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Mushroom extracts with antioxidant activity against lipid peroxidation

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Abstract

Methanol and water extracts, with antioxidant activity, from two edible mushrooms (*Lentinus edodes* and *Volvariella volvacea*) were subfractionated by liquid–liquid partition using organic solvents and by membrane ultrafiltration, respectively. The dichloromethane subfraction of the methanol extract of *V. volvacea* and the low molecular weight (LMW) subfraction of the water extract of *L. edodes* had the highest antioxidant activity against lipid peroxidation of rat brain homogenate with IC_{50} values of 0.109 and 1.05 mg/ml, respectively. The ethyl acetate subfraction of the methanol extract of *V. volvacea* was found to have comparable antioxidant activity ($p > 0.05$) to caffeic acid against the oxidation of human low-density lipoprotein (LDL). The antioxidant activities against lipid peroxidation in the above assays were found to correlate with the phenolic content in different subfractions of mushroom extracts.

Keywords: Antioxidant activity; LDL oxidation; Phenolic compound; Mushrooms

1. Introduction

Lipid peroxidation is a major cause of food deterioration, affecting colour, flavour, texture and nutritional value. Besides, it has been suggested that oxidative modification of low-density lipoproteins (LDLs) may play a role in the development of atherosclerosis (Jialal & Devaraj, 1996). The oxidative modification depends on a common initiating step – the peroxidation of polyunsaturated fatty acid components in the LDLs (Steinberg, Parthasarathy, Carew, Khoo, & Witztum, 1989). Such modification of LDLs can be inhibited by antioxidants (Frankel & Kanner, 1993; Mangiapane et al., 1992). There is also increasing clinical, experimental and epidemiological evidences that dietary antioxidants are important in the prevention of coronary heart diseases (Adams & Wermuth, 1999; Maxwell & Lip, 1997; Singh & Downing, 1995; Witztum, 1994). In order to minimize the oxidative damage, various dietary sources of antioxidants, such as white cabbage and eggplant, have been studied (Gazzani, Papetti, Massolini, & Daglia, 1998). Phenolic compounds were found to have antioxidant activity in the inhibition of LDL oxidation (Vinson, Dabbagh, Serry, & Jang, 1995; Teissedre & Waterhouse, 2000). Proteins, such as whey protein (Tong, Sasaki, McClements, & Decker, 2000) and protein hydrolyzates (Shahidi & Amarowicz, 1996) or amino acids (e.g., proline, methionine) (Amarowicz & Shahidi, 1997) were also found to have antioxidant activity. Recently, mushrooms are considered to be a good source of protein (Breene, 1990) and phenolic antioxidants, such as variegatic acid and diboviquinone, which have been found in mushrooms (Kasuga, Aoyagi, & Sugahara, 1995). Based on our previous study, the methanol and water extracts from two mushroom varieties (*Lentinus edodes* and *Volvariella volvacea*) were found to have antioxidative activities which correlated with their phenolic contents (Cheung, Cheung, & Ooi, 2003). In this report, the subfractions of the methanol and water extracts from these two mushrooms were further investigated, specifically for their inhibition of lipid peroxidation.

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2. Materials and methods

2.1. Extraction

The two mushroom samples, *Lentinus edodes* and *Volvariella volvacea*, were purchased from a local market and treated as previously described (Cheung et al., 2003). Mushroom powders (140 g) were extracted in a Soxhlet extractor (5 l) sequentially with 2 l of petroleum ether, ethyl acetate and methanol for 24 h. The extraction solvents were removed using a rotary evaporator (BÜCHI Rotavapor R-144, Switzerland) and the residues were collected as the extracts and stored at 4 °C. The insoluble materials in the thimble were air-dried before each solvent extraction was started.

The methanol-insoluble residues were extracted for 4 h with boiling water, four times. The sample to solvent ratio (v/v) in the aqueous extraction was 1:2. After the extraction, the water extract was freeze-dried (Labconco, MO). The methanol and water extracts were then fractionated into different subfractions by liquid–liquid partition and membrane ultrafiltration, respectively.

2.2. Fractionation

2.2.1. Fractionation of the methanol crude extract

The methanol extract (~1 g) of the two mushrooms was dissolved in distilled water (100 ml for *V. volvacea* and 150 ml for *L. edodes*). The dissolved methanol extract was then partitioned sequentially in three different solvents, dichloromethane, ethyl acetate and n-butanol in the ratio 1:1 (v/v), three times at room temperature, resulting in four different subfractions.

2.2.2. Fractionation of boiling water crude extract

Two grammes of the water extract of the two mushrooms were dissolved in 50 ml of distilled water and then the solutions were subjected to ultrafiltration (Amicon 8400) with a membrane of 10,000 Da molecular weight cut-off. After filtration, the retentate (high molecular weight) and filtrate (low molecular weight) were freeze-dried separately as the two subfractions of the crude water extract.

2.3. Antioxidant activity assays

2.3.1. Assays of lipid peroxidation using rat brain tissue

All the subfractions of the methanol and water extracts were evaluated for their inhibition of lipid peroxidation according to the procedures described by Ng, Liu, and Wang (2000) with some modifications.

Brains of Sprague–Dawley (SD) rats were dissected and homogenized with a Polytron in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate which was centrifuged at 3000*g* for 10 min. A 0.1 ml aliquot of the supernatant was incubated with 0.2 ml of the subfractions of the methanol (0.05–20 mg/ml) and water (0.5–20 mg/ml) crude extracts in the presence of 0.1 ml of 10 μM $FeSO_4$ (AnalaR) and 0.1 ml of 0.1 mM ascorbic acid (Sigma) at 37 °C for 1 h. The reaction was stopped by the addition of 0.5 ml trichloroacetic acid (Univar) (TCA, 28%, w/v), followed by 0.38 ml thiobarbituric acid (Sigma) (TBA, 2%, w/v), and the mixture was then heated at 80 °C for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm using a spectrophotometer (Spectronic Genesys, Milton Roy CO., Ivyland, PA, USA). Butylated hydroxyanisole (BHA, Sigma, St. Louis, MO, USA) (0.2 mg/ml) was used as a positive control. The inhibition ratio (%) was calculated using the following formula:

$$\text{Inhibition ratio } (\%) = [(A - B)/A] \times 100\%, \quad (1)$$

where A and B were the absorbance of the control and the sample, respectively.

2.3.2. Human LDL oxidation

The antioxidant activities of the subfractions of the methanol and crude water extracts were further assessed by human LDL oxidation. The preparation of human LDL was carried out according to the procedures described by Zhang, Chan, Luk, Ho, and Chen (1997). Fresh blood samples were collected from healthy human subjects at the Prince of Wales Hospital in Hong Kong.

The extent of LDL oxidation was monitored by measuring the production of thiobarbituric acid reactive substances (TBARS), as described by Buege and Aust (1978). LDL stock solution was dialyzed against 50–100 volumes of the degassed dialysis solution (0.01 M sodium phosphate, 0.9% NaCl, 10 μM EDTA and 0.05% NaN_3 at pH 7.4) for 24 h in the dark, four times. A 0.4 ml aliquot of dialyzed LDL (250 μg/ml) was incubated with 50 μl of 50 μM $CuSO_4$ and 50 μl mushroom subfractions (1 mg/ml) or water (as control) at intervals of 4, 12 and 36 h at 37 °C. The reaction was stopped by addition of 25 μl of 1% EDTA and the mixture was cooled to 4 °C. Two millilitres of TBA–TCA–HCl solution (0.67 g of thiobarbituric acid and 15 g of trichloroacetic acid in 100 ml of 0.1 N HCl) were added to the mixture and it was heated at 95 °C for 1 h before cooling in an ice-bath. TBARS formed were then determined by measuring the absorbance at 532 nm. The value of TBARS was expressed in nmol MDA/mg LDL by a calibration curve constructed from malondialdehyde (MDA) (0–25 nmol/ml) in tetramethoxypropane with caffeic acid as standard.

2.4. Determination of total phenolic content

The concentrations of phenolic compounds in the subfractions of the methanol and water extracts were measured according to the method of Singleton and Rossi (1965) with some modifications described previously (Cheung et al., 2003). Phenolic content in mushroom subfractions was expressed as gallic acid equivalents (GAE).

2.5. Crude protein content

Total nitrogen was determined by the Kjeldahl method (AOAC, 1995) using a digestion apparatus (Kjeldahl 6040 Digestion Unit, Gerhardt) and a titration system (Kjeltec System 1002 Distilling Unit, Tecator). The protein content was calculated with a conversion factor of 4.38, taking into account the non-protein nitrogen in mushroom (Breene, 1990).

2.6. Thin-layer chromatography

Three thin-layer chromatography (TLC) plates, coated with silica gel G (Fluka Chemie, Switzerland) to 0.25 mm thickness, were each spotted with 20 μl of the three subfractions (dichloromethane, ethyl acetate and butanol), at a concentration of 2.5%, obtained from the methanol extract of the two mushrooms. All plates were then developed in a solvent system of ethyl acetate/methanol/water (10:2:1; v/v/v). After drying, one of the developed plates was first observed under UV light at a wavelength of 365 nm and sprayed with 0.4 mM DPPH radical in methanol (Espin, Soler-Rivas, & Wichers, 2000). Furthermore, the other two developed TLC plates were sprayed separately with Spray 1 solution [1% solution of iron(III) chloride in water mixed immediately before use with an equal volume of a 1% solution of potassium hexacyanoferrate(III) in water (Barton's Reagent)] which gave a blue colour in the presence of phenolic compounds, and Spray 2 solution [2% iron(III) chloride in ethanol] which, when heated to 105 °C for 5–10 min, gave either a blue colour, indicating the presence of phenolics with trihydroxy groups or a green colour, indicating phenols with dihydroxy groups or a red/brown colour indicating the presence of other phenolics.

2.7. Statistical analysis

All analyses were performed in triplicate except for the protein content of the subfractions which were done in duplicate. The data were expressed as means ± standard deviations and one-way analysis of variance (ANOVA) and Student's t test were carried out to test for any significant differences between the means by SPSS (version 10.1 for Windows 98, SPSS Inc.). Correlations were obtained by Pearson correlation coefficient in bivariate correlations. Differences between means at 5% ($p \leqslant 0.05$) level were considered significant.

3. Results and discussion

3.1. Yield of extraction and fractionation

The yield of the petroleum ether and the ethyl acetate extracts were less than 10% (data not shown) and no further work was carried out on these two extracts. The yield of methanol extract (% dry weight of mushroom) from the two mushrooms was 36.1% for *L. edodes* and 30.8% for *V. volvacea*. The yield (% dry weight of mushroom) of water extract of *L. edodes* and *V. volvacea* were 18.0% and 21.4%, respectively. The yields of the methanol and water extracts in the present large-scale extraction were similar to those of the previous small-scale extraction (Cheung et al., 2003).

The chemical components in the methanol extract were fractionated by solvents of increasing polarity to give the dichloromethane, ethyl acetate, butanol and water subfractions. The proportion of the subfractions of the methanol extract is shown in Table 1. The distributions of chemical components in the four solvents were different in the two mushrooms. In *L. edodes*, nearly half (49% and 29%) of the components were extracted by the butanol and water subfractions, respectively. In *V. volvacea*, most of the solutes in the methanol crude extract remained in the water subfraction with a very small amount of substances being found in the other subfractions (Table 1). The major chemical components in the methanol extract of both mushrooms seemed to be high in polarity.

The water extract was fractionated by ultrafiltration according to the molecular size of the chemical components, which were divided into high molecular weight (HMW) and low molecular weight (LMW) subfractions (Table 1). The two mushrooms had a relatively larger amount of HMW materials than LMW ones, probably due to the extraction of the low molecular weight compounds by methanol prior to the water extraction.

Table 1
Yield (% of crude extract) of the subfractions of the water and methanol extracts from *L. edodes* and *V. volvacea*

Fractions	*L. edodes*	*V. volvacea*
Methanol extract		
Dichloromethane subfraction	16.3	4.15
Ethyl acetate subfraction	6.04	1.02
Butanol subfraction	49.0	9.94
Water subfraction	28.7	84.9
Water Extract		
High molecular weight subfraction (>10,000 Da)	56.7	80.4
Low molecular weight subfraction(<10,000 Da)	43.2	19.6

3.2. Chemical characterization of subfractions

3.2.1. Protein content

Hydrolysed proteins from many animal and plant sources, individual peptides and amino acids have been found to possess antioxidant activity (Amarowicz & Shahidi, 1997). The antioxidant activity of the HMW fraction (MWt > 3500) of whey from pasteurized milk increased with its concentration, as determined by its ability to inhibit TBARS and lipid peroxide formation (Tong et al., 2000). The protein contents of the HMW and LMW subfractions were therefore evaluated as a potential antioxidant source of the two mushrooms. The protein contents of the LMW subfraction for *L. edodes* and *V. volvacea* after ultrafiltration were 28.7% and 22.6%, respectively, which were significantly ($p < 0.05$) higher than those of the HMW subfraction (3% and 6%, respectively). The LMW subfraction of these two mushrooms might contain considerable amounts of free amino acids or small peptides, as reported previously (Mau, Chyau, Li, & Tseng, 1997; Terashita, Kono, Mishima, Obata, & Yamauchi, 1990).

3.3. Total phenolic content

The total phenolic contents of most of the subfractions (except LMW) of both the methanol and water extracts of *V. volvacea* were significantly higher than those of *L. edodes* (Table 2). The phenolic compounds in these mushrooms were preferentially extracted in the low-polarity fractions, such as the dichloromethane and ethyl acetate subfractions, with the highest concentration being found in the ethyl acetate subfraction of *V. volvacea* (Table 2).

3.4. Antioxidant activity

3.4.1. Assay for lipid peroxidation of rat brain

The inhibition of lipid peroxidation on rat brain homogenates, as measured by the colour intensity of MDA-TBA complex, by the six subfractions of the two mushrooms showed similar concentration-dependence in the tested concentration range, except for the water subfraction, of *L. edodes* which showed no inhibition at all (data not shown).

The subfractions of the methanol extract of *V. volvacea* generally showed a higher inhibition percentage of lipid peroxidation than those of *L. edodes*, except for the butanol subfraction, as indicated by a lower IC_{50} value in the former (Table 2). In contrasts the subfractions of the water extract of *L. edodes* had a lower IC_{50} value than those of *V. volvacea*.

The lowest IC_{50} value (0.109 mg/ml) was found in the dichloromethane subfraction of *V. volvacea*, suggesting that this subfraction could be a potential source of hydroxyl radical scavenger in inhibiting lipid peroxidation.

A strong negative correlation was found between total phenolic contents (GAE) in the subfractions for both mushrooms (Table 2) and their IC_{50} values (Table 2), especially in the dichloromethane subfraction ($R = -1.00$, $p < 0.001$) and ethyl acetate subfraction ($R = -0.998$, $p < 0.001$). These showed that the antioxidant activity of these mushroom subfractions was related to their phenolic content.

There was also a strong negative correlation ($R = -0.962$, $p < 0.05$) between the IC_{50} value for inhibition of lipid peroxidation (Table 2) and protein content in the LMW subfractions of the crude water extracts for both mushrooms. The higher antioxidant activities of the LMW subfractions seemed to correlate with their larger amount of protein/ free amino acids.

Although no mushroom extract could give a similar percentage of inhibition of lipid peroxidation at the same concentration (0.1 mg/ml) as BHA (93.6%, data not shown), some subfractions exhibited a similar inhibition percentage to BHA, though at a higher concentration. For example, the dichloromethane subfractions of *V. volvacea* at 0.109 mg/ml could exhibit 50% inhibition percentage, which indicated that the presence of some potent antioxidants in this subfraction.

Table 2
Concentrations of total phenolics (μmoles of GAEs[a]/mg of extract) in different subfractions of methanol and water extracts of *L. edodes* and *V. volvacea*[b] and their IC_{50} values (mg/ml) of inhibition of lipid peroxidation of rat brain homogenate

Subfraction	Concentration of total Phenolics		IC_{50} value	
	L. edodes	*V. volvacea*	*L. edodes*	*V.volvacea*
Dichloromethane subfraction	0.060 ± 0.002c	0.253 ± 0.003d	0.297	0.109
Ethyl acetate subfraction	0.085 ± 0.016c	0.502 ± 0.016d	0.434	0.221
Butanol subfraction	0.063 ± 0.006c	0.160 ± 0.023d	0.825	1.47
Water subfraction	0.024 ± 0.001c	0.041 ± 0.002d	>20.0	11.9
LMW subfraction	0.118 ± 0.021c	0.098 ± 0.017c	1.05	5.26
HMW subfraction	0.016 ± 0.004c	0.068 ± 0.003d	11.0	14.9

[a] GAEs, gallic acid equivalents.
[b] Values expressed are means ± SD of triplicate measurements. Means in the same row with different letters (c, d) were significantly different ($p < 0.05$, Student's *t*-test).

3.4.2. LDL oxidation

Antioxidant effects of various mushroom subfractions on the production of TBARS were examined by incubating human LDL in the presence of 5 μM $CuSO_4$ as an oxidation initiator. A lower level of TBARS implied that LDL oxidation was inhibited. The levels of TBARS (nmol MDA/mg of LDL protein) produced in the presence of the LMW subfraction from *L. edodes* (12.2 ± 0.630), the dichloromethane subfraction (9.62 ± 0.801), the ethyl acetate subfraction (10.2 ± 1.07) as well as the butanol subfraction from *V. volvacea* (10.3 ± 3.20) were all significantly lower than that of the control (43.0 ± 19.2) ($p < 0.05$) at the 4th h (Figs. 1 and 2). After incubating for 36 h, only the ethyl acetate subfraction from *V. volvacea* (17.1 ± 3.33) had a TBARS level that was as low as the standard caffeic acid (15.3 ± 6.02), and lower than the control (48.0 ± 1.24) ($p < 0.05$) and the rest of the other subfractions (Fig. 2). These results showed that the inhibitory effect of the ethyl acetate subfraction of *V. volvacea* on the production of TBARS in Cu^{2+}-mediated oxidation of human LDLs was the most persistent among all mushroom subfractions which was similar to epicatechin at 20 μM of jasmine green tea (Zhang et al., 1997). The results also showed that LMW subfractions of both mushrooms gave strong inhibition of LDL oxidation, though the inhibitory effect was not so long lasting as that of the ethyl acetate subfraction (Figs. 1 and 2). The subfractions from *V. volvacea* seemed to exhibit an overall stronger inhibition of LDL oxidation than those from *L. edodes* (Figs. 1 and 2).

An inverse correlation between total phenolic content of the subfractions, for both mushrooms, and the level of TBARS production was found. The level of TBARS in the presence of the dichloromethane subfractions at the 4th h showed a negative correlation ($R = -0.918$, $p < 0.05$) with the total phenolic content (GAE). At the same time, the level of TBARS in the presence of butanol subfractions at the 4th h showed a negative correlation with the total phenolic content in the subfractions ($R = -0.958$, $p < 0.01$). Also, the ethyl acetate subfractions are found to have negative correlation with phenolic content throughout the incubation period with an *R*-value of –0.971 at 4th h ($p < 0.01$). The results showed that the inhibitory effect on LDL oxidation in these fractions might be related to their content of phenolic compounds. However, there was no correlation shown between the other subfractions with either their total amount of phenolics or protein content ($p > 0.05$).

As oxidatively modified LDL may play a role in the pathogenesis of atherosclerosis, the prevention of LDL oxidation may reduce the risk of this disease. In this study, it has been demonstrated that the polyunsaturated fatty acids in LDL could be protected from oxidation by the dichloromethane, ethyl acetate and butanol subfractions of the methanol crude extract, especially the ethyl acetate subfraction obtained from *V. volvacea*. The presence of phenolic compounds in these subfractions might account for the prevention of LDL oxidation.

3.5. Thin-layer chromatography

The chemical components, especially the phenolic compounds in the subfractions, of the methanol extracts were detected by TLC with the use of UV absorption and specific spraying reagents. A solvent system, ethyl acetate:methanol:water (EMW) (10:2:1, v/v/v) was used to separate the chemical components in each subfrac-

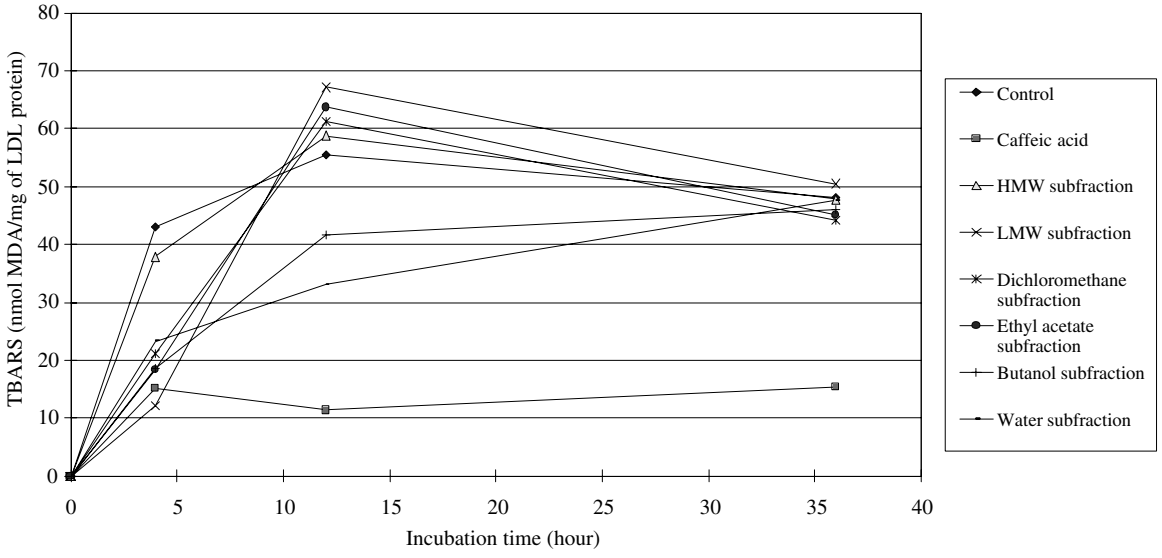

Fig. 1. Inhibitory effects of different subfractions from *L. edodes* on production of TBARS in LDL oxidation.

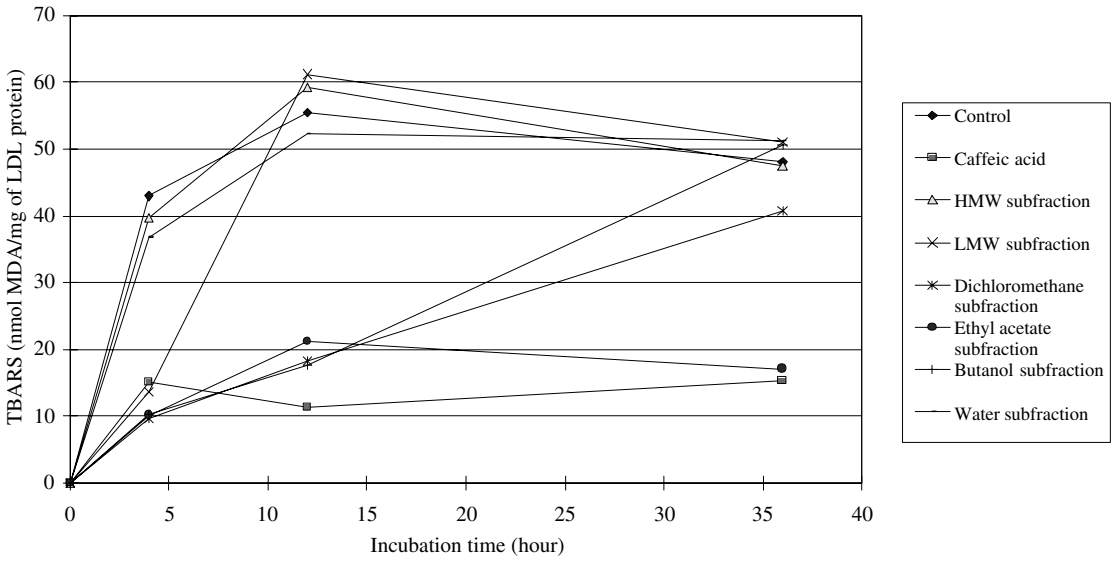

Fig. 2. Inhibitory effects of different subfractions from *V.volvacea* on production of TBARS in LDL oxidation.

Table 3
The R_f values of the chemical compounds of the subfractions of the mushroom methanol extracts which showed positive results to tests of antioxidation activity and presence of phenolics in TLC under a mobile system of ethyl acetate:methanol:water (10:2:1,v/v/v)

Sample	DPPH test	Spray 1[a]	Spray 2[b]
Dichloromethane subfraction			
L. edodes	0.95	0.95	0.95 (brown)
V. volvacea	0.88, 0.94	0.94	0.94 (brown)
Ethyl acetate subfraction			
L. edodes	0.86, 0.94	0.94	0.94 (brown)
V. volvacea	0.32, 0.90	0.79, 0.90	0.90 (brown)
Butanol subfraction			
L. edodes	0.95	0.90	0.82,0.88,0.96 (brown)
V. volvacea	0.94	–	–

[a] Spray 1 solution: 1% solution of iron(III) chloride in water mixed immediately before use with an equal volume of a 1% solution of potassium hexacyanoferrate(III) in water (Barton's Reagent).
[b] Spray 2 solution: 2% iron(III) chloride in ethanol.

tion. With the EMW solvent system, each fraction was separated into three to six UV-distinct spots (data not shown). The results of further testing of these UV-positive TLC spots for their antioxidation activity (DPPH radical-scavenging) and phenolic compound identification (spray tests) are shown in Table 3. All six subfractions had one to two TLC spots that showed antioxidant activity and the presence of phenolic compounds except for the butanol subfraction of *V. volvacea* which showed negative in the test for phenolics (Table 3).

4. Conclusions

As rat brain homogenate and LDL are rich in lipid, including polysaturated fatty acids, we can deduce that the dichloromethane and ethyl acetate subfractions of *V. volvacea* contained antioxidants that could inhibit lipid oxidation via a radical-scavenging effect. As indicated by the high phenolic content, by chemical analysis and the presence of antioxidative phenolic compounds in the TLC analysis, the ethyl acetate subfraction of *V. volvacea* could be a potential source of antioxidant. In our previous results, the methanol extract of *V. volvacea* did not show any substantial antioxidant activity against lipid peroxidation (Cheung et al., 2003). This could be due to the fact that the concentration of antioxidants in the methanol extract was too low. Further investigations, to isolate and characterize individual mushroom phenolic compounds from these subfractions, are underway.

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References

Adams, A. K., & Wermuth, E. O. (1999). Antioxidant vitamins and the prevention of coronary heart disease. *American Family Physician, 60*, 895–902.

Amarowicz, R., & Shahidi, F. (1997). Antioxidant activity of peptide fractions of capelin protein hydrolysates. *Food Chemistry, 58*, 355–359.

AOAC (1995). *Official Methods of Analysis*. 15th ed. Washington, DC, USA: Association of Official Analytical Chemists.

Breene, W. M. (1990). Nutritional and medicinal value of specialty mushrooms. *Journal of Food Protection, 53*, 883–894.

Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology, 52*, 302–309.

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

Espin, J. C., Soler-Rivas, C., & Wichers, H. J. (2000). Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. *Journal of Agricultural and Food Chemistry, 48*, 648–656.

Frankel, E. N., & Kanner, J. (1993). Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet, 341*, 454–457.

Gazzani, G., Papetti, A., Massolini, G., & Daglia, M. (1998). Anti- and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. *Journal of Agricultural and Food Chemistry, 46*, 4118–4122.

Jialal, I., & Devaraj, S. (1996). Low-density lipoprotein oxidation, antioxidants, and atherosclerosis: A clinical biochemistry perspective. *Clinical Chemistry, 42*, 498–506.

Kasuga, A., Aoyagi, Y., & Sugahara, T. (1995). Antioxidant activity of fungus *Suillus bovines* (L:Fr.) O. Kuntze. *Journal of Food Science, 60*, 1113–1115.

Mangiapane, H., Thomson, J., Salter, A., Brown, S., Bell, G. D., & White, D. A. (1992). The inhibition of the oxidation of low density lipoprotein by (+)-catechin, a naturally occurring flavonoid. *Biochemical Pharmacology, 43*, 445–450.

Mau, J. L., Chyau, C. C., Li, J. Y., & Tseng, Y. H. (1997). Flavor compounds in straw mushrooms *Volvariella volvacea* harvested at different stages of maturity. *Journal of Agricultural and Food Chemistry, 45*, 4726–4729.

Maxwell, S. R. J., & Lip, G. Y. H. (1997). Free radicals and antioxidants in cardiovascular disease. *British Journal of Clinical Pharmacology, 44*, 307–317.

Ng, T. B., Liu, F., & Wang, Z. T. (2000). Antioxidative activity of natural products from plants. *Life Science, 66*, 709–723.

Shahidi, F., & Amarowicz, R. (1996). Antioxidant activity of protein hydrolyzates from aquatic species. *Journal of the American Oil Chemists' Society, 73*, 1197–1199.

Singh, R. B., & Downing, D. (1995). Antioxidants and coronary artery disease. *Journal of Nutritional and Environmental Medicine, 5*, 219–224.

Singleton, V. L., & Rossi, J. A., Jr. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C., & Witztum, J. L. (1989). Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *The New England Journal of Medicine, 320*, 915–924.

Teissedre, P. L., & Waterhouse, A. L. (2000). Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils varieties. *Journal of Agricultural and Food Chemistry, 48*, 3801–3805.

Terashita, T., Kono, M., Mishima, N., Obata, T., & Yamauchi, M. (1990). The proximate components free and protein-bound amino acids in protein and 5′ GMP in fruit-bodies of *Lentinus-edodes* singer Shiitake mushroom grown on artificial bed-blocks. *Journal of the Japanese Society for Food Science and Technology Nippon Shokuhin Kogyo Gakkaishi, 37*, 528–532.

Tong, L. M., Sasaki, S., McClements, D. J., & Decker, E. A. (2000). Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. *Journal of Agricultural and Food Chemistry, 48*, 1473–1478.

Vinson, J. A., Dabbagh, Y. A., Serry, M. M., & Jang, J. (1995). Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *Journal of Agricultural and Food Chemistry, 43*, 2800–2802.

Witztum, J. L. (1994). The oxidation hypothesis of atherosclerosis. *Lancet, 344*, 793–795.

Zhang, A., Chan, P. T., Luk, Y. S., Ho, W. K. K., & Chen, Z. Y. (1997). Inhibitory effect of jasmine green tea epicatechin isomers on LDL-oxidation. *Nutritional Biochemistry, 8*, 334–340.