Changes in antioxidant activities and compounds during cultivation of shiitake (Lentinula edodes)

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CHANGES IN ANTIOXIDANT ACTIVITIES AND COMPOUNDS DURING CULTIVATION OF SHIITAKE (*LENTINULA EDODES*)

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ABSTRACT

Mushrooms contain a variety of secondary metabolites, including various phenolic compounds, which have been shown to act as excellent antioxidants. Recently, a specific antioxidant, ergothioneine (ERG) has been identified in various genera of mushrooms, including shiitake (*Lentinula edodes*). Shiitake is the second most highly consumed mushroom in the world, and present several functional properties, such as antitumor, hypocholesterolemic, antioxidant and antimicrobial potentials that have been intensively investigated. Although research was focused on its therapeutic effects, little information is available about the compounds responsible of the antioxidant properties. Our objectives were to identify changes in quality of shiitake as antioxidant source during cultivation cycles on oak woodships. The antioxidant activities and contents of potential antioxidant components, including total phenolic (TP) compounds and ERG, were analyzed in two parts of mushrooms (stipe and cap) from the successive flushes. In addition, the seasonal variations were studied. The Folin-Ciocalteu reagent was used to quantify the TP, and ERG was quantified by HPLC-DAD. The antioxidant activity was measured using the ORAC assay. Our analysis revealed only minor seasonal variations in TP and ERG contents as well as the ORAC value, excepted during the winter where these contents were 50% higher in the caps. Regarding the age of the culture, we observed a progressive decrease in the contents of TP and ERG all along the flushes, whatever the part of the mushroom. Surprisingly, the ORAC value increased in the whole mushrooms, whereas it decreased in the caps and strongly increased in the stipes. We concluded that the antioxidant activities could be largely dependent on other molecules than ERG, perhaps specific phenolic compounds. Having established the main variations in the antioxidant activity of shiitake, the chemical characteristics of the antioxidative components will be now further investigated. This study could provide valuable new opportunities for mushroom growers, since shiitake can serve as a good source of antioxidants in the human diet.

Keywords: *Lentinula edodes*; Shiitake; Antioxidants; Ergothioneine; Phenolic compounds

INTRODUCTION

Shiitake, *Lentinula edodes* (Berk) Pegler, is the second largest cultivated and most popular edible mushroom in the world, comprising about 25% worldwide production. Shiitake mushroom contains several therapeutic actions such as antioxidant, antitumoral and antimicrobial properties, carried by the diversity of its components. Shiitake mushrooms are a very good source of three key antioxidant minerals: manganese, selenium and zinc. They also contain some

unusual phytonutrient antioxidants. One of the best studied is ergothioneine (ERG). This antioxidant is derived from the amino acid histidine, and acts as an antioxidant by scavenging most reactive oxygen species, chelating various divalent metallic cations and suppressing the oxidation of homoproteins. Polyphenolic compounds have also been detected in shiitake, and may contribute to the antioxidant potential of this mushroom.

There is a growing interest to measure the antioxidant capacity in foods, since the compounds exhibiting such properties could be isolated and used for the prevention of free radicals mediated pathologies, such as cardiovascular and neurodegenerative diseases. In this study, the antioxidant capacity (radical scavenging) of shiitake cultivated on oak sawdust from the successive flushes was investigated in two parts of mushrooms (stipe and cap) using the oxygen radical absorbance capacity assay (ORAC). In addition, total phenolic compounds (TP) and ERG were quantified in order to evaluate their contribution to the antioxidant capacity of mushrooms.

MATERIALS AND METHODS

Chemicals. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), fluorescein, 2,2'-azobis (2-methylpropionamidine) dihydrochloride (AAPH), ergothioneine, phosphate buffer and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (France). All the solvents were of HPLC grade (Scharleau).

Sample collection and preparation.
The *Lentinula edodes* strain 3706 (Mycelia) was cultivated by EuroShiitake Company in insulated green houses in a temperate region, Dordogne, France. The composition of the cultivation substrate was: 85 Kg of oak woodchips and sawdust, 10 kg of wheat bran, 1 kg of gypsum, water to reach 62-65% water content. Substrate sterilization was performed with live steam for 4 hours in a tanker. After cooling 4 % (w/w) of spawn were added aseptically and mixed in the tanker. Polyethylene bags were filled with 6 Kg of spawned substrate. Incubation was at 22 ± 2 °C for 3 to 4 weeks. After removing of the bags, browning was induced by and placing them on shelves in a greenhouse with temperature from 16 to 19 °C and 92-98 % relative humidity, for 3 to 4 weeks. Fruiting was induced by soaking the blocks into cooled tap water (12-15 °C) until they reached their initial weight (6 Kg). This was done after each flushed of harvest.

Mushrooms were harvested at the French commercial stage of development (before veil had broken). For the studies of seasonal variations, mushrooms were randomly harvested one day of collect in October, January, April and July (3 Kg). For the studies of culture cycles variations, mushrooms were collected in winter during 4 successive flushes.

Harvested mushrooms immediately placed at 4°C and then freeze within 4 hours. In the laboratory they were freeze-dried, ground to a fine powder and stored in the dark at room temperature prior to analysis. Mushroom extracts were performed as followed: 100 mg of dried powder were extracted with 5 ml of MeOH overnight. The extract was centrifuged at 3000 rpm for 10 min. and the supernatant was used for analysis. All the extractions were performed in triplicates.

Determination of antioxidant capacity. The ORAC assay was applied as described previously [1]. The reaction was carried out in 75 mM phosphate buffer (pH 7.4) in a 96-well plate. 30 µl of mushroom extract or pure phenolic compounds solutions and 180 µl of fluorescein solution (70 nM final concentration) were mixed and preincubated for 5 minutes at 37°C. 90 µl of APPH solution (12 mM final concentration) were then added and the fluorescence was recorded for 60

minutes at excitation and emission wavelengths of 485 nm and 520 nm respectively using a Fluostar Optima plate reader (BMG Labtech, Germany). A blank sample and six calibration solutions of Trolox (0.1 to 4 µM, final concentration) were also tested in each assay. All samples were analyzed in triplicate. Area under curve (AUC) was calculated for each sample by integrating the fluorescence curve. Net AUC was calculated by subtracting the AUC of the blank. Regression equation between net AUC and Trolox concentration was determined and ORAC values were expressed as equivalent concentration of Trolox per dry weight.

Determination of total phenolic content. The total phenolic concentration in mushroom extracts was determined according to the Folin-Ciocalteu method using gallic acid as standard. 20 µl solution of mushroom extract and 80 µl of sodium carbonate solution (7.5% in deionized water) were added to 100 µl of Folin-Ciocalteu reagent (diluted 10 fold in deionized water) in a 96-well plate. A blank sample and six calibration solutions of gallic acid (0.625 to 20 µg/ml, final concentration) were analyzed under the same conditions. After incubation for 30 min. at room temperature, the absorbance was measured at 760 nm using a Fluostar Optima plate reader (BMG Labtech, Germany). All determinations were carried out in triplicate and results were expressed as mg gallic acid equivalent/100 g of dry weight.

Determination of ergothionein content. The ergothionein content of mushroom extracts was analyzed by reverse-phase analytical HPLC using a Varian Prostar HPLC. The separation was performed using a Prontosil C18 reverse phase column (250 mm × 4 mm, 5 µm particle size), protected with a C18 guard column. Mushroom extracts were injected (100 µl) and the elution (1 ml/min) was performed using a solvent system comprising solvents A (0.1 % trifluoroacetic acid (TFA) in water) and B (0.1 % TFA in acetonitrile) mixed using a gradient starting with 0% B and linearly increasing to 72% B in 50 min. ERG was quantified by monitoring absorbance at 254 nm and comparing the peak area of the sample to peak areas obtained from different concentrations of the authentic standard.

RESULTS AND DISCUSSION

Seasonal variations. Variations in yields are commonly observed in mushroom farms during the year. They might be due to the outside climatic conditions having consequences on how are maintained the temperature and humidity in the cultivation rooms, and to variations in the quality of raw ingredients used to produce the substrate throughout the year. Several studies have shown that the antioxidant activity of mushrooms was correlated with the content of their phenolic compounds [2-4]. Thus, it was important to consider the total phenolic (TP) in relation to the antioxidant activity (ORAC) of methanolic mushroom extracts at various periods of the year.

Figure 1 shows that the amounts of total phenolic compounds in methanolic extracts are in the range of 0.8-1.5 % dry weight (dw) in the caps and 0.8-1.1 % dw in the stipes. There were minor seasonal variations in TP and ORAC values, but a significant difference between spring and summer was observed. The winter values in the caps were twice as high as those at spring.

Differences between the parts of mushrooms had previously been observed with other mushrooms [5] and should be taken into account for the extraction of antioxidants from mushrooms. On another hand, the level of variations in *L. edodes* composition and quality as source of bioactive molecules observed here in a same farm throughout a year of cultivation are significant and should affect the efficiency of mushrooms as source of antioxidant. That also stresses why it is so difficult to compare the data obtained by various authors on samples harvested at only one time under different cultivation conditions.

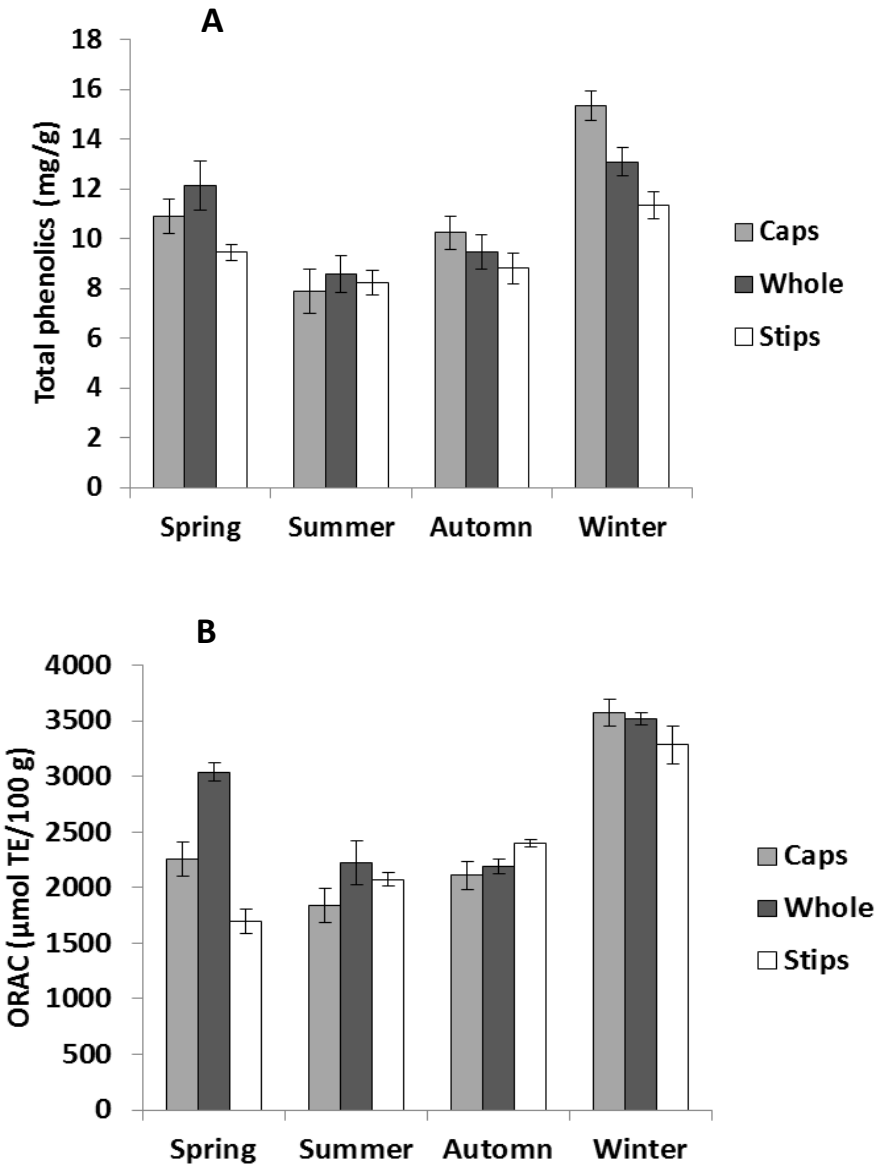

Figure 1: Effects of seasonal variations in the total phenolic contents (A) and antioxidant activities (B) of methanolic extracts from different parts of the mushroom.
Results are expressed as means ± SD (n=3 extractions).

Effect of culture aging on mushroom contents and antioxidant activity. Changes in mushroom quality with fruiting body maturity stage are documented [5, 6]. The changes between flushes of harvest that linked to culture aging are commonly suspected, but less documented.

Regarding the age of the culture, we observed a strong decrease in the contents of TP in the caps during the culture cycles, whereas those in the stipes were constant (Fig. 2). Surprisingly, the ORAC value increased of 30% in whole mushroom, whereas it decreased in the caps and strongly increased in the stipes.

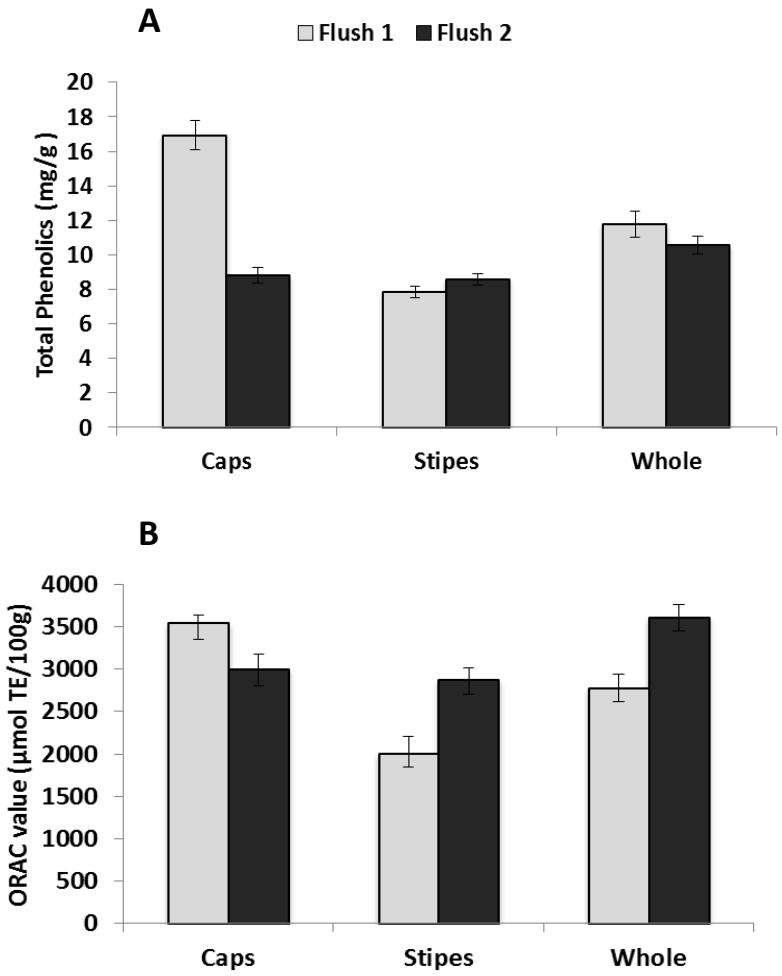

Figure 2: Effect of culture aging on total phenolic compounds (A) and antioxidant activity (B) in different parts of the mushroom.
Results are expressed as means ± SD (n=3 extractions).

A recent study showed that ERG was responsible for the majority of the DPPH scavenging activity of shiitake mushrooms [7]. In this study, we investigated this hypothesis by analyzing the compounds in whole mushroom extracts during successive flushes using HPLC-DAD (280 nm). As shown in Fig.3, the profiles of the different samples are similar but the intensity of several peaks increased or decreased during the culture cycle. Indeed, the ERG content decreased of 45% between flush 1 (36,4 mg/100 g) and 2 (19,7 mg/100 g), whereas the intensities of peaks A and B increased.

These results could explain the increase of the antioxidant activity during aging, and we suggest that the antioxidant activities of mushrooms could be largely dependent on other molecules than ERG, perhaps specific compounds which could be synthetized in the stipes during aging. Work is in progress to identify the compounds responsible for the antioxidant activity of aged cultures.

CONCLUSIONS

In this study, we have established the main variations in the antioxidant activity and selected compounds (ERG and TP) of shiitake during cultivation cycles on oak sawdust. We concluded that the antioxidant activities could be largely dependent on other molecules than ERG, perhaps

specific phenolic compounds. The chemical characteristics of the antioxidative components will be now further investigated. This study could provide valuable new opportunities for mushroom growers, since shiitake can serve as a good source of antioxidants in the human diet.

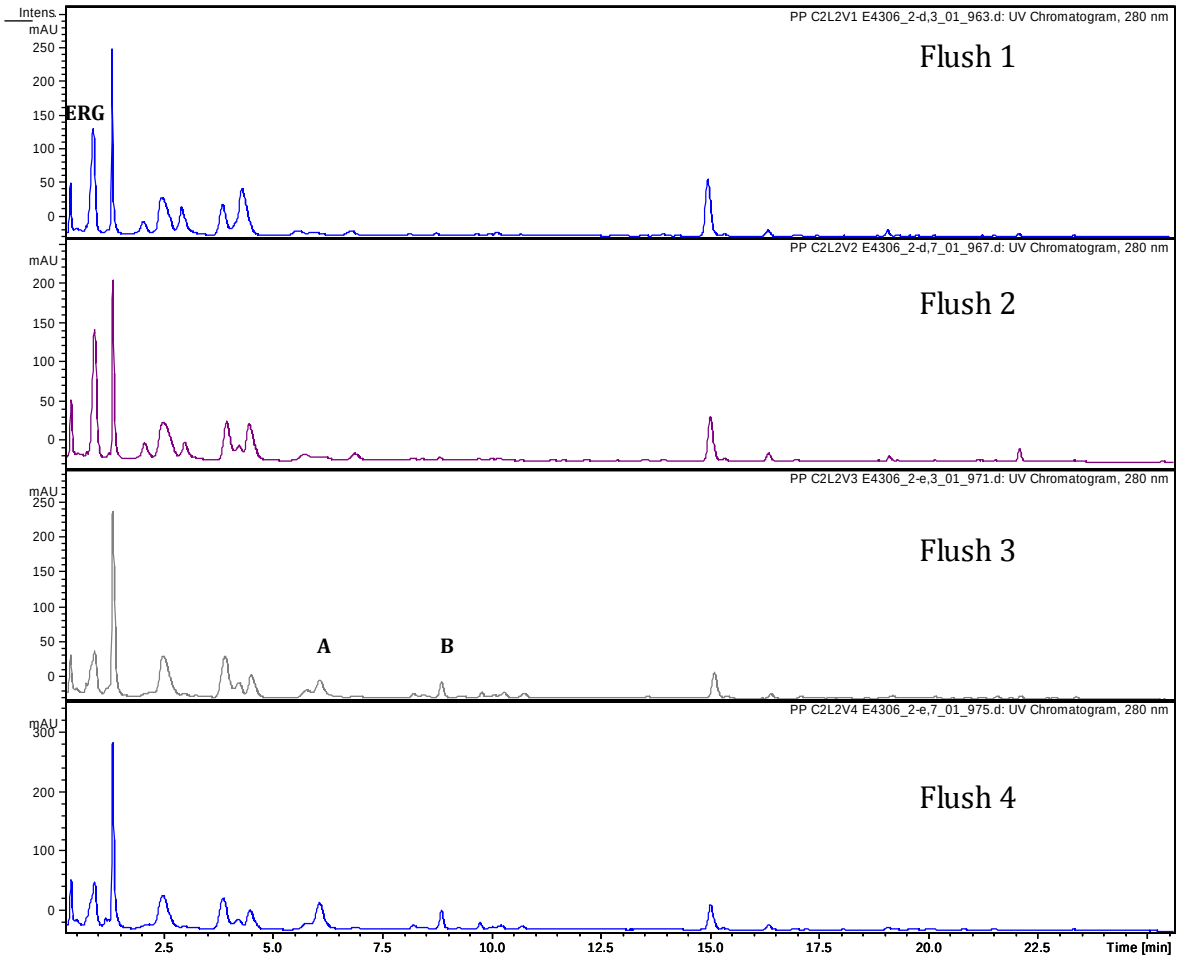

Figure 3: HPLC chromatograms (280 nm) of whole mushroom methanolic extracts from successive culture cycles. ERG: ergothioneine.

ACKNOWLEDGEMENTS

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