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Antioxidant activity and total phenolic content of *Agaricus brasiliensis* (*Agaricus blazei* Murril) in two stages of maturity

Andréia Assunção Soares [a], Cristina Giatti Marques de Souza [a,c], Francielle Marina Daniel [b], Gisele Pezente Ferrari [b], Sandra Maria Gomes da Costa [d], Rosane Marina Peralta [a,b,c,*]

[a] *Programa de Pós graduação em Ciências Biológicas, Universidade Estadual de Maringá, 87020-900, Maringá, PR, Brazil*
[b] *Programa de Pós graduação em Ciências da Saúde, Universidade Estadual de Maringá, 87020-900, Maringá, PR, Brazil*
[c] *Departamento de Bioquímica, Universidade Estadual de Maringá, 87020-900, Maringá, PR, Brazil*
[d] *Departamento de Biologia, Universidade Estadual de Maringá, 87020-900, Maringá, PR, Brazil*

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The antioxidant capability and total phenolic contents of methanolic extracts of *Agaricus brasiliensis* in two stages of maturity, young (YB) and mature (MB), were evaluated in this work. Four complementary assays, reducing power, radical scavenging capacity, inhibition of lipid peroxidation and chelating ability for ferrous ions were used to screen the antioxidant properties of extracts. Minor differences in the composition of phenolic compounds were detected, but the extracts showed similar antioxidant activities, except for the chelating ability for ferrous ions, higher in MB than in YB. Our results support the use of both young and mature fruiting bodies of *Agaricus blazei* as sources of antioxidant compounds.

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1. Introduction

Free radicals are produced in normal and pathological cell metabolism considering that oxidation is essential to most living organisms for the production of energy to fuel biological processes. Oxygen-centered free radicals and other reactive oxygen species (ROS) have been associated with the beginning of many diseases and degenerative processes in ageing. Almost all organisms are well protected against free radical damage by oxidative enzymes such as superoxide dismutase and catalase or chemical compounds such as α-tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione (Niki, Shimaski, & Mino, 1994). However, these systems are frequently insufficient to totally prevent the damage, resulting in diseases and accelerated ageing. Natural products with antioxidant activity may be used to help the human body to reduce oxidative damage. Many fruits, vegetables, herbs, cereals, sprouts, seeds and edible mushrooms have been investigated for their antioxidant activities in the last years (Dimitrios, 2006; Kahkonen et al., 1999; Mau, Chao, & Wu, 2001; Rice-Evans, Miller, & Paganga, 1997).

Mushrooms are world wide appreciated for their taste and flavour and are consumed both in fresh and processed form. From a nutritional point of view, mushrooms are not rich in protein or fat but they contain appreciable amounts of dietary fibre, particularly important for the regulation of physiological functions in the human organism (Manzi, Gambelli, Marconi, Vivanti, & Pizzoferrato, 1999). Studies have demonstrated that the regular consumption of mushrooms or consumption of isolated bioactive constituents present in mushrooms is beneficial to health. Mushrooms may thus be considered as functional food or nutraceutical product (Chang & Buswell, 2003; Mau, Lin, & Chen, 2002a; Mau, Lin, & Song, 2002b). Mushrooms accumulate a variety of secondary metabolites such as phenolic compounds, polyketides, terpenes and steroids possibly involved in their medicinal effects and functional values (Turkoglu, Duru, Mercan, & Gezer, 2007). It is important to note that the accumulation of these compounds depends on management, processing and maturity at the time of harvest (Barros, Baptista, & Ferreira, 2007; Choi, Lee, Chun, Lee, & Lee, 2006).

Agaricus brasiliensis Wasser & Didukh, formely known as *Agaricus blazei* Murril ss. Heinemann, is a basidiomycete popularly known in Brazil as *Cogumelo do Sol* and *Cogumelo Piedade*. It was brought to Japan where it is known as *Himematsutake*, *Agarikusutake* or *Kawarihiratak*. It is widely used today in several Oriental countries both as an edible mushroom, considered as functional

* Corresponding author. Address: Programa de Pós graduação em Ciências Biológicas, Universidade Estadual de Maringá, 87.020-900, Maringá, PR, Brazil. Tel./fax: +55 4432614715.
E-mail addresses: rmperalta@uem.br, rmperalta@wnet.com.br (R.M. Peralta).

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food, and natural therapy in the form of a medicinal extract used mostly for prevention and treatment of cancer. In Brazil it is consumed as concentrated extract or tea and popularly used against a variety of diseases such as diabetes, atherosclerosis, hypercholesterolemia and heart disease (Firenzuoli, Gori, & Lombardo, 2007; Mizuno, 1995). A β-glucan isolated from *A. brasiliensis* has been described to stimulate proliferation of lymphocyte T-cells in mice (Mizuno, Morimoto, Minate, & Tsuchida, 1998), to exhibit antitumour and antimicrobial activities in animals (Yan et al., 1999) and to protect against chemically induced DNA damage in human lymphocytes (Angeli et al., 2007). However, most medicinal effects attributed to *A. brasiliensis* are not supported by any scientific evidence (Firenzuoli et al., 2007).

Due to commercial requirements, the basidiocarps (fruiting bodies) of *A. blazei* are normally harvested in a young (immature) stage, when the cap is still closed (Fig. 1). Although young fruiting bodies have not yet achieved their highest biomass, it is at this stage that they reach the highest market value. The mature fruiting bodies (mature basidiocarps) have lower commercial value, and are usually discarded by the farmers. However, a recent study has found that in this stage the basidiocarps are equally rich in β-glucans (Camelini et al., 2005). If additionally the mature fruiting body proves to be as rich as the young as a source of antioxidant agents, its higher yield in terms of biomass could become eventually advantageous for both farmers and consumers. Based on this concept, the aim of the present work was to compare the total phenolic contents of *A. blazei* in both stages of fruiting body maturity, young and mature, and to evaluate its antioxidant potential. Because different antioxidant compounds may act *in vivo* through different mechanisms, no single method can fully evaluate the total antioxidant capacity of foods. For this reason, several complementary test systems, namely β-carotene–linoleic acid assay, free radical scavenging activity, reducing power and ferrous ion chelating ability were used in this study.

2. Materials and methods

2.1. Basidiocarp (fruiting body) selection

Fruiting bodies (basidiocarps) of *A. brasiliensis* were obtained from a local producer in Maringá, PR, Brazil in Spring, 2006. The basidiocarps were harvested and dried in two stages of maturity, young (cap closed), designated in this work as YB and mature (cap opened) with immature spores, designated here as MB (Fig. 1). In this stage, maximum yield (weight of fresh mushrooms) is obtained in the cultures.

Fig. 1. *Agaricus blazei* fruiting bodies (basidiocarps) in two stages of maturity: young (YB) and mature (MB).

2.2. Methanolic extracts

Prior to use the basidiocarps were milled until obtaining a fine powder. The samples (5 g) were extracted by stirring with 100 ml of methanol, at room temperature and at 130 rpm for 24 h, and filtered through Whatman no. 1 paper. The filtrates were concentrated with a rotary vacuum evaporator at 40 °C. The resultant extracts were stored in freezer until use.

2.3. Antioxidant properties

2.3.1. β-Carotene–linoleic acid assay (lipid peroxidation inhibition assay)

The antioxidant activity of extracts was evaluated by the β-carotene–linoleate model system (Miller, 1971), as described in Shon, Kim, & Sung, 2003 with some modification. Firstly, β-Carotene (0.2 mg) was dissolved in 1.0 ml of chloroform. After, 0.02 ml of linoleic acid plus 0.2 ml of Tween 80 was added and the mixture was left standing at room temperature for 15 min. After evaporation of chloroform, 50 ml of oxygenated distilled water was added and the mixture was shaken to form an emulsion (β-carotene–linoleic acid emulsion). Aliquots of 3.0 ml of this emulsion were transferred into test tubes containing 0.2 ml of different concentrations of extracts. The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance (A_0) was measured at 470 nm using a spectrophotometer. A second absorbance (A_1) was measured after 120 min. A blank, without β-carotene was prepared for back-ground subtraction. Lipid peroxidation (LPO) inhibition was calculated using the following equation: LPO inhibition (%) = $A_1/A_0 \times 100$. The assays were carried out in triplicate and the results expressed as means values ± standard deviations. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration. BHT (2,6-di-tert-butyl 4-methyl phenol), a synthetic antioxidant used generally for food, cosmetics and pharmaceuticals was used as standard.

2.3.2. DPPH radical scavenging activity

The free radical scavenging activities of extracts were measured by using 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$) (Blois, 2002). Briefly, 150 μl of each extract at various concentrations was added to 2.850 ml of DPPH solution (0.1 mM), vigorously shaken and maintained for 24 h at room temperature in the dark. Methanol was used instead of mushroom extract as a control. Then the absorbance was measured at 515 nm. The capability to scavenge the DPPH radical was calculated using the following equation: DPPH scavenging effect (%) = $[(A_0 - A_1/A_0) \times 100]$, where A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of DPPH scavenging effect against extract concentration. BHT was used as standard.

2.3.3. Ferrous ion chelating ability

The ferrous ion chelating ability of the methanolic extracts was determined as described in Senevirathne et al. (2006) with some modifications. A sample (0.7 ml) of each extract was diluted in 0.7 ml of distilled water and mixed with 0.175 ml of $FeCl_2$ (0.5 mM) and the absorbance (A_0) was measured at 550 nm. After, the reaction was initiated by the addition of 0.175 ml ferrozine (0.5 mM). The mixture was shaken vigorously for 1 min and left standing at room temperature for 20 min when the absorbance (A_1) was measured at 550 nm. The percentage of inhibition of the ferrozine–Fe^{2+} complex formation was calculated as follows: chelating ability (%) = $[(A_0 - A_1)/A_0] \times 100$. A lower absorbance indicates higher chelating ability. The extract concentration providing 50% chelating ability (EC_{50}) was calculated from the graph

of antioxidant activity percentage against extract concentration. EDTA (ethylenediaminetetraacetic acid) at 0.5 mM was used as the positive control.

2.3.4. Reducing power

The reducing power was assayed as described in Kuda, Tsunekawa, Goto, and Araki (2005) with some modifications. Different concentrations of methanolic extracts of mushrooms (1.0 ml) were mixed with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min. After, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml $FeCl_3$ solution (0.1%, w/v). The absorbance was measured at 700 nm. The assays were carried out in triplicate and the results were expressed as mean values ± standard deviations. Increased absorbance values indicate a higher reducing power. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against the extract concentration. BHT was used as standard.

2.4. Determination of total phenolic contents

Total soluble phenolic compounds in the methanolic extracts were measured according to the method of Singleton and Rossi (1965) and expressed as gallic acid equivalents. A sample of the methanolic extract was added to distilled water for a final volume of 2 ml. After, it was mixed with 0.3 ml of a saturated sodium carbonate (Na_2CO_3) solution and 0.1 ml of 1 N Folin–Ciocalteu's phenol reagent. The mixture was placed for 1 h at room temperature in the dark. The absorbance was measured at 725 nm against the blank.

2.5. Phenolic compounds analysis by thin-layer chromatography

Thin-layer chromatography was performed on plates of 10 cm × 10 cm silica gel Polygram Sil G (Macherey-Nagel, Germany). Methanolic extracts (10 µl) were spotted on silica gel plates and developed in a horizontal chamber saturated with ethyl acetate:water:formic acid (85:15:10). After drying, the plates were developed using two sprays. Spray A (ferric chloride–potassium ferricyanide [$FeCl_3$–$K_2Fe(CN)_6$] was used to locate phenolic compounds. This spray was prepared at moment of the use by mixing equal volumes of each salt solution at 1%. The reagent has an orange–brown colour and the phenolics present in the plate are detected by the formation of blue spots (Barton, Evans, & Gardner, 1952). Spray B (0.04% DPPH in methanol) was used to locate antioxidant compounds. The use of purple reagent detected the presence of antioxidant compounds in the plate by the formation of white spots. Upon the development and viewing of a TLC plate, the starting point and solvent front (the level the solvent reached when the plate was removed from the developing tank) were marked and all spots observed on the plate were circled in lead pencil. The location of each spot on the plate was then represented numerically by calculating a retention factor (R_f).

2.6. Fractionation of phenolic compounds by silica gel column chromatography

A sample of 200 µl of the methanolic extract was applied to a column (1.0 cm diameter and 10 cm height) of silica gel 60 (100–200 mesh), grade 634, Aldrich-Germany. The material was eluted using an ethyl acetate:water:formic acid solvent (85:15:10), followed by an elution using the same system in the proportion 50:50:10. Fractions (500 µl each) were collected in test tubes and the presence of phenolics was determined by using Folin–Ciocalteu's phenol reagent. DPPH reagent was used to evaluate the antioxidant activities in each phenolic fraction.

2.7. HPLC analysis

A HPLC system (Shimadzu, Tokyo) with a LC-20AT Shimadzu system controller, Shimadzu SPD-20 A UV–vis detector, equipped with a reversed phase Shimpak C18 column (4.6 × 250 mm), maintained at 30 °C, was used for analysis of phenolic compounds. All samples in duplicate were filtered through a 0.22 µm filter unit (Millex® – GV, Molsheim, France) before injection and the solvents were filtered through a 0.45 µm filter (Whatman, Maidstone, England). A solvent system consisting of water:acetic acid:methanol (80:5:15) was used as mobile phase at a flow rate of 1 ml/min and the injection volume was 20 µl. Detection of phenolic compounds was carried out at 280 nm.

2.8. Statistical analysis

All analyses were performed in triplicate. The data were expressed as means ± standard deviations and one-way analysis of variance (ANOVA) and Tukey test were carried out to assess for any significant differences between the means. Differences between means at the 5% ($P < 0.05$) level were considered significant.

2.9. Chemicals

DPPH, β-carotene, linoleic acid, gallic acid, BHT, Folin–Ciocalteu's phenol reagent were obtained from Sigma Chemical Co. Other chemicals were analytical degree.

3. Results and discussion

3.1. Extraction yield

The yields of methanol extracts (% dry weight of mushroom) from both stages of maturity of *A. brasiliensis* were 28.14 ± 1.97% for YB and 31.65 ± 3.67% for MB. The yield of the extraction in the young stage in the present work was similar to that obtained in previous work also using methanol as solvent (Huang & Mau, 2006). Tsai, Tsai, and Mau (2007) used ethanol and hot water to extract soluble materials and obtained yields of 15.6% and 47.3%, respectively. Methanol appears, thus, to be a better solvent than ethanol, but it was less efficient than hot water. However, the extraction of high amounts of soluble materials was not directly related to a high extraction of antioxidant activity. In several antioxidant tests, for instance, the ethanolic extracts of *A. blazei* were more efficient than its aqueous extracts (Tsai et al., 2007). The yields obtained by us in the present work were superior to those obtained from other medicinal mushrooms such as *Coriolus versicolor* (9.16%) and *Ganoderma lucidum* (5.61%) (Mau et al., 2002a) using methanol as solvent, but they were similar to those found for several edible mushrooms with yields varying from 15.9% to 43.9% (Mau et al., 2002b; Yang, Lin, & Mau, 2002).

3.2. Antioxidant properties

3.2.1. β-Carotene–linoleic acid assay (LPO inhibition)

Fig. 1A shows the antioxidant activity of the mushroom extracts as measured by the bleaching of the β-carotene–linoleate system. The free radical linoleic acid attacks the highly unsaturated β-carotene, and the presence of different antioxidants can hinder the extent of β-carotene-bleaching by neutralising the linoleate free radical and other free radicals formed in the system. The absorbance decreased rapidly in samples without antioxidant, whereas in the presence of an antioxidant the colour was retained for a long

time. BHT, the positive control used in this test, had 92% of antioxidant activity at 0.2 mg/ml.

The LPO inhibition by YB and MB extracts increased with concentration. At 2, 5 and 10 mg/ml, the LPO inhibitions by both extracts were around 45%, 80% and 90%, respectively. Similar values of LPO inhibition were found for methanolic extracts of *Lentinula edodes* (Cheung, Cheung, & Ooi, 2003). In a previous study, where the antioxidant activity of *Lactarius piperatus* was evaluated as a function of the fruiting body maturity stages, no significant differences between LPO inhibition by immature and mature fruiting body (with immature spores) extracts were found (Barros et al., 2007). However, these authors describe differences between immature and mature (with mature spores) fruiting bodies.

3.2.2. DPPH radical scavenging activity

ROS produced *in vivo* include superoxide radical, hydrogen peroxide and hypochlorous acid. Hydrogen peroxide and superoxide can interact in the presence of certain transition metal ions to yield a highly-reactive oxidising species, the hydroxyl radical (Halliwell, 1991). The antioxidants react with the stable free radical DPPH (deep violet colour) and convert it to 1,1-diphenyl-2-picryl hydrazine with decoloration. The scavenging effects of both extracts increased with their concentrations to similar extents, around 90% at the concentration of 6 mg/ml (Fig. 1B). This value was considerably lower than that reported by Huang, Huang, Chen, and Mau (1999), who found a high scavenging ability of 97.1% at 2.5 mg/ml for the methanolic extract from *A. blazei*. However, the radical free scavenging capacities of our extracts were superior to those found for several other edible mushroom methanolic extracts (Cheung et al., 2003; Ferreira, Baptista, Vilas-Boas, & Barros, 2007). The standard BTH presented a scavenging effect of 92% at the concentration of 0.2 mg/ml.

3.2.3. Ferrous ion chelating ability

In this assay, the chelating agents disrupt the ferrozine–Fe^{2+} complex, thus decreasing the red colour. The measurement at 550 nm of the rate of colour reduction, therefore, allows estimation of the chelating activity. A lower absorbance indicates higher chelating ability. The chelating actions of the methanolic extracts from YB and MB of *A. brasiliensis* on ferrous ions increased with their concentrations (Fig. 1C). The chelating abilities of the methanolic extracts of MB were higher than those of extracts of YB. At 10 and 20 mg/ml, MB chelated 62 ± 7.8% and 78 ± 6.1% of ferrous ions whereas YB chelated 46 ± 6.3% and 61 ± 6.9%, respectively ($P < 0.05$). EDTA (positive control) showed a high chelating ability of 99.3% at 0.2 mg/ml. Tsai et al. (2007) reported that ethanolic extracts of *A. blazei* had chelating abilities of 58.8% at 20 mg/ml. These authors suggest that moderate to high ferrous-ions chelating abilities showed by mushrooms could be beneficial to health. Iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Halliwell, 1991).

3.2.4. Reducing power

Fig. 1D shows the reducing power of the YB and MB methanolic extracts as a function of their concentration. In this assay, the yellow[1] colour of the test solution changes to various shades of green and blue, depending on the reducing power of each compound (Barros et al., 2007). The presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. By measuring the formation of Perl's Prussian blue at 700 nm, it is possible to determine the Fe^{2+} concentration. The reducing power of the YB and MB extracts increased with their concentrations. At 5, 10 and 20 mg/ml, reducing powers of both extracts were around 0.3, 0.6 and 0.8, respectively, while a solution of 0.2 mg/ml of BHT, the positive control used in this test, had a reducing power value of 0.771. In previous studies, a methanolic extract from *A. brasiliensis* showed a reducing power of 0.86 at 10 mg/ml (Huang et al., 1999), while a reducing power around 0.7 was found for an ethanolic extract of the mushroom at the same concentration (Tsai et al., 2007). With regard to reducing powers, the methanolic extracts of YB and MB of *A. brasiliensis* obtained in the present study, were appreciable and comparable to those of edible and medicinal mushroom in general (Cheung et al., 2003; Mau et al., 2002b). On the other hand, exceptionally high reducing power has been described for some medicinal mushrooms. For example, 4 mg/ml methanolic extracts of *Ganoderma tsugae* and *G. lucidum*, present reducing powers of 2.38 and 2.28, respectively (Mau et al., 2002a).

3.3. EC_{50} values and total phenolic contents

In Table 1, we present EC_{50} values obtained in the antioxidant activity assays for YB and MB extracts. The mushroom in both stages revealed very similar antioxidant properties (no significant difference between EC_{50} values, $P > 0.05$), except for the chelating ability, where MB presented a lower value of EC_{50} ($P < 0.05$). The antioxidant properties are inversely correlated with their EC_{50} values and values lower than 10 mg/ml are indicative of the effective antioxidant activity (Lee, Ming-Tsung, & Mau, 2007). The total phenolic contents did not change significantly in both maturity stages, and were quite high: 29.64 ± 0.91 mg/g dry YB extract and 28.82 ± 2.04 mg/g MB extract ($P > 0.05$). The antioxidant activity of plant materials is well correlated with their content in phenolic compounds (Velioglu, Mazza, Gao, & Oomah, 1998). More recently, several researchers have shown a correlation between total phenolic content and antioxidant activity in mushroom extracts (Cheung et al., 2003; Turkoglu et al., 2007). Considering the fact that similar amounts of phenolics were found in both extracts, but MB presented a higher chelating ability, it is reasonable to conclude that other factors, not related with the total phenolic contents, may be implicated in this property. In this respect it has been reported that compounds with structures containing two or more of the following functional groups –OH, –SH, –COOH, $-PO_3H_2$, –C=O, $-NR_2$, –S– and –O– in a favourable structure-function configuration can show metal chelation activity (Lindsay, 1996). This includes, for example, ubiquitous compounds such as citric, malic, tartaric, oxalic, succinic, lipoic and phytic acids Fig. 2.

Table 1
EC_{50} values of young and mature *Agaricus blazei* methanolic extracts from antioxidant properties

	EC_{50} values[a] (mg extract/ml)	
	Young *A. blazei* extract	Mature *A. blazei* extract
Scavenging ability on DPPH radicals	3.00 ± 0.10 A	3.20 ± 0.15 A
Reducing power	8.05 ± 0.35 A	8.10 ± 0.41A
LPO inhibition	2.43 ± 0.03 A	2.12 ± 0.05 A
Ferrous ion chelating ability	13.03 ± 0.67 A	5.00 ± 0.36 B

[a] EC_{50} values: the effective concentration at which the antioxidant activity using the β-carotene–linoleic acid assay (LPO inhibition) was inhibited by 50%; the absorbance was 0.5 for reducing power; DPPH radicals was scavenged by 50%; and ferrous ions were chelated by 50%, respectively. EC_{50} values were obtained by interpolation from linear regression analysis. Each EC_{50} value is expressed as mean ± standard deviation ($n = 3$). Means with different capital letters within a row are significantly different ($P < 0.05$).

[1] For interpretation of colour in Figs. 1 and 3, the reader is referred to the web version of this article.

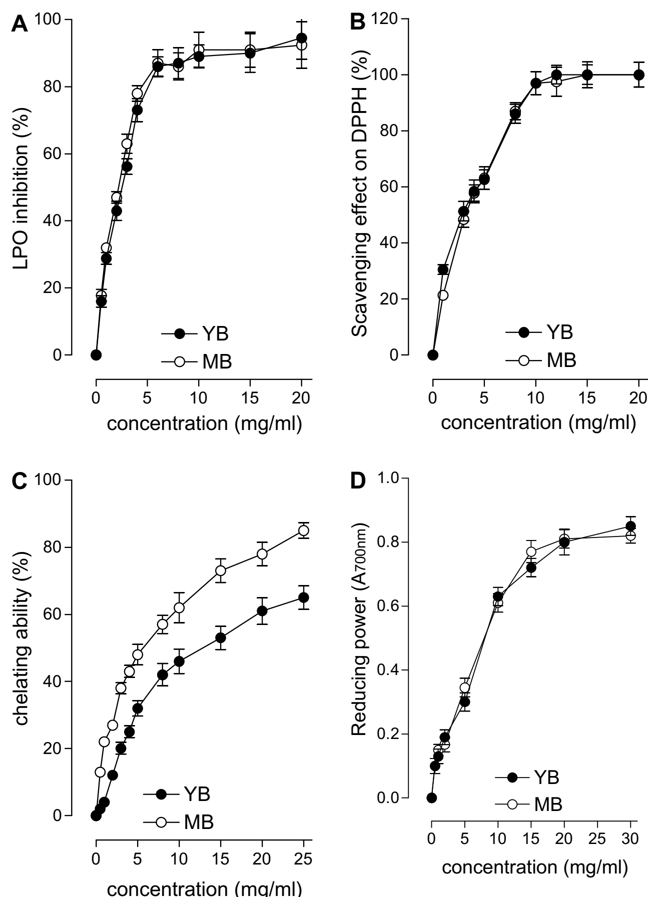

Fig. 2. Antioxidant activities of methanolic extracts from *Agaricus blazei* in two maturity stages. YB = young basidiocarp (young fruiting body); MB = mature basidiocarp (mature fruiting body).

3.4. Cromatographic analysis of phenolic compounds in the extracts

The phenolics present in both methanolic extracts were analysed firstly by TLC using two specific spraying reagents. The revelation with $FeCl_3$ showed the presence of at least six spots, with *rf*'s of 0.77, 0.63, 0.47, 0.35, 0.22 and 0.05, respectively identified from the top as 1–6 in both extracts (Fig. 3). All of them showed antioxidant activities. Moderate antioxidant activities were detected in spots 2 and 4, and strong antioxidant activity was detected in spot 6. Identical volumes of both extracts were then applied to a silica gel column. The phenolic compounds were fractionated using ethyl acetate:water:formic acid solvent (Fig. 4). The two major peaks of phenolic compounds and antioxidant activities (F_1 and F_2) were then analysed by HPLC. The F_2 fractions of both extracts were very similar. F_1 fractions of YB and MB presented small differences, especially in the amount of the phenolic compound with a retention time near 5 min, more evident in YB extracts (Fig. 5). Comparison of Figs. 3–5, allows conclude, concerning their phenolic contents, that the methanolic extracts of young *A. blazei* fruiting bodies differ only slightly from the methanolic extracts of mature fruiting bodies.

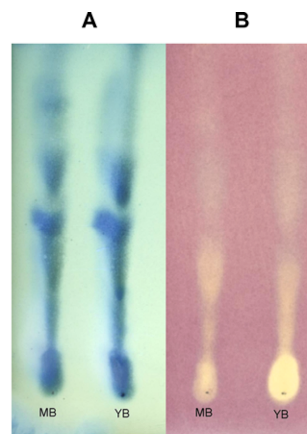

Fig. 3. TLC of methanolic extracts of young basidiocarp (YB) and mature basidiocarp (MB) from *A. blazei*. (A) Revelation with $FeCl_3$ showed at least six different phenolic compounds identified as 1–6 in both extracts. (B) DPPH revelation to antioxidant activity.

4. Conclusions

In conclusion, our analysis revealed only minor differences in the total contents of phenolic compounds in the two maturity stages, young and mature, of *A. brasiliensis*. Consistently, both extracts showed similar antioxidant activities, except the chelating ability for ferrous ions, higher in MB than in YB. As discussed, the latter property could be largely dependent on other molecules than phenolics, such as dicarboxylic acids, for example. On the basis of the results obtained, consumption of both young and mature fruiting

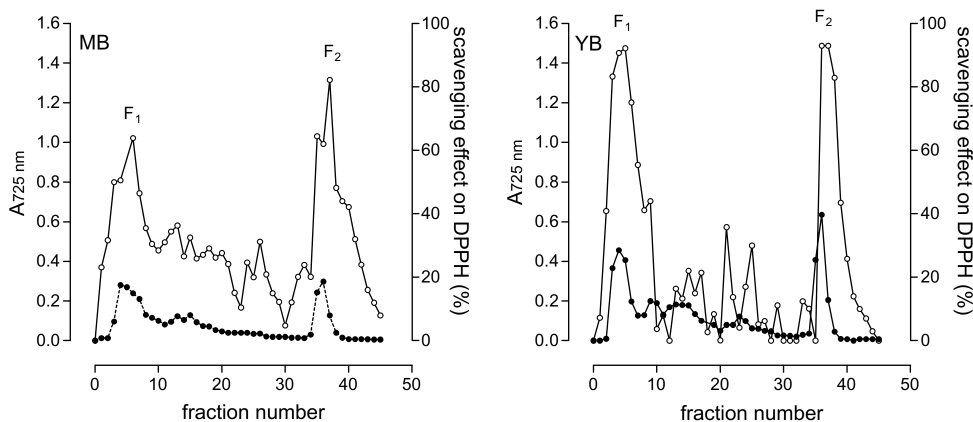

Fig. 4. Silica gel column profiles of methanolic extracts of young basidiocarp (YB) and mature basidiocarp (MB) from *A. blazei*. A volume of 200 µl of each methanolic extract was applied to silica gel 60 column. The material was eluted firstly using an ethyl acetate:water:formic acid solvent (85:15:10) (up to fraction number 20), followed by an elution using the same system in the proportion 50:50:10. (●) phenolic compounds; (○) DPPH radical scavenging activity.

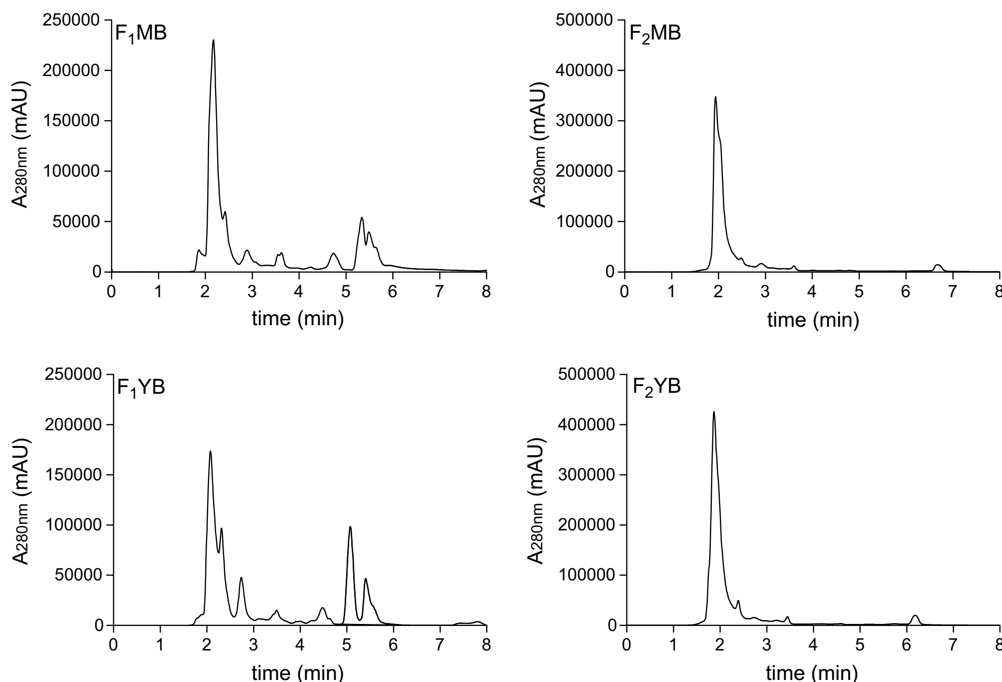

Fig. 5. HPLC-UV profiles of F_1 and F_2 fractions from young basidiocarp (YB) and mature basidiocarp (MB) extracts of *A. blazei*.

bodies of *A. blazei* might be equally beneficial to the human antioxidant protection system against oxidative damage.

Up to now the individual phenolic components of *A. blazei* have not been precisely identified and quantified. Identification of the phenolics in different maturity stages of *A. blazei* is thus highly desirable since it is also a precondition for a more extensive understanding of the mechanisms involved in the antioxidant capability.

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