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# Ergosterol and Amino Acids Contents of Culinary-Medicinal Shiitake from Various Culture Conditions <sup>†</sup>

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**Abstract:** *Lentinus edodes* (Shiitake) is an edible mushroom cultivated and marketed due to its nutritional and medicinal values. *L. edodes* is appreciated for its unique fragrant taste and its high dietetic potential. Its bioactive molecules explain its interest as medicinal mushroom. This mushroom can be grown using various substrates and culture conditions. Thus, our work focused on the comparison of chemical constituents (i.e., amino acids and ergosterol) of *L. edodes* fruit bodies cultivated in organic or nonorganic growing conditions in the French region of Occitanie. Sequential extraction was performed on freeze-dried fungal materials. Quantitative evaluation of amino acids was done using high performance thin layer chromatography. Assay of ergosterol was carried out using high performance liquid chromatography. For both ergosterol and amino acids, differences were highlighted between extracts (depending on the nature of the solvents) and between growing conditions (organic versus nonorganic). Extracts from organic producer contained the highest content of ergosterol, isoleucine and alanine. In conclusion, this work demonstrated that culture conditions influence the chemical profile of *L. edodes* as for ergosterol and amino acids, which could improve nutrition and human health.

**Keywords:** *Lentinus edodes*; food health benefit; medicinal mushrooms; culture conditions

## 1. Introduction

*Lentinus edodes* (Berk.) Singer (= *Lentinula edodes* [Berk.] Pegler, *Marasmiaceae*, *Agaricomycetes*) is an edible mushroom cultivated and marketed due to its nutritional and medicinal values [1–4]. The Shiitake mushroom is appreciated for its unique fragrant taste; its high dietetic potential is valuable for health and its bioactive molecules explain its interest as medicinal mushroom [5–7]. Shiitake can be grown using various substrates and culture conditions [8,9]. Our work focused on the comparison of amino acids and ergosterol contents from *L. edodes* fruit bodies cultivated in organic or nonorganic growing conditions in the French region of Occitanie.

## 2. Materials and Methods

### 2.1. Mushroom Materials

Shiitake fruit bodies were cultivated by three mushroom producers [9] in the French region of Occitanie using the strain Mycelia-3782 in various growing conditions (organic: producer B; nonorganic: producers A and C). Mycelia-3782 strain of *L. edodes* (Mycelia©, Deinze, Belgium) was cultivated on sterilized substrate blocks from his own engineering (producer B) or provided from commercial substrate (producers A and C).

The *L. edodes* sporophores from producer A (Fontiès-d'Aude, France) grew on a mixture of wood chips and straw (nonorganic conditions; substrate of Eurosubstrat©, Callac, France) with temperatures ranging from 10 °C to 20 °C and 80% humidity.

The mushrooms generated by producer B (Saint-André-de-Lancize, France) were cultivated in the Cevennes National Park on organic sawdust of chestnut, wheat bran, and rye (organic conditions) with temperatures ranging from 18 °C to 21 °C and 60–70% humidity.

The mushrooms from producer C (Saint-Bonnet-de-Salendrinque, France) grew on a mixture of wood chips, oak sawdust, and straw (nonorganic conditions, substrate of "Lentin de la buche" SA©, Monétay-sur-Loire, France) and temperatures ranged from 15 °C to 17 °C and 100% humidity.

### 2.2. Sample Preparation

Five kg of *L. edodes* from each producer were cleaned, sliced, gauged, and carefully packaged in plastic bags and snap frozen. Then, they were lyophilized in a RP2V lyophilizer (Groupe Serail, Le Coudray Saint Germer, France).

### 2.3. Extraction Conditions

Sequential extraction (cyclohexane, chloroform, ethanol, water) under sonication was performed on freeze-dried fungal materials. First, 50 g of each lyophilized mushroom sample were crushed with a Thermomix© Vorwerk crusher (Vorwerk, Wuppertal, Germany). Then, 5 g of crushed mushroom were placed in 50 mL cyclohexane, sonicated for 90 min at 30 °C, and then filtered using a Büchner device. The cyclohexanic filtrate was stored for subsequent evaporation procedure. Retentate was then submitted successively to extractions with chloroform (50 mL), ethanol (50 mL), and water (50 mL) under the same conditions.

### 2.4. Quantification of Ergosterol

Quantification of ergosterol was performed as previously described by Barreira et al. [10] using high performance liquid chromatography (Ultimate U3000 Thermo Fisher Scientific Inc., San Jose, CA, USA). The system was operated using the Chromeleon software, version 7.0. Chromatographic separation was achieved on an ODS Hypersyl C18 column (250 mm × 4.6 mm, 5 µm, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), with a column temperature maintained at 30 °C. Mobile phase consisted of a mixture of methanol and acetonitrile (isocratic 30:70 during 30 min, then methanol 100% during 10 min, flow rate: 1 mL/min). 20 µL of the extracts (5 mg/mL) were analysed. Ergosterol was quantified by comparison of the area of its peak with the calibration curve (250 µg/mL, 62.5 µg/mL, 15.6 µg/mL and 3.9 µg/mL) obtained from a commercial standard (Acros Organics, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA; 98% purity). The UV/V is spectra were recorded in the 200–400 nm range and chromatograms were acquired at 280 nm for quantification of ergosterol. The results are expressed in mg/mg of extract and in mg/100 g of dry material.

### 2.5. Quantification of Free Amino Acids

Quantitative evaluation of amino acids was carried out for the ethanolic extracts using high performance thin layer chromatography Camag Automatic TLC sampler using HPTLC Silica gel 60 glass plates (Merck) as stationary phase and butanol/acetic acid/water (3:1:1) as mobile phase; detection of amino acids was performed at 550 nm (Camag TLC scanner 3) after spraying ninhydrin

reagent. Calibration curves were established with the amino acids standards (aspartic acid, glutamic acid, alanine, arginine, glycine, histidine, isoleucine, methionine, phenylalanine, serine, threonine and valine; Sigma-Aldrich, Saint-Louis, Missouri, USA; 99% purity) at 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.12 µg/mL; 0.75 µg/mL. The ethanolic extracts were analysed at 1 mg/mL.

### 3. Results

#### 3.1. Ergosterol, a Provitamin of Vitamin D<sub>2</sub>

Quantification of ergosterol was made only for cyclohexane, chloroform and ethanol Shiitake extracts from the three producers. Indeed, all aqueous extracts did not contain ergosterol (detection limit 0.001 mg/mg of extract).

Higher concentration was observed in the most apolar solvents, especially in cyclohexane extract of nonorganic producer C ( $C > B > A$ ).

In terms of total amount of ergosterol, Table 1 shows that producer B (478.21 mg/100 g dry weight of Shiitake) has a higher content than producer A (294.43 mg/100 g of dry weight) and producer C (341.29 mg/100 g of dry weight). Indeed, Shiitake fruit bodies from organic producer B have the highest ergosterol content and thus it is an appreciated source of precursor of vitamin D<sub>2</sub> (Table 1).

**Table 1.** Ergosterol concentration (mg/mg of Shiitake extract,  $n = 3$ ) and ergosterol content (mg/100 g dry weight of Shiitake).

Producers	Extracts	Ergosterol Concentration	Ergosterol Content
A	Cyclohexane	0.169 ± 0.012	294.43
	Chloroform	0.095 ± 0.006	
	Ethanol	0.001 ± 0.001	
	Water	0.001	
B	Cyclohexane	0.183 ± 0.016	478.21
	Chloroform	0.15 ± 0.019	
	Ethanol	0.002 ± 0.001	
	Water	0.001	
C	Cyclohexane	0.235 ± 0.016	341.29
	Chloroform	0.093 ± 0.013	
	Ethanol	0.001 ± 0.001	
	Water	0.001	

#### 3.2. Free Essential and Non Essential Amino Acids

Ethanolic extracts of Shiitake were investigated for twelve free amino acids. Compared to the retention factor of the standards, three free amino acids were identified and quantified following the scan of the HPTLC chromatoplates. These were two essential amino acids (valine and isoleucine) and a non-essential amino acid (alanine).

The highest value of the essential amino acid valine was reported for nonorganic producer C (5.17 µg/mg of extract; Table 2). The highest concentration of essential amino acid isoleucine was reported for organic producer (7.29 µg/g of extract).

The highest values of alanine (non-essential amino acid) were demonstrated for organic producer B and nonorganic producer A as 8.53 µg/mg and 8.21 µg/g of Shiitake extracts, respectively.

**Table 2.** Amino acids concentration ( $\mu\text{g}/\text{mg}$  of Shiitake ethanolic extract,  $n = 3$ ).

Ethanolic Extracts	Amino Acids Concentration ( $\mu\text{g}/\text{mg}$ of Extract)		
	Alanine	Valine	Isoleucine
Producer A	$8.21 \pm 0.46$	$3.77 \pm 0.28$	$5.42 \pm 0.17$
Producer B	$8.53 \pm 0.25$	$3.71 \pm 0.15$	$7.29 \pm 0.97$
Producer C	$5.92 \pm 0.50$	$5.17 \pm 0.60$	$6.19 \pm 0.71$

#### 4. Discussion

It is difficult to compare the results that we obtained for ergosterol and amino acids contents from cultivated *L. edodes* fruit bodies (strain Mycelia-3782) in organic and nonorganic culture conditions with those previously reported based on different strains and culture conditions as well as various extraction methodologies [10–12].

Ergosterol content as 107.9 mg/g of fresh weight cultivated Shiitake mushrooms produced in Sweden (unspecified strain, unspecified culture conditions) was reported in 2007 [13]. Later, ergosterol content was reported as 84.9 mg/g of fresh weight from commercially distributed Shiitake in U.S. markets (unspecified strain, unspecified culture conditions) [14]. This year, ultraviolet irradiation was reported to increase the concentration of Vitamin D<sub>2</sub> [15] and decrease the concentration of ergosterol in Shiitake powder in an ethanolic suspension but the authors did not precise the exact value of the controls (around 5000  $\mu\text{g}/\text{g}$  of dry weight of Shiitake bought in a Chinese market, unspecified strain, unspecified culture conditions). Similar study [16] was previously carried out with Shiitake fruit bodies purchased from a local farm in Thailand (unspecified strain or culture conditions; the ergosterol content was evaluated around 3.8 mg/g of dry weight. In these two last studies the ergosterol contents were comparable to ours.

Recently, the *L. edodes* strain WX1 (ACCC 50926) was investigated for amino acids contents based on five mushroom cultivation substrates prepared as follows [17]: control (80% oak sawdust; wheat brane 18%; lime 1%; saccharose 1%), RS20 (20% rice straw; 60% oak sawdust; wheat bran 18%; lime 1%; saccharose 1%), RS40 (40% rice straw; 40% oak sawdust; wheat bran 18%; lime 1%; saccharose 1%), RS60 (60% rice straw; 20% oak sawdust; wheat bran 18%; lime 1%; saccharose 1%), RS80 (80% rice straw; wheat bran 18%; lime 1%; saccharose 1%). The contents of total free amino acids varied from 16.29 to 24.59 mg/g of dry weight and the highest level of free amino acids was found in mushrooms cultivated from RS20 and RS40 [17]. The highest level of alanine (3.38 mg/g), valine (0.55 mg/g) and isoleucine (0.22 mg/g) were produced on RS20.

#### 5. Conclusions

Our study focused on the chemical composition of *L. edodes* fruit bodies cultivated by nonorganic and organic mushroom professionals using the same strain. This work demonstrated that culture conditions of *L. edodes* influence the chemical profile of the harvested sporophores for the chemical constituents as ergosterol and free amino acids. Several Shiitake strains and various growing substrates will need to be tested under both organic and nonorganic conditions to improve biosynthesis of those fungal chemical constituents. That could contribute to improve nutrition and human health.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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