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QUALITATIVE ANALYSIS OF FATTY ACIDS COMPOSITION IN DIFFERENT COLLECTIONS OF COPRINOID MUSHROOMS

J. A. MKRTCHYAN*

Laboratory of Fungal Biology and Biotechnology YSU, Armenia

Qualitative analysis of fatty acids composition of mycelial extracts obtained from 18 species 30 strains of coprinoid mushrooms (CMs) from different clades (*Coprinellus* (*C. bisporus*, *C.* curtus, *C. disseminatus*, *C. domesticus*, *C. ellisii*, *C. flocculosus*, *C. micaceus*, *C.* aff. *radians* I, III, *C. xanthothrix*, *C. sp.*), *Coprinopsis* (*C. cinerea*, *C. coth*urnata, *C. gonophylla*, *C. lagopides*, *C. maysoidispora*, *C. strossmayeri*), *Coprinus* (*C. comatus*)), as well as not yet reclassified species *Coprinus patouillardii* was realized by Gas chromatographic analysis. Two unsaturated (lineolic, oleic) and three saturated (palmitic, stearic, myristic) fatty acids were detected in tested samples. Differences in fatty acid composition between clades from different families (*Agaricaceae*, *Psathyrellaceae*) were observed. All five fatty acids were detected in *Coprinus clade* (family *Agaricaceae*) in opposite to *Coprinellus* and *Coprinopsis* clades (family *Psathyrellaceae*).

***Keywords*:** coprinoid mushrooms, mycelium, fatty acids, Gas chromatographic analysis.

Introduction. Over 20 different fatty acids of great structural variation might be present in different species of mushrooms [1]. The most common fatty acids in mushrooms are linoleic, palmitic and stearic acids, which were reported for several mushroom species from different groups [2, 3]. There have been numerous studies of the fatty acid composition of fungi and many included the use of fatty acids in taxonomic and phylogenetic investigations [1]. It is reported that unsaturated fatty acids predominate over the saturated in the total fatty acids content in mushrooms [2]. It was reported that composition of lipophilic compounds, particularly fatty acids significantly depend on cultivation conditions, such as medium, cultivation time and agitation [4]. Thus, the available data on species lipid profiles are principally related to fruiting bodies, with surprisingly little information on the fungal lipid composition of the mycelium [5].

Dietary and medicinal significance of fatty acids is known. The knowledge of fatty acid composition in cellular lipids produced, would serve as a useful database for taxonomic, nutritional and medicinal evaluation of mushroom species [6, 7].

The genus *Coprinus* Pers. (*Coprinus* s.l.) is the largest group of the former family of *Coprinaceae* Overeem (Homobasidiomycetes) with more than 200 species. Data regarding to morphological and ecological characteristics of mycelia

* E-mail: mkrtchyanjon@gmail.com

of this group of mushrooms are reported [8, 9]. Medicinal properties (antifungal, antibacterial, antioxidant, fibrinolytic and proteolytic activities) of several coprini, from different clades particularly *Coprinus comatus* and *Coprinopsis cinerea* were known [10–12]. As far as we know data related to fatty acids composition of these mushrooms is absent.

In this paper results regarding to qualitative analysis of fatty acids composition of mycelia of several collections of coprinoid mushrooms (CMs) are reported.

Materials and Methods.

Fungal Materials and Culture Conditions. Collections of 30 strains of 18 coprini species (*Coprinellus* (*C. bisporus*, *C. curtus*, *C. disseminatus*, *C. domesticus*, *C. ellisii*, *C. flocculosus*, *C. micaceus*, *C. radians*, *C. xanthothrix*, *C. sp.*), *Coprinopsis* (*C. cinerea*, *C. cothurnata*, *C. gonophylla*, *C. lagopides*, *C. maysoidispora*, *C. strossmayeri*), *Coprinus* (*C. comatus*) and not yet reclassified species *Coprinus patouillardii*) with different origination from Culture Collection of the Laboratory of Fungal Biology and Biotechnology, Yerevan State University (FCC-YSU) were used in this study [13]. Mycelial strains were grown on malt-extract medium (200 *mL,* 8° malt-extract and 800 *mL* distilled water, pH 6.0) in 200 *mL* Erlenmeyer flasks (60 *mL* malt-extract, 5 inocula in each flask) in static culture conditions at 25°*C*, in darkness during 21days.

Standards and Reagents. Hexane, sodium methylate and hydrochloric methanol were obtained from "Sigma-Aldrich". Caproic, caprylic, capric, undecanoic, lauric, tridecanoic, myristic, myristoleic, pentadecanoic, palmitic, palmitoleic, heptadecanoic, cis-10-heptadecenoic, stearic, oleic, linoleic, *γ*-linolenic, cis-11--eicosenoic, *α*-linolenic, arachidic, cis-11,14-eicosadienoic, cis-8,11,14-eicosatrienoic, arachidonic, cis-,8,11,14,17-eicosapentaenoic, heneicosanoic, behenic, erucic, cis-4,7,10,13,16,19-docosahexaenoic, tricosanoic, lignoceric and nervonic acid methyl esters were purchased from Supelco ("Sigma-Aldrich").

Gas Chromatography Analysis. At the end of cultivation mycelial biomass was separated from cultural liquid by filtration, washed by distilled water, homogenized and extracted by ethanol (1:2, *v*/*v*) during 1 day at 37°*C*. The supernatants were evaporated at room temperature. From obtained samples of dry mycelial extracts 100 *mg* of each were further diluted by hexane (1.9 *mL*) and added 0.1 *mL* 2 *M* sodium methylate. After filtration the samples were used for Gas chromatographic (GC) analyses [14]. Cellular lipids were converted into their methyl-esters in a two-steps reaction with methanolic sodium and hydrochloric methanol in order to avoid generation of trans fatty acid isomers. Then fatty acid methyl-esters were analyzed by GC device equipped with a capillary column Omega-WAX 320 CB (Shimadzu GC 2010, Japan, Chrompack; 30 *m*, ID=0.32, film thickness 0.25 *μm*) and a flame ionization detector. Analysis conditions were as follows: oven temperature 200°*C* for 13 *min*, increase to 220°*C* (2° per *min*) for 4 *min* and further increase to 240°*C* for 3 *min*; injector temperature 250°*C*; detector temperature 260°*C*; carrier gas helium; flow 1.4 $mL \cdot min^{-1}$. Identification of methyl esters was based on the comparison of retention times with Supelco FAME Chemical standards ("Sigma-Aldrich").

Results and Discussion. Five fatty acids – palmitic (C16:0), stearic (C18:0), myristic (C14:0), oleic (Δ9C18:1) and lineolic (Δ9, 12C18:2), were detected in mycelia of tested coprini collections (see Table). Among them lineolic and oleic

acids belong to unsaturated and palmitic, stearic, myristinic acids to saturated groups of fatty acids.

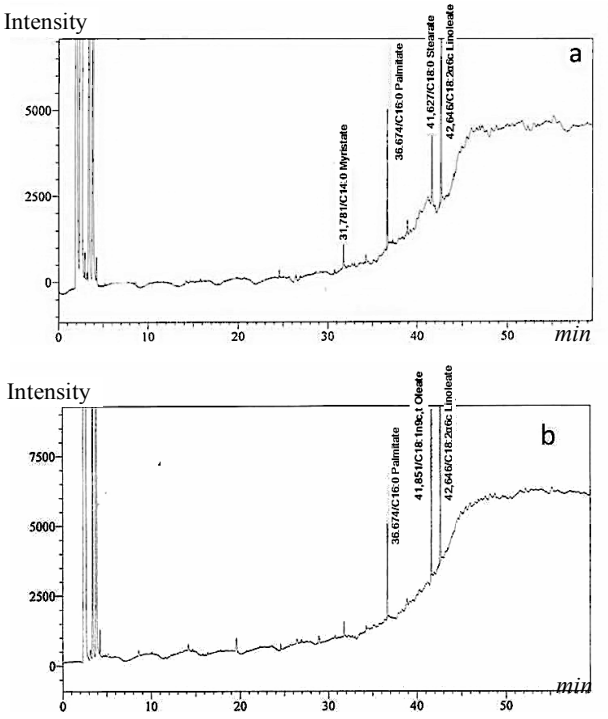
Qualitative analysis of fatty acid composition in mycelia of coprini collections

Clade/Species	Strain	Fatty Acid Composition				
		unsaturated		saturated		
		lineolic	oleic	palmitic	stearic	myristic
Coprinellus						
C. bisporus	C406	+	+	+	+	–
C. curtus	C71	+	–	+	+	–
	C311	+	–	+	+	+
C. disseminatus	C50	+	–	+	+	–
	Cd–30	+	–	+	+	–
	Gp8Da	+	+	+	–	–
C. domesticus	C72	+	+	+	–	–
C. ellisii	C140	+	–	+	+	–
C. micaceus	15–2C	+	–	+	+	+
	9–1C	+	–	+	+	+
C. aff. *radians* I	1–1C	+	–	+	+	–
	L_2C	+	+	+	–	–
C. aff. *radians* II	C35	+	–	+	+	–
C. aff. *radians* III	Cb1	+	+	+	+	–
C. xanthothrix	C144	+	+	+	–	–
	C482	+	+	+	+	+
Coprinellus sp.	G2–1S	+	+	+	–	–
Coprinopsis						
C. cinerea	Amut Bmut	+	–	+	+	–
C. cothurnata	C145	+	–	+	+	–
C. gonophylla	C399	+	–	+	+	+
C. lagopides	C262	+	–	+	+	–
C. maysoidispora	C219	+	–	+	+	–
C. strossmayeri	17–C	+	–	+	–	–
	15–2C	+	+	+	+	–
	I–1S	+	–	+	+	–
	15–1C	+	+	+	+	–
Coprinus						
C. comatus	6S	+	+	+	+	–
	10–C	+	+	+	+	–
	C108	+	+	+	+	+
	IV	+	+	+	+	+
Not yet reclassified species						
C. patouillardii	C9	+	+	+	+	–

Unsaturated lineolic acid was detected in all tested species/strains, whereas oleic acid was found in 7 species and 8 strains from *Coprinellus* clade (*C. bisporus*, *C. disseminatus*, *C. domesticus*, *C.* aff. *radians* I and III, *C. xanthothrix*, *C. sp*.) and 1 species 2 strains of *Coprinopsis strossmayeri* (family *Psathyrellaceae*). Lineolic acid was present in 4 tested strains of *Coprinuss comatus* (family *Agaricaceae*), as well as not yet reclassified species/strain of *C. patouillardii*.

Unsaturated oleic acid was often detected in *Coprinellus*, rather than *Coprinopsis* species. It was only absent in 3 *Coprinellus* (*curtus*, *disseminatus*,

micaceus), but almost all *Coprinopsis* species, except 2 strains of *C. strossmayeri*, which contained oleic acid (see Table and Figure).



GC profile of methyl esters of free fatty acids of
Coprinellus micaceus, str. 9-1C (a), *C. xanthothrix, str.* C144 (b).

Saturated palmitic acid was also detected in all tested species/strains of CMs from different clades. The stearic acid was detected in species from all clades, except two species and 3 strains of *Coprinellus* (*C. domesticus* and *C. sp*.) and 1 strain (17-C) of *C. strossmayeri*. Meanwhile, it was present in all tested strains of *C. comatus*. Saturated stearic acid was often in both *Coprinellus*/*Coprinopsis* collections, but mainly in *Coprinus* (see Table).

Myristic acid in tested samples was detected in three *Coprinellus* (*C. curtus*, *C. micaceus*, *C. xanthothrix)*, one *Coprinopsis* (*C. gonophylla*) species and in two strains (C108, IV) of *C. comatus* [15]. It was found rarely, but relatively often in *Coprinellus*, rather than in *Coprinopsis* species (see Table).

All five fatty acids were detected in *Coprinus* clade (family *Agaricaceae*) opposite to *Coprinellus* and *Coprinopsis* clades (family *Psathyrellaceae*).

Thus, by content of unsaturated and saturated FAs the species from clades *Coprinellus* differ from *Coprinopsis* species and *Coprinus comatus*.

Regarding to not yet reclassified species *Coprinus patouiilardii* it contains all fatty acids, except myristic acid.

Conclusion. Received data on qualitative analysis of fatty acids composition of several coprini mushrooms confirmed that linoleic, palmitic and stearic acids are predominant in mushrooms. All five fatty acids were detected in *Coprinus* clade

(family *Agaricaceae*) in contrast to *Coprinellus* and *Coprinopsis* clades (family *Psathyrellaceae*). The presence of unsaturated linoleic and oleic acids in almost all tested coprini samples makes them perspective for further studies to formulate novel mushroom-based dietary supplements.

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