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Fatty acid profiles of polar and non-polar lipids of *Pleurotus ostreatus* and *P. cornucopiae* var. '*citrino-pileatus*' grown at different temperatures

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ABSTRACT

The application of fatty acid (FA) composition data has now extended to studies of physiology, chemotaxonomy, and intrageneric differentiation, as well as to studies of human nutrition. Environmental factors such as nutritional components, oxygen, and temperature are known to affect lipid content and composition in living organisms, including fungi. In the present study, the polar and non-polar lipid content of *Pleurotus ostreatus* and *P. cornucopiae* var. *citrino-pileatus* fruiting bodies produced at temperatures ranging from 12–27 °C and from 17–27 °C, respectively, were analysed to evaluate the effect of temperature on lipid composition in these mushrooms. Results showed that lowering the growth temperature below 17 °C generally provided an expected increase in FA unsaturation in polar and non-polar lipids of *P. ostreatus*. Although raising the temperature above 17 °C did not show any clear-cut tendency in FA unsaturation, it did reveal that growth temperature had a differential effect on the FA profiles in fruiting bodies of *P. ostreatus* and *P. cornucopiae*. This study suggests that care should be taken when using FA content and unsaturation data for physiological, chemotaxonomic, and intrageneric differentiation studies, and that it may be possible to manipulate lipid unsaturation in *Pleurotus* spp. through modified growth temperatures.

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Introduction

Fatty acids (FAs) are important constituents of fungal cells with recognized roles as storage material and as components of plasmalemma and cell organelle membranes. In fungi, the major FAs that typically occur in membrane phospholipids and storage triacylglycerols are palmitic and stearic acids and their unsaturated derivatives palmitoleic, oleic, linoleic and linolenic acids (Suutari 1995).

Many environmental factors may affect lipid content in living organisms including fungi (Lösel 1988). Indeed, the composition of mycelial FAs varies greatly depending on growth conditions such as nutritional factors, oxygen, and temperature (Suutari 1995). With regard to temperature, it is generally recognized that FA unsaturation increases as temperature decreases, although reports to the contrary exist (Chavant *et al.* 1981; Lösel 1988; Sumner *et al.* 1969; Suutari 1995; Suutari & Laakso 1994; Suutari *et al.* 1990; Wilson & Miller 1978).

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Interest in lipids, especially in FA composition, is currently expanding. Such data are used for physiological, chemotaxonomic, and intrageneric differentiation studies of many organisms such as bacteria, algae, fungi, and flowering plants (Bagci *et al.* 2003; Dimou *et al.* 2002; Erwin 1973; Stahl & Klug 1996; Wolff *et al.* 2002). Regarding human nutrition, linoleic (18:2 Δ9,12) and α-linolenic (18:3 Δ9,12,15) acids are essential for basal metabolism, whereas long-chain polyunsaturated FAs are now believed to have many beneficial effects on human health (Parikh *et al.* 2005). Therefore, knowledge of the FA content of edible mushrooms is of interest.

Pleurotus ostreatus and *P. cornucopiae* var. *citrino-pileatus* are two edible mushroom species grown commercially in many countries (Royse *et al.* 2004). Interest in these species has increased considerably in the last decade because of their gastronomic value and their nutraceutical properties (Bao *et al.* 2004; Chang & Miles 2004). Although FA profiles of different *Pleurotus* species including *P. ostreatus* and *P. cornucopiae* have been characterized (Dimou *et al.* 2002), little is known about the effect of temperature on lipid content of these mushrooms.

In the present study, the polar and non-polar lipid content of *P. ostreatus* and *P. cornucopiae* var. *citrino-pileatus* fruiting bodies produced at temperatures ranging from 12–27 °C and from 17–27 °C, respectively, were analysed to evaluate the effect of temperature on the FA composition of these mushrooms.

Materials and methods

Organisms

Pleurotus ostreatus (strain no. 123) and *P. cornucopiae* var. *citrino-pileatus* (strain no. 140) pre-spawned bags (fully colonized by the mycelium and ready to fruit) were used in this study and were purchased from Lambert Spawn Company (Coatesville, PA). The bags contained cottonseed hulls (average weight 22 lb) spawned [15 % (w/w) D.W.] with either *P. ostreatus* or *P. cornucopiae* that were incubated under stringently controlled conditions to obtain a full colonization of cottonseed hulls.

Voucher materials of the studied fungi are permanently preserved and available at the following locations: Canadian Collection of Fungal Cultures (CCFC); American Type Culture Collection (ATCC), and Centraalbureau voor Schimmelcultures (CBS).

Experimental design

In order to evaluate the effect of temperature on *Pleurotus ostreatus* and *P. cornucopiae* morphology and FA profiles, the pre-spawned bags were placed in growth chambers (90 % relative humidity) at the following temperatures: 12, 17, 21, 27 °C (*P. ostreatus*) and 17, 21, 27 °C (*P. cornucopiae*). Experiments were performed independently according to a completely randomized design with five replicates. Each spawn bag was considered as an experimental unit. Samples (four to ten basidiocarps with a pileus diam averaging 2–3.5 cm) were collected from each spawn bag after appropriate periods of incubation in the growth chambers. Basidiome morphological characteristics were determined and the samples were then lyophilized and homogenized for further analyses.

Basidiome morphological characteristics

Stipe length and pileus diam were measured with a ruler. The stipe length:pileus diam ratio was then calculated.

Lipid extraction

Lyophilized samples (0.5 g) were extracted for 2 h, at room temperature and protected from light, using hexane as the extraction solvent. Extracts were centrifuged, the supernatants were filtered, dried over anhydrous sodium sulphate, concentrated (−30 °C) in a rotary evaporator under vacuum, weighed and identified as the non-polar lipid extracts. The residual mushroom materials were extracted for 2 h, at room temperature, using chloroform ($CHCl_3$):methanol (MeOH) (2:1) as the extraction solvent. Extracts were centrifuged, the supernatants were filtered, dried over anhydrous sodium sulphate, concentrated (−30 °C) in a rotary evaporator under vacuum, weighed and identified as the polar lipid extracts.

Fatty acid methyl ester analysis

Non-polar lipid extracts (in 500 μl hexane) were methylated in sealed tubes with BF_3-MeOH (500 μl; Aldrich, St Louis, MO) at 70 °C for 45 min with occasional stirring. The hexane layer was withdrawn, partitioned against water, dried over anhydrous sodium sulphate, and kept at −35 °C until analysis. Polar lipid extracts (1 ml, in $CHCl_3$:MeOH 2:1), were evaporated to dryness and methylated in sealed tubes with BF_3-MeOH (1 ml) at 70 °C for 45 min with occasional stirring. FA methyl esters (FAMEs) were extracted with hexane (1 ml). The hexane layer was withdrawn, partitioned against water, dried over anhydrous sodium sulphate and kept at −35 °C until analysis.

FAME samples (1 ml) were analysed by gas chromatography (GC) on a Hewlett-Packard 5890 Series II (Hewlett-Packard, Palo Alto, CA) gas chromatograph connected to a flame ionization detector (FID) and to a computer with Hewlett-Packard Chemstation software. Samples were injected into an open tubular BPX-70 capillary column (70 % cyanopropyl polysilphenylene-siloxane, 60 m × 0.25 mm i.d. × 0.25 μm film thickness; SGE, Austin, TX), in splitless mode. The injector and FID temperatures were maintained at 215 and 230 °C, respectively. The oven temperature program was as follows: 35 to 180 °C at a rate of 20 °C min^{-1}, held for 3 min, increased to 230 °C at a rate of 3 °C min^{-1} and held for 13 min. Hydrogen was used as the carrier gas under constant flow (1.4 ml min^{-1}). FAME standards (Glc-60, Glc-76 and Glc-502; Nu-Chek-Prep, Elysian, MN) were used for confirmation of retention time. FAMEs were also analysed by GC-mass spectrometry (MS) on a Hewlett-Packard 6890 Series II with a selective quadrupole mass detector (model 5973 N, Agilent Technologies, Palo Alto, CA) and connected to a computer with Hewlett-Packard Chemstation software, with the previous column and under conditions previously described, with helium as carrier gas and electron impact (EI) set at 70 eV.

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