Delta T}{\Delta A'_{420}/\Delta T} \times 100$$

In this formula, $\Delta A'_{420}/\Delta T(V_0)$ is the auto-oxidation rate of pyrogallol (OD/min), and $\Delta A_{420}/\Delta T(V)$ is the auto-oxidation rate of sample (OD/min).

*2.7. Inhibition of lipid peroxidation on liver and kidney tissues of mice*

The adult male and female mice were housed at 24 ± 2 °C and relative humidity of 30–70%. After the mice were fasted for 16 h and rapidly sacrificed by decapitation. The inhibition of lipid peroxidation was evaluated in accordance with the method of Kessler, Ubeaud, and Jung (2003). Briefly, 5 g of liver or kidney tissues were homogenised in 25 mL of 20 mM tris–HCl buffer (pH 7.4). About 1 mL of 0.5% (w/v) liver or kidney homogenate and 0.1 mL of different concentration lotus germ oil emulsification and the respective controls were incubated at 37 °C for 120 min. After incubation, the mixture was tested for the formation of thiobarbituric acid reactive substances (TBARS) (Sakanaka, Tachibana, Ishihara, & Juneja, 2005). About 1 mL of 15% trichloroacetic acid (TCA) (w/v) and 1 mL of 0.67% thiobarbituric acid (TBA) were added in the test samples and mixed vigorously, then heated in a boiled water bath for 15 min and cooled to room temperature. The samples were centrifuged at 1006*g* for 10 min. The absorbance of the supernatant was measured at 532 nm. The average of quadruplicate analyses was given as result. The inhibition rate (%) of lotus germ oil on the lipid peroxidation was calculated by the following formula:

$$\text{Inhibition rate } (\%) = (A_2 - A_1)/A_2 \times 100\%$$

In which, $A_1$ is absorbance of sample tube with lotus germ oil, and $A_2$ is absorbance of control tube.

*2.8. Inhibition of the haemolysis of red blood cells (RBCs) induced by hydrogen peroxide*

The anti-haemolysis activity was assayed according to the method described by Miki, Tamia, Mino, Yamamoto, and Nki (1987). Blood was collected from male mice eyeballs. The RBCs were separated from plasma by centrifugation at 1006*g* for 20 min. The RBCs were then washed five times with 10 mL of phosphate-buffered saline (PBS, pH 7.4). About 1 mL of 0.5% (v/v) suspension of RBC in PBS was mixed with 0.1 mL of lotus germ oils emulsification of different concentrations. The incubation mixture was shaken gently in a water bath at 37 °C for 10 min. After incubation, 0.5 mL of 50 mM $H_2O_2$ was added to the mixture and incubated in a water bath at 37 °C for 60 min. After that, 4 mL of PBS solution were added to the reaction mixture, followed by centrifugation at 1006*g* for 5 min. The absorbance of the supernatant at 415 nm was recorded in a spectrophotometer. Percentage inhibition was calculated by the following equation:

$$\text{Inhibition rate } (\%) = (A_2 - A_1)/(A_2 - A_3) \times 100\%$$

where $A_1$ is absorbance of sample tube with lotus germ oil, $A_2$ is absorbance of control tube without lotus germ oil, and $A_3$ is absorbance of PBS.

*2.9. Inhibition of lipid peroxidation on lard peroxidation*

Lipid peroxidant value (POV) was measured according to AOAC methods (1990). Lard without additives was used as the substrates to evaluate the antioxidant activity of lotus germ oil. Lotus germ oil was dissolved in 1 mL of 95% ethanol and added to the fats (100 g/treatment) at different concentrations. The control sample contained 1 mL of 95% ethanol only. The lipid system was thoroughly homogenised and stored at 60 ± 1 °C in a water bath for 12 days with occasional stirring with a glass rod.

*2.10. Statistical analysis*

Results were reported as mean ± SD for at least three analyses for each sample. Statistical analyses were performed according to the SPSS-PC package. Analyses of variance were performed using the ANOVA procedure. Significant differences ($p < 0.05$) between the means were determined using Duncan's multiple range test.

## 3. Results and discussion

*3.1. The components of the lotus germ oil*

Fig. 1 and Table 1 show that a total of 21 components are identified as constituents of the lotus germ oil extracted by SFE, comprising about 92.13% of the total oil. The main components were found to be linoleic acid (30.03%), followed by phytol (11.49%), phenolic compounds (9.06%), hexadecanoic acid (7.92%), oleic acid (6.79%), linolenic acid (4.39%), carotene (4.30%), gamma-sitosterol (3.16%) and caryophyllene (3.02%). Clearly, the remarkable high content of unsaturated fatty acids, phytol, phenolic compounds and plant sitosterols constitute the four distinguished characteristics of lotus germ oil. In addition, lotus germ oil also has a distinctly high total tocopherol content of 485.1 ± 50 mg/100 g. Tocopherol is a fat-soluble vitamin that generally functions as a potent antioxidant via chain-breaking reactions during peroxidation of unsaturated lipids and maintains the integrity of cell membranes (Franke, Murphy, Lacyey, & Custer, 2007). The higher phenolic acids contents have been confirmed to be responsible for the stronger antioxidant activity and identified as gallic acid, *p*-hydroxybenzoic acid, chlorogenic acid, and caffeic acid (Yen et al., 2005). Moreover, the lotus germ oil contains high content carotene (4.30%) and gamma-sitosterol (3.16%) which inhibits cholesterol absorption and might reduce the risk of atherosclerosis and gallstones in vivo (Ostlund, 2002). These results show that the lotus germ oil might be used as healthcare food.

*3.2. Hydroxyl free radical scavenging activities*

The hydroxyl free radical scavenging activities of lotus germ oil are shown in Fig. 2. Five different concentrations of lotus germ oil were investigated. In the range of 10–90 mg/mL, the lotus germ oil displayed a significant dose-dependent inhibition on the hydroxyl free radical. However, the $IC_{50}$ value for hydroxyl free radical is found to be about 50 mg/mL, which is two orders of magnitude higher than that of water extracts and organic solvents extracts of lotus seeds (the $IC_{50}$ value is found to be about 0.1 mg/mL (Yen et al., 2005)). For the lotus seeds extracts, the antioxidant activity was found to be in the order: water extracts > ethyl acetate extracts > *n*-hexane extracts (Yen et al., 2005). Obviously, the antioxidant activity of lotus germ oil extracted by SFE is weaker than that of water extracts and organic solvents extracts. This is attributed to the differences of active components in the different solvents extract and the high content of unsaturated fatty in the lotus germ oil extracted by SFE. According to our previous study, both lotus germ water and ethanol extracts could scavenge the oxygen free radicals and have antioxidation ability (Zheng & Zhang, 2005). However, in comparison with our previous results, the lotus germ oil extracted by SFE could scavenge the oxygen free radicals and prevent the lipid peroxidation in the lower dose and has stronger antioxidation ability. The hydroxyl free radical in the biological systems can lead to lipid peroxidation, damage of cells and tissues and a variety of diseases including ageing, cancer, diabetes and rheumatoid arthritis. It has been shown that hydroxyl free radical is the major active oxygen species causing lipid oxidation (Namiki, 1990). The extensive investigations on antiradical and antioxidant activities of phenolic compounds, including flavonoids and phenolic acids have been reported (Manian, Anusuya, Siddhuraju, & Manian, 2008). The total phenolic compounds of lotus seeds showed a significant chelating binding on ferrous ions and marked scavenging of reactive oxygen species (Yen et al., 2005). The high content

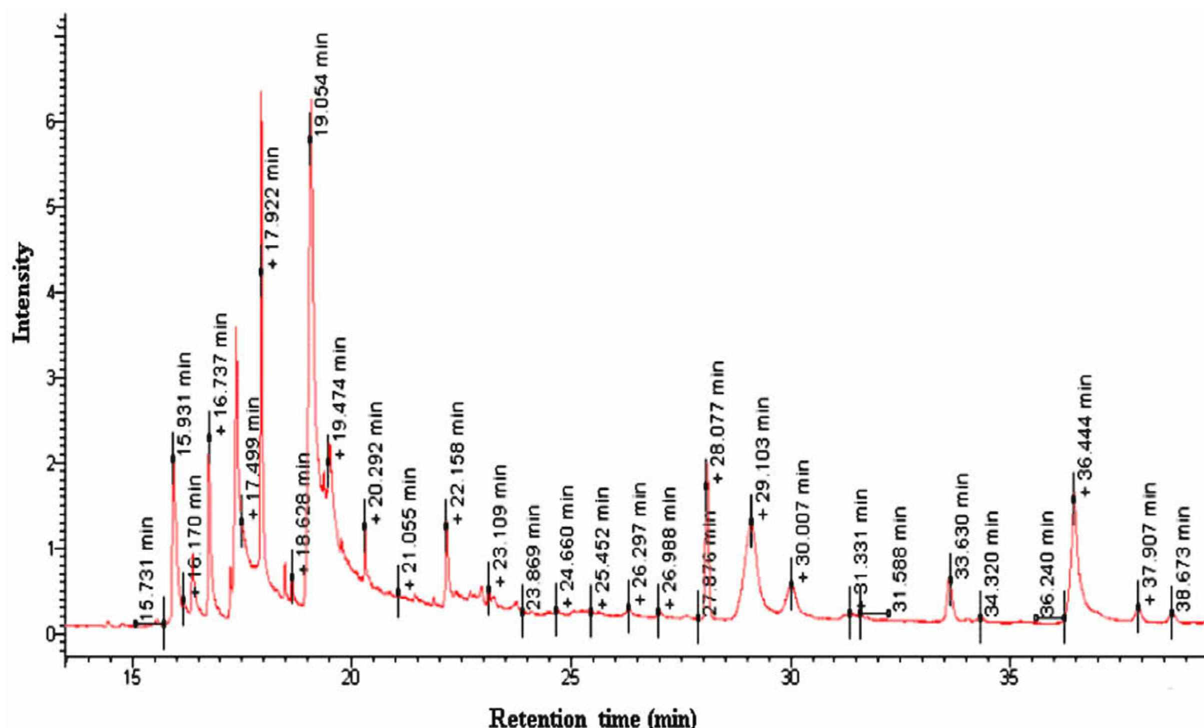



**Fig. 1.** GC–MS chromatogram of the lotus germ oil. A CP3800-Saturn 2200 gas chromatography–mass spectrometry (GC–MS) system was used to separate and identify the components. The temperature programs were 80–260 °C at a rate of 3 °C/min and held at 260 °C for 20 min. The injector and ion source temperatures were 250 and 200 °C, respectively.

**Table 1**
Chemical composition of the lotus germ oil.

| Pick no. | Relative retention times (min) | Components[a] | Relative percentage[b] (%) |
|---|---|---|---|
| 1 | 15.931 | Phytol | 11.49 ± 0.16 |
| 2 | 16.170 | 11-Hexadecyn-1-ol | 2.08 ± 0.02 |
| 3 | 16.538 | 6,9,12,15-Docosatetraenoic acid | 1.02 ± 0.01 |
| 4 | 16.737 | Caryophyllene | 3.02 ± 0.03 |
| 5 | 17.175 | Aromedendrene | 0.58 ± 0.01 |
| 6 | 17.940 | Hexadecanoic acid | 7.92 ± 0.16 |
| 7 | 18.452 | Tetradecen acid | 0.95 ± 0.01 |
| 8 | 18.628 | Estra-1,3,5-trienol | 1.74 ± 0.02 |
| 9 | 19.054 | Linoleic acid | 30.03 ± 0.31 |
| 10 | 19.745 | Linolenic acid | 4.39 ± 0.04 |
| 11 | 20.292 | Oleic acid | 6.79 ± 0.06 |
| 12 | 21.055 | Stearic acid | 0.99 ± 0.02 |
| 13 | 21.436 | Eicosapentaenoic acid | 0.86 ± 0.01 |
| 14 | 22.932 | Ethyl iso-allocholate | 0.68 ± 0.01 |
| 15 | 24.660 | Ursodesoxycholic acid | 0.56 ± 0.01 |
| 16 | 28.077 | Carotene | 4.30 ± 0.05 |
| 17 | 29.103 | Gamma-sitosterol | 3.16 ± 0.04 |
| 18 | 30.007 | Beta-sitosterol | 1.51 ± 0.02 |
| 19 | 31.331 | Betulin | 0.61 ± 0.01 |
| 20 | 36.444 | Phenolic compounds | 9.06 ± 0.11 |
| 21 | | Total tocopherol | 485.1 ± 50 mg/100 g |
| | | Total identification | 92.13 |

[a] Components were identified by the comparison of each retention time with authentic standards or diagnosed by the mass spectral databases from the Wiley, NIST and NBS and the previous publications, at least with a library fit factor >90%.
[b] Area percentage of individual profiles was used as the relative content of the corresponding components. Values are mean ± standard deviation of triplicate determinations.

phenolic compounds and tocopherol in the lotus germ oil could partially account for the antioxidant activity. The lotus germ oil extracted by SFE could quench hydroxyl radical. The results farther support the previous report (Bi et al., 2006; Yen et al., 2005, 2006). Other important parts of the antioxidant activity for lotus germ oil, which may not be neglected, may result from the high content of unsaturated fatty and carotene. The detailed mechanism of antioxidant activity for lotus germ oil is being studied in our laboratory.

### 3.3. Superoxide anion free radical scavenging activities

The scavenging effect of lotus germ oil on superoxide anion free radical is shown in Fig. 3. In the range of 10–70 mg/mL, the extract

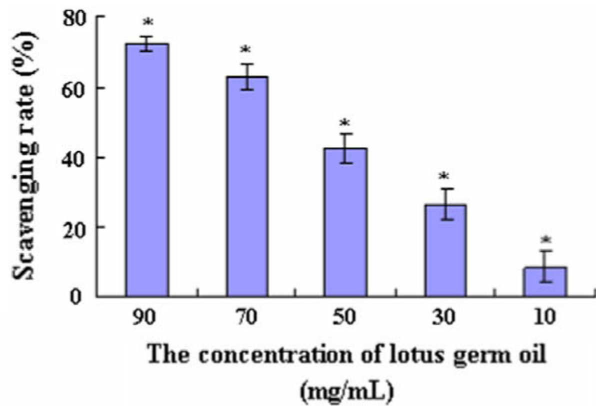



**Fig. 2.** Scavenging effects of lotus germ oil on hydroxyl free radical. Results are means ± SD for $n = 6$. An asterisk (*) indicates $p < 0.05$ compared to control group (without the lotus germ oil).

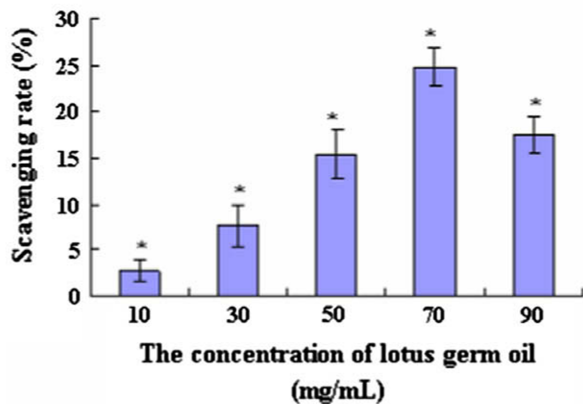



**Fig. 3.** Scavenging effects of lotus germ oil on superoxide anion free radical. Results are means ± SD for $n = 6$. An asterisk (*) indicates $p < 0.05$ compared to control group (without the lotus germ oil).

also showed a dose-dependent inhibition on the superoxide anion free radical. The scavenging ratio on superoxide anion free radical reached a plateau (about 25%) at the 70 mg/mL dose and then declined. In comparison with the scavenging effects on the hydroxyl radical, the scavenging effects of louts germ oil on the superoxide anion free radical was rather low.

### *3.4. Inhibition effects on lipid peroxidation in liver and kidney tissues of mice*

Mice liver and kidney were used to evaluate antioxidant activities of lotus germ oil in vitro. Malondialdehyde (MDA) is one of cytotoxic products of lipid peroxidation reaction and a widely used marker of the oxidation. The inhibition of MDA formation in liver and kidney tissue homogenates by the lotus germ oil is shown in Fig. 4. With the increasing concentration of the lotus germ oil from 0.05 to 1.0 mg/mL, the inhibition ratio of MDA formation both in liver and kidney tissue homogenates presented a dose-dependent manner. Lotus germ oil could inhibit the MDA level by 83% and 94% in the liver and kidney tissue homogenates at the highest dose (1 mg/mL), respectively. The $IC_{50}$ value is about 0.15 mg/mL for inhibition of MDA formation in liver and kidney tissue homogenates. The data indicated that lotus germ oil significantly inhibited MDA formation in liver and kidney tissue and could protect the cell membrane lipid oxidation.

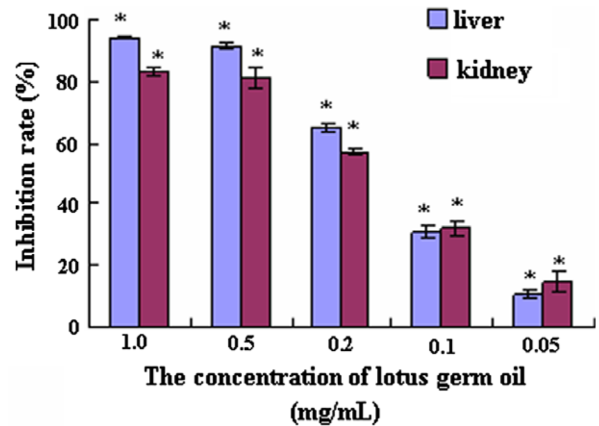



**Fig. 4.** Inhibition effects of lotus germ oil on the lipid peroxidation in liver and kidney tissues of mice. Results are means ± SD for $n = 6$. An asterisk (*) indicates $p < 0.05$ compared to control group (without the lotus germ oil).

### *3.5. Inhibition effects on the haemolysis of RBC induced by hydrogen peroxide*

The lipid peroxidative degradation of biomembrane is one of the cytotoxic principles. The accumulated $H_2O_2$ in vivo can result in lipid peroxidation, RBC auto-haemolysis. The in vitro model of RBC haemolysis was generally used to evaluate the antiradical and antioxidant activities by mimicking this situation. The inhibition effects of lotus germ oil on the RBC haemolysis induced by $H_2O_2$ are shown in Fig. 5. The results indicated that the lotus germ oil could prevent RBC haemolysis in a dose-dependent manner. At the dose >2.0 mg/mL, lotus germ oil displayed a significant inhibition of RBC haemolysis ($p < 0.05$). The $IC_{50}$ value for inhibition of RBC haemolysis is 8.0 mg/mL. These results furthermore validated that the lotus germ oil could protect the cell membrane lipid oxidation in biological system and possesses antioxidant activity.

### *3.6. Inhibition effects on lard peroxidation*

To farther investigate the antioxidation activities effect of lotus germ oil, the lard auto-oxidation system was tested under controlled conditions and POV was measured according to AOAC (1990). Pure lard without any additives has been widely used as

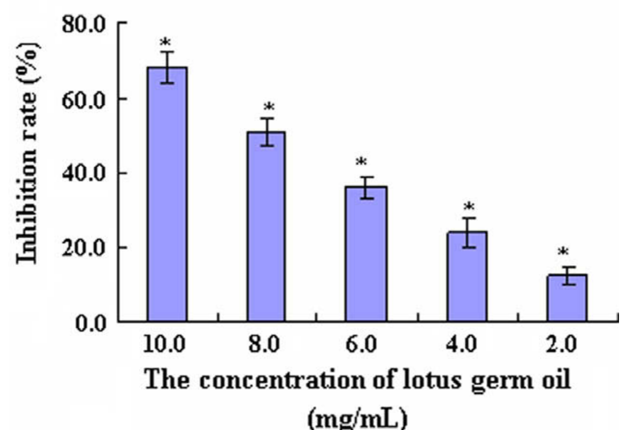



**Fig. 5.** Inhibition effects of lotus germ oil on the haemolysis of RBC induced by $H_2O_2$. Results are means ± SD for $n = 6$. An asterisk (*) indicates $p < 0.05$ compared to control group (without the lotus germ oil).

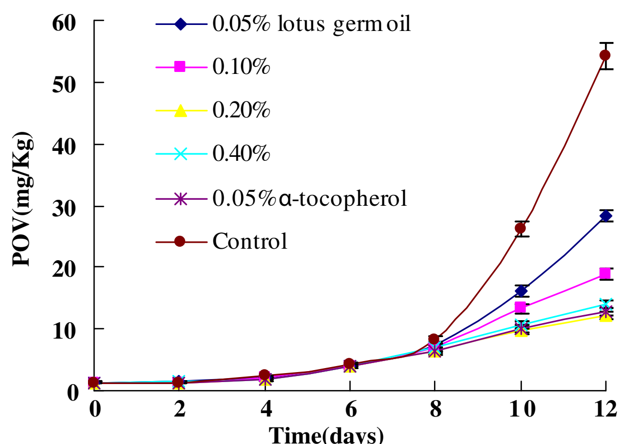



**Fig. 6.** Inhibition effects of lotus germ oil on the lard oxidation. Results are means ± SD for $n = 3$. The control does not contain lotus germ oil.

oxidation substrate to evaluate the antioxidant activity in lipid system. The results showed that the addition of lotus germ oil have significant antioxidation ability after eight days incubation (Fig. 6) ($p < 0.05$). The antioxidant activity of lotus germ oil reached the maximum at 0.2% dose after 12 days incubation ($p < 0.05$). There were significant differences between 0.4% and 0.2% of lotus germ oil after eight days incubation ($p < 0.05$). In comparison with the 0.05% (w/w) α-tocopherol, the same concentration (w/w) lotus germ oil obviously has a low antioxidation ability in the lard auto-oxidation system. This may be attributed to low concentration α-tocopherol in the 0.05% lotus germ oil and its high content of unsaturated fatty.

In summary, lotus germ oil extracted by SFE showed distinct characteristics in composition which were abundant in phenolic compounds and tocopherols. The antioxidant activities revealed that lotus germ oil could obviously scavenge ROS and inhibit the lipid peroxidation in liver and kidney homogenate, decrease the haemolysis of RBC induced by $H_2O_2$ and prevent the lard peroxidation. The antioxidant activity of lotus germ oil may be attributed to the high content phenolic compounds and tocopherols. These results suggest the lotus germ oil is a high quality natural oil source and can be developed as healthcare oil. This paper is the first report for antioxidant activity of lotus germ oil. However, further studies on the antioxidative components of lotus germ oil and more in vivo evidence are required.

## Acknowledgment

This work was supported by the Project of the Natural Science Foundation of the Jiangsu Higher Education Institution of China (Grant No. 07KJD550115).

## References

AOAC (1990). *Methods of analysis* (15th ed.). Washington, DC: Association of Official Analytical Chemists. p. 72.

Bi, Y. L., Yang, G. L., Li, H., Zhang, G. W., & Guo, Z. (2006). Characterization of the chemical composition of lotus plumule oil. *Journal Agricultural and Food Chemistry, 54*, 7672–7677.

Borgi, W., Reciob, M. C., Ríos, J. L., & Chouchane, N. (2008). Anti-inflammatory and analgesic activities of flavonoid and saponin fractions from *Zizyphus lotus* (L.) Lam. *South African Journal of Botany, 74*, 320–324.

Caillet, S., Yu, H., Lessard, S., Lamoureux, G., Ajdukovic, D., & Lacroix, M. (2007). Fenton reaction applied for screening natural antioxidants. *Food Chemistry, 100*, 542–552.

Diaaz-reinoso, B., Moure, A. S., Domianguez, H., & Parajoa, J. C. (2006). Supercritical $CO_2$ extraction and purification of compounds with antioxidant activity. *Journal Agricultural and Food Chemistry, 54*, 2441–2469.

Franke, A. A., Murphy, S. P., Lacyey, R., & Custer, L. (2007). Tocopherol and tocotrienol levels of foods consumed in Hawaii. *Journal Agricultural and Food Chemistry, 55*, 769–778.

Guo, J., Li, M., & Meng, Y. (2003). Fluormetric method for the determination of tocopherol in food. *Chinese Journal of Health Laboratory Technology (Chinese), 13*(3), 348–349.

Hu, M., & Skibsted, L. H. (2002). Antioxidative capacity of rhizome extract and rhizome knot extract of edible lotus (*Nelumbo nuficera*). *Food Chemistry, 76*, 327–333.

Hu, Q. H., Xu, J., Chen, S. B., & Yang, F. M. (2004). Antioxidant activity of extracts of black sesame seed (*Sesamum indicum* L.) by supercritical carbon dioxide extraction. *Journal Agricultural and Food Chemistry, 52*, 943–947.

Kashiwada, Y., Aoshima, A., Ikeshiro, Y., Chen, Y. P., Furukawa, H., Itoigawa, M., et al. (2005). Anti-HIV benzylisoquinoline alkaloids and flavonoids from the leaves of *Nelumbo nucifera*, and structure–activity correlations with related alkaloids. *Bioorganic and Medicinal Chemistry, 13*, 443–448.

Kessler, M., Ubeaud, G., & Jung, L. (2003). Anti- and pro-oxidant activity of rutin and quercetin derivatives. *Journal Pharmacy Pharmacology, 55*, 131–142.

Liu, C. P., Tsai, W. J., Shen, C. C., Lin, Y. L., Liao, J. F., Chen, C. F., et al. (2006). Inhibition of (S)-armepavine from *Nelumbo nucifera* on autoimmune disease of MRL/MpJ-lpr/lpr mice. *European Journal Pharmacology, 531*, 270–279.

Lou, H. X., Yuan, H. Q., & Ji, M. (1995). Studies on the composition of the lotus plumule. *Journal Shandong Medicine University (Chinese), 33*, 347–348.

Manian, R., Anusuya, N., Siddhuraju, P., & Manian, S. (2008). The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. *Food Chemistry, 107*, 1000–1007.

Marklund, S., & Marklund, G. (1974). Involvement of superoxide anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal Biochemistry, 47*, 469–472.

Miki, M., Tamia, H., Mino, M., Yamamoto, Y., & Nki, E. (1987). Freeradical chain oxidation of rat read blood cells by molecular oxygen and its inhibition by a-tocopherol. *Archives of Biochemistry Biophysics, 258*, 373–380.

Moskovitz, J., Yim, M. B., & Chock, P. B. (2002). Free radicals and disease. *Archives of Biochemistry Biophysics, 397*(2), 354–359.

Mukherjee, P. K. (2002). *Quality control of herbal drugs – An approach to evaluation of botanicals* (1st ed.). New Delhi, India: Business Horizons. pp. 604–608.

Mukherjee, P. K., Das, J., Balasubramanian, R., Saha, K., Pal, M., & Saha, B. P. (1995). Antidiarrhoeal evaluation of *Nelumbo nucifera* rhizome extract. *Indian Journal of Pharmacology, 27*, 262–364.

Mukherjee, P. K., Pal, S. R., Saha, K., & Saha, B. P. (1995). Hypoglycemic activity of *Nelumbo nucifera* rhizome (methanolic extract) in streptozotocin induced diabetic rats. *Phytotherapy Research, 9*, 522–524.

Mukherjee, P. K., Saha, K., Das, J., Giri, S. N., Pal, M., & Saha, B. P. (1996). Antipyretic activity of *Nelumbo nucifera* rhizome extract. *Indian Journal of Experimental Biology, 82*, 274–276.

Mukherjee, P. K., Das, J., Saha, K., Pal, M., & Saha, B. P. (1996). Diuretic activity of the rhizomes of *Nelumbo nucifera* Gaertn. (Fam. Nymphaeaceae). *Phytotherapy Research, 10*, 424–425.

Namiki, M. (1990). Antioxidants/antimutagens in foods. *CRC Critical Reviews in Food Science Nutrition, 29*, 273–300.

Ono, Y., Hattori, E., Fukaya, Y., Imai, S., & Ohizumi, Y. (2006). Anti-obesity effect of *Nelumbo nucifera* leaves extract in mice and rats. *Journal of Ethnopharmacology, 106*, 238–244.

Ostlund, R. E. Jr., (2002). Phystosterols in humane nutrition. *Annual Review Nutrition, 22*, 533–549.

Pahlavani, M. A., & Harris, M. D. (1998). Effect of *in vitro* generation of oxygen free radicals on T cell function in young and old rats. *Free Radical Biology Medicine, 25*(8), 903–913.

Qian, J. Q. (2002). Cardiovascular pharmacological effects of bisbenzylisoquinoline alkaloid derivatives. *Acta Pharmacologica Sinica, 23*, 1086–1092.

Rai, S., Wahile, A., Mukherjee, K., Pada, B. S., & Mukherjee, P. K. (2006). Antioxidant activity of *Nelumbo nucifera* (sacred lotus) seeds. *Journal of Ethnopharmacology, 104*, 322–327.

Sakanaka, S. J., Tachibana, Y. M., Ishihara, O., & Juneja, L. R. (2005). Antioxidant properties of casein calcium peptides and their effects on lipid oxidation in beef homogenates. *Journal Agricultural and Food Chemistry, 53*, 464–468.

Sohn, D. H., Kim, Y. C., Oh, S. H., Park, E. J., Li, X., Lee, B. H., et al. (2003). Hepatoprotective and free radical scavenging effects of *Nelumbo nucifera*. *Phytomedicine, 10*, 165–169.

Yen, G. C., Duh, P. D., & Su, H. J. (2005). Antioxidant properties of lotus seed and its effect on DNA damage in human lymphocytes. *Food Chemistry, 89*, 379–385.

Yen, G. C., Duh, P. D., Su, H. J., Yeh, C. T., & Wu, C. H. (2006). Scavenging effects of lotus seed extracts on reactive nitrogen species. *Food Chemistry, 94*, 596–602.

Zheng, T. S., & Zhang, M. (2005). Scavenging effects of lotus germ extraction on superoxide anion and hydroxyl radical. *Shi Pin Ke Xue (Chinese), 26*(8), 357–360.