Triacylglycerol Profile as a Chemical Fingerprint of Mushroom Species: Evaluation by Principal Component and Linear Discriminant Analyses

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Abstract

Mushrooms are becoming relevant foods due to their nutritional, gastronomic and pharmacological properties, namely antioxidant, antitumor and antimicrobial properties. However, despite several mushroom species have been chemically characterized, the evaluation of triacylglycerol (TAG) profile remained nearly discarded. Since TAG was formerly used to assess the authentication of highly valued commercial oils, and the distribution of fatty acids on the glycerol molecule is genetically controlled, the potential of TAG profile to act as taxonomical marker was evaluated in 30 wild mushroom species. Principal component analysis and linear discriminant analysis were used to verify the taxonomical rank (order, family, genus or species) more related with the detected TAG profile. The results pointed out that the ability of TAG profile to discriminate mushroom samples increased for the lower taxonomical ranks, reaching a maximal performance for species discrimination. Since there is high resemblance among mushroom species belonging to the same genus and considering that conservation techniques applied to mushrooms often change their physical properties, this might be considered as a valuable outcome with important practical applications.

Keywords: Wild mushrooms; triacylglycerols; PCA; LDA.

INTRODUCTION

The Northeast of Portugal, with its climatic conditions and flora diversity, is one of the European regions with higher wild edible mushrooms diversity, some of them with great gastronomic relevance. Studies conducted on mushrooms proved their antioxidant (*1*), antitumor (*2*) and antimicrobial properties, as well as their interesting contents in nutraceuticals (*3*). Furthermore, mushrooms are becoming important in our diet for their nutritional and organoleptic characteristics (*4*). Our research group has been interested in the bioactive properties and chemical profile of wild and commercial mushrooms; regarding chemical characterization, special attention has been dedicated to the determination of proteins, fat, ash, carbohydrates, individual sugars, fatty acids, phenolic compounds, carotenoids, ascorbic acid and tocopherols (*5-11*). Other authors also analyzed ergosterol, vitamin D2, nucleosides and nucleobases in mushrooms (*12-15*).

The determination of selected lipid species is of considerable interest because it allows conclusions on metabolic processes (*16*). Furthermore, the lipidic fraction of a natural product has a characteristic pattern of triacylglycerols (TAG), comprising highly specific information due to the genetic control of the stereospecific distribution of fatty acids (FA) on the glycerol molecule, which is typical for each species (*17*). Analysis of TAG in oils and fats has gained increasing attention in the last decades. In food research, it is used to study crystallization phenomena, to detect adulteration of specialty fats and oils, and for recognition of oils origin (*18*). Nevertheless, the studies dealing with TAG in mushrooms are rather scarce and based on highly specific features, for instance the neurolysin inhibitory ability of agaricoglycerides (a class of aromatic triacylglycerols) produced by some Basidiomycetes (*19*). The evaporative light-scattering detector (ELSD) is a mass-sensitive detector that responds to any analyte less volatile than the mobile phase, which is a suitable solution for TAG analysis. It has a low background signal, a non-specific response (unlike a flame ionization detector), is compatible

with gradient elution (unlike a refraction index (RI) detector) and with a broad range of solvents, besides having a signal independent of the degree of saturation and chain length (unlike an ultraviolet detector). From a theoretical point of view, the response of the ELSD is sigmoidal upon increasing analyte concentrations (*18*). Partition number, equivalent carbon number, theoretical carbon number, and matrix models are proposed methods to identify TAG peaks from HPLC-ELSD analysis. These methods are relatively well fit when reversed-phase (RP)-HPLC is used as analytical tool (*20*).

Due to the high commercial value of mushrooms, finding an analytical parameter that might act as a chemical fingerprint is a mandatory subject. Herein, thirty different species of mushrooms are characterized regarding their TAG profile, in order to define this parameter as a taxonomical marker. The results were scrutinized trough an analysis of variance, a principal component analysis as pattern recognition unsupervised classification method, and a stepwise based linear discrimination analysis as a supervised classification technique.

MATERIALS AND METHODS

Standards and reagents

Triacylglycerols 1,2,3-tripalmitoylglycerol (PPP), 1,2,3-tristearoylglycerol (SSS), 1,2,3-trilinolenoylglycerol (LnLnLn), and 1,2,3-tripalmitoleoylglycerol (PoPoPo), of purity >98%, and 1,2,3-trioleoyglycerol (OOO), 1,2,3-trilinoleoyglycerol (LLL), 1,2-dilinoleoyl-3-palmitoyl-*rac*-glycerol (PLL), 1,2-dilinoleoyl-3-oleoyl-*rac*-glycerol (OLL), 1,2 -dipalmitoyl-3-oleoyl-*rac*-glycerol (PPO), 1,2-dioleoyl-3-stearoyl-*rac*-glycerol (OOS), 1-palmitoyl-2-oleoyl-3-linoleoylglycerol (POL), and 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol (POO), of ≈99% purity, were purchased from Sigma (St. Louis, MO, USA). Petroleum ether was of analytical grade and obtained from Fisher Scientific (Leicestershire, UK). Acetonitrile and acetone were

of HPLC grade and obtained from Merck (Darmstadt, Germany). The code letters used for the fatty acids are: Po, palmitoleic; L, linoleic; Ln, linolenic; M, myristic; O, oleic; P, palmitic; S, stearic.

Samples

Samples of thirty different wild edible mushrooms (**Table 1**; their composition in fatty acids was previously reported in the cited references) were collected in Bragança (Northeast Portugal) between 2005 and 2010. Taxonomical identification of sporocarps was made and representative voucher specimens were deposited at the herbarium of Escola Superior Agrária of Instituto Politécnico de Bragança. All the samples were lyophilised (Ly-8-FM-ULE, Snijders, Holland), reduced to a fine dried powder (20 mesh) and mixed to obtain a homogenate sample.

Triacylglycerols analysis

The sample (~3 g) was submitted to an extraction with petroleum ether (40-60 ºC) performed in Soxhlet apparatus for 1.5 h. The chromatographic analyses were carried out according to the procedure previously described *(21)*, with a Jasco (Tokyo, Japan) HPLC system, equipped with a PU-1580 quaternary pump and a Jasco AS-950 automatic sampler with a 10 µL loop. The chromatographic separation of the compounds was achieved with a Kromasil 100 C_{18} (5 µm; 250 × 4.6 mm) column (Teknokroma, Barcelona, Spain) operating at room temperature (≈20 °C). The mobile phase was a mixture of acetone and acetonitrile (70:30), in an isocratic mode, at an elution rate of 1 mL/min. Detection was performed with an evaporative light-scattering detector (ELSD) (model 75-Sedere, Alfortville, France) with the following settings: evaporator temperature 40 ºC, air pressure 3.5 bar and photomultiplier sensitivity 6. Taking into account the selectivities (R, relative retention times to LLL), peaks were identified

according to the logarithms of R in relation to homogeneous TAG standards. Quantification of the peaks was made by internal normalization of chromatographic peak area, and the results were expressed in relative percentage, assuming that the detector response was the same for all the compounds. Data were analyzed using the Borwin-PDA Controller Software (JMBS, France).

Statistical analysis

Two samples of each mushroom species were used. For each mushroom sample, two extractions were performed, and each extract was injected twice in the HPLC system. Data were expressed as means±standard deviations. All the statistical tests were performed at a 5% significance level using the SPSS software, version 18.0 (SPSS Inc).

Analysis of variance

The fulfillment of the one-way ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Kolmogorov-Smirnov with Lilliefors correction and the Levene's tests, respectively. In the cases where statistical significance differences were identified, the dependent variables were compared using Tukey's honestly significant difference (HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively.

Principal component analysis (PCA)

PCA was applied as pattern recognition unsupervised classification method. PCA transforms the original, measured variables into new uncorrelated variables called principal components. The first principal component covers as much of the variation in the data as possible. The second principal component is orthogonal to the first and covers as much of the remaining

variation as possible, and so on (*18*). The number of dimensions to keep for data analysis was evaluated by the respective eigenvalues (which should be greater than one), by the Cronbach's alpha parameter (that must be positive) and also by the total percentage of variance (that should be as higher as possible) explained by the number of components selected.

Stepwise linear discriminant analysis (LDA)

LDA was used to classify the mushroom species according to their TAG profiles. A stepwise technique, using the Wilks' λ method with the usual probabilities of F (3.84 to enter and 2.71 to remove), was applied for variable selection. This procedure uses a combination of forward selection and backward elimination procedures, where before selecting a new variable to be included, it is verified whether all variables previously selected remain significant (*19, 20*). Discriminant analysis defines a combination of varieties in a way that the first function furnishes the most general discrimination between groups, the second provides the second most, and so on (*25*). To verify which canonical discriminant functions were significant, the Wilks' λ test was applied. To avoid overoptimistic data modulation, a leaving-one-out cross-validation procedure was carried out to assess the model performance. Moreover, the sensibility and specificity of the discriminant model were computed from the number of individuals correctly predicted as belonging to an assigned group (*24*). Sensibility was calculated by dividing the number of samples of a specific group correctly classified by the total number of samples belonging to that specific group. Specificity was calculated by dividing the number of samples of a specific group classified as belonging to that group by the total number of samples of any group classified as belonging to that specific group.

RESULTS AND DISCUSSION

In the absence of similar publications reporting TAG profile of wild edible mushrooms, **Table 1** presents the FA with higher representativeness among the TAG of the studied species. **Table 2** shows the mean values obtained for TAG profiles of each mushroom species. Besides the evaluated compounds, OLLn was also found in *Laccaria amethystina*. The values are presented in relative percentage, because in the particular case of TAG, the existence of high purity standards with a mixed FA composition is limited. However, even if reference material was commercially available, the diversity of TAG molecules in each oil would make virtually impossible to construct a calibration curve for each TAG. Accordingly, the relative peak areas might be readily converted into relative TAG concentration, assuming linearity and uniformity of the detector signal, regardless of the TAG molecules and absolute concentration (*18*). Denoting S = saturated, M = monoenoic, D = dienoic and T = trienoic acids, the following order of chromatographic separation is generally obtained: SSS>SSM>SMM> SSD>MMM>SMD>MMD>SDD>SST>MDD>SMT>MMT>DDD>SDT>MDT>DDT>STT> MTT>DTT>TTT (*26*). TAG found in this work (presented in **Table 2** according with their elution time) followed the expected order: PPO (SSM)>POO (SMM)>OOO (MMM)>POL (SMD)>OOL (MMD)>PLL (SDD)>OLL (MDD)>LLL (DDD)>LLLn (DDT)>LLnLn (DTT). Furthermore, and despite this conclusion cannot be drawn so directly, the obtained profiles are generally in agreement with the FA percentages (**Table 1**) quantified by several researchers in these mushroom species.

Analysis of variance (ANOVA). Regarding the main purpose of this work, *i.e.* assessing TAG profile as mushroom taxonomical markers, the Levene test showed that the assumption of equality among variances could only be assumed for OLL and OOL. Even so, to facilitate the analysis, and since the statistical differences ($p < 0.05$) were always significant (as detected by the one-way ANOVA test), the differences among mushroom species were

classificated by means of the Tamhanes' T2 test. The multiple comparisons allowed the conclusion that TAG profiles of the evaluated species were quite dissimilar. For instance, the maximal values for each TAG were exclusive for a single mushroom, except in the case of PPO (LLnLn, *Fistulina hepatica*: 22±1; LLLn, *Lycoperdon umbrinum*: 27±1; LLL, *Leucoagaricus leucothites*: 57±1; OLL, *Leucopaxillus giganteus*: 36±1; PLL, *Chlorophyllum rhacodes*: 35±1; OOL, *Sarcodon imbricatus*: 42±1; POL, *Clavariadelphus pistillaris*: 28.9±0.4; OOO, *Lycoperdon molle*: 60±1; POO, *Amanita caesarea*: 33±1; PPO, *Macrolepiota procera*: 19±1 and *Boletus edulis*: 18±1). As an example, the HPLC-ELSD TAG profiles of *Lycoperdon molle* (A) and *Ramaria aurea* (B) can be observed in **Figure 1**. The significant differences found among the mean values for each TAG are signalized by different letters in each column, and as it can be seen, most of the values could be differentiated from each other. These differences were a good preliminary indicator of the ability of TAG profile to act as taxonomical marker. This assumption was checked through a Principal Component Analysis (PCA), as an unsupervised classification technique, and a Linear Discriminant Analysis (LDA), as a supervised classification technique.

Principal Component Analysis (PCA). PCA was applied using different labeling variables: order, family or genus. In each case, the first two dimensions were considered. The reliability of these dimensions was assured by the value of the Cronbach's alpha parameter (first dimension: 0.769; second dimension: 0.558) and the related eigenvalue (first dimension: 3.251; second dimension: 2.008). The selected dimensions account for most of the variance of all quantified variables (32.5% and 20.1%, respectively). Third and fourth dimensions were also reliable (Cronbach's alpha- third dimension: 0.286; fourth dimension: 0.090; eigenvalue- third dimension: 1.347; fourth dimension: 1.088) and would include 77% of the variance instead of 53%, but the correspondent output would not allow a meaningful interpretation. The effects of the variables more correlated with each considered dimension (LLL, PLL,

OOO, OOL and POO, for the first; OLL, POL, LLLn and OOO for the second) allowed higher separation when genus was used as a labeling variable. Regarding the relation between the objects and variables (**Figure 2**), it is clear that *Lycoperdon*, *Clavariadelphus* and *Chlorophylum* are characterized for having high LLLn, POL and PLL percentages, respectively (dashed ellipses), but the remaining genera are somehow difficult to characterize. Although the lower dimensional solutions often conceal differences among variables, PCA results were satisfactory, and there was no need to increase the number of dimensions. In fact, the results plotted in **Figure 2** show that, in general, the TAG profiles recorded for different mushroom genera evaluated in this study contain valuable information that may be used as an effective tool for their diferentiation. Actually, the spatial distribution of the object points was improved with the lowering of taxonomical rank, indicating that TAG profile is most related with the lowest ranks. This is in accordance with the the genetic control of the stereospecific distribution of fatty acids (FA) on the glycerol molecule, which is typical for each species (*17*).

Linear Discriminant Analysis (LDA). To confirm this hypothesis a LDA was also performed, attempting to separate the assayed mushroom species based on their taxonomical ranks. The significant independent variables (TAG) were selected using the stepwise procedure of the LDA, according to the Wilks' λ test. Only those that showed a statistical significant classification performance ($p < 0.05$) were kept for analysis. The analysis was applied considering order, family, genus or species as grouping variables. As it would be expected after the performed PCA, the classification performance decreased from lower to higher taxonomical ranks (**Table 3**). In fact, when mushrooms were grouped by species, 100.0% of the samples were correctly classified for the originally grouped cases, as well as for the cross-validated grouped cases, but due to practical reasons, the presented output (**Figure 3**) is the one obtained using genus as grouping variable. The three plotted functions

integrated 89.2% of the observed variance (first: 59.1%; second: 15.8%; third: 14.3%). As it can be observed, besides the clusters are well individualized, the model joined (dot and dashed ellipses) genera belonging to the same family (*Armillaria*, *Calocybe*, *Lepista*, *Leucopaxillus* and *Tricholoma* belonging to *Tricholomataceae*; *Agaricus*, *Chlorophylum* and *Leucoagaricus* belonging to *Agaricaceae*; *Bovista* and *Lycoperdon* belonging to *Lycoperdaceae*).

In summary, the set of analyzed mushrooms presented very particular intrinsic differences in their TAG profile. Hence, chemical assessment linked to stereospecific analysis of TAG can be very useful in checking mushroom species. In fact, the usefulness of stereospecific analysis of TAG as a potential species discriminator was already indicated in vegetable oils (*32*). Herein, the results obtained for TAG analysis showed the ability to assemble the tested mushroom species within single groups, indicating a high degree of specificity possibly derived from the genetic control of the stereospecific distribution of FA on the glycerol molecule (*17*). Therefore, TAG profile seems to be related with the most specific taxonomical rank, proving that it might be used as a practical tool to identify a particular mushroom species. Since the conservation techniques applied to mushrooms often change their physical properties, TAG profile might be a feature for the species identification.

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LITERATURE CITED

(1) Ferreira, I. C. F. R.; Barros, L.; Abreu, R. M. V. Antioxidants in wild mushrooms. *Curr. Med. Chem.* **2009**, *16*, 1543-1560.

(2) Ferreira, I. C. F. R.; Vaz, J. A.; Vasconcelos, M. H.; Martins, A. Compounds from wild mushrooms with antitumor potential. *Anti-cancer Agent Me.* **2010**, *10*, 424-436.

(3) Barros, L.; Cruz, T.; Baptista, P.; Estevinho, L.; Ferreira, I. C. F. R. Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food Chem. Toxicol.* **2008a**, *46*, 2742-2747.

(4) Barros, L.; Baptista, P.; Correia, D. M.; Casal, S.; Oliveira, M. B. P. P.; Ferreira, I. C. F. R. Fatty acid and sugar compositions, and nutritional value of five wild edible mushrooms from Northeast Portugal. *Food Chem.* **2007**, *105*, 140-145.

(5) Barros, L.; Venturini, B. A.; Baptista, P.; Estevinho, L. M.; Ferreira, I. C. F. R. Chemical composition and biological properties of Portuguese wild mushrooms: A comprehensive study. *J. Agr. Food Chem.* **2008b**, *56*, 3856-3862.

(6) Grangeia, C.; Heleno, S. A.; Barros, L.; Martins, A.; Ferreira, I. C. F. R. Effects of trophism on nutritional and nutraceutical potential of wild edible mushrooms. *Food Res. Int.* **2011**, *44*, 1029-1035.

(7) Heleno, S. A.; Barros, L.; Sousa, M. J.; Martins, A.; Ferreira, I. C. F. R. Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and high performance liquid chromatography. *Microchem. J.* **2009**, *93*, 195-199.

(8) Heleno, S. A.; Barros, L.; Sousa, M. J.; Martins, A.; Santos-Buelga, C.; Ferreira, I. C. F. R. Targeted metabolites analysis in wild Boletus species. *LWT-Food Sci. Technol.* **2011**, *44*, 1343-1348.

(9) Pereira, E.; Barros, L.; Martins, A.; Ferreira, I. C. F. R. Towards chemical and nutritional inventory of Portuguese wild edible mushrooms in different habitats. *Food Chem.* **2012**, *130*, 394-403.

(10) Reis, F. S.; Heleno, S. A.; Barros, L.; Sousa, M. J.; Martins, A.; Santos-Buelga, C.; Ferreira, I. C. F. R. Towards the antioxidant and chemical characterization of mycorrhizal mushrooms from Northeast Portugal. *J. Food Sci.* **2011**, *76*, 824-830.

(11) Vaz, J. A.; Barros, L.; Martins, A.; Santos-Buelga, C.; Vasconcelos, M. H.; Ferreira, I. C. F. R. Chemical composition of wild edible mushrooms and antioxidant properties of their water soluble polysaccharidic and ethanolic fractions. *Food Chem.* **2011**, *126*, 610-616.

(12) Yuan, J.-P.; Kuang, H.-C.; Wang, J.-H.; Liu, X. Evaluation of ergosterol and its esters in the pileus, gill, and stipe tissues of agaric fungi and their relative changes in the comminuted fungal tissues. *Appl. Microbiol. Biotechnol.* **2008**, *80*, 459-465.

(13) Jasinghe, V.J.; Perera, C.O. Distribution of ergosterol in different tissues of mushrooms and its effect on the conversion of ergosterol to vitamin D2 by UV irradiation. *Food Chem.* **2005**, *92*, 541-546.

(14) Li, S.P.; Li, P.; Lai, C.M.; Gong, Y.X.; Kan, K.W.; Dong, T.T.X.; Tsim, K.W.K.; Wang, Y.T. Simultaneous determination of ergosterol, nucleosides and their bases from natural and cultured Cordyceps by pressurised liquid extraction and high-performance liquid chromatography. *J. Chromatog. A* **2004**, *1036*, 239-243.

(15) Yuan, J. P.; Zhao, S. Y.; Wang, J. H.; Kuang, H. C.; Liu, X. Distribution of nucleosides and nucleobases in edible fungi. *J. Agric. Food Chem.* **2008**, *56*, 809-815.

(16) Fuchs, B.; Schiller, J. Lysophospholipids: their generation, physiological role and detection. Are they important disease markers? *Mini-Rev. Med. Chem.* **2009**, *9*, 368-378.

(17) Buchgraber, M.; Ulberth, F.; Emons, H.; Anklam, E. Triacylglycerol profiling by using chromatographic techniques. *Eur. J. Lipid Sci. Tech.* **2004**, *106*, 621-648.

(18) Rombaut, R.; De Clercq, N.; Foubert, I.; Dewettinck, K. Triacylglycerol Analysis of Fats and Oils by Evaporative Light Scattering Detection. *J. Am. Oil Chem. Soc.* **2009**, *86*, 19-25.

(19) Stadler, M.; Hellwig, V.; Mayer-Bartschmid, A.; Denzer, D.; Wiese, B.; Burkhardt, N. Novel analgesic triglycerides from cultures of *Agaricus macrosporus* and other Basidiomycetes as selective inhibitors of neurolysin. *J. Antibiot.* **2005**, *58* (12), 775-786.

(20) Cunha, S. C.; Oliveira, M. B. P. P. Discrimination of vegetable oils by triacylglycerols evaluation of profile using HPLC/ELSD. *Food Chem.* **2006**, *95*, 518-524.

(21) Barreira, J. C. M.; Casal, S.; Ferreira, I. C. F. R.; Oliveira, M. B. P. P.; Pereira, J. A. Nutritional, fatty acid and triacylglycerol profiles of *Castanea sativa* Mill. cultivars: a compositional and chemometric approach. *J. Agr. Food Chem.* **2009**, *57*, 2836-2842.

(22) Patras, A.; Brunton, N. P.; Downey, G.; Rawson, A.; Warriner, K.; Gernigon, G. Application of principal component and hierarchical cluster analysis to classify fruits and vegetables commonly consumed in Ireland based on in vitro antioxidant activity. *J. Food Compos. Anal.* **2011**, *24*, 250-256.

(23) Hill, T.; Lewicki, P. Statistics: methods and applications. A comprehensive reference for science, industry, and data mining, StatSoft, **2006**.

(24) López, A.; García, P.; Garrido, A. Multivariate characterization of table olives according to their mineral nutrient composition. *Food Chem.* **2008**, *106*, 369-378.

(25) Benitez, E.; Nogales, R.; Campos, M.; Ruano, F. Biochemical variability of olive-orchard soils under different management systems. *Appl. Soil Ecol.* **2006**, *32*, 221-231.

(26) Fuchs, B.; Rosmarie, Süβ R.; Teuber, K., Eibisch, M.; Schiller J. Lipid analysis by thin-layer chromatography - a review of the current state. *J. Chromatogr. A* **2011**, *1218*, 2754-2774.

(27) Pedneault, K.; Angers, P.; Gosselin, A.; Weddell, R. J. Fatty acid composition of lipids from mushrooms belonging to the family *Boletaceae*. *Mycol. Res.* **2006**, *110*, 1179-1183.

(28) Kavishree, S.; Hemavathy, J.; Lokesh, B. R.; Shashirekha, M. N.; Rajarathnam, S. Fat and fatty acids of Indian edible mushrooms. *Food Chem.* **2008**, *106*, 597-602.

(29) Liu, Y.-T.; Sun, J.; Luo, Z.-Y.; Rao, S.-Q.; Su, Y.-J.; Xu, R.-R.; Yang, Y.-J. Chemical composition of five wild edible mushrooms collected from Southwest China and their antihyperglycemic and antioxidant activity. *Food Chem. Toxicol.* **2012**, *in press*.

(30) Senatore, F.; Dini, A.; Marino, A. Chemical constituents of some Basidiomycetes. *J. Sci. Food Agr.* **1988**, *45*, 337-345.

(31) Díez, V.A.; Alvarez, A. Compositional and nutritional studies on two wild edible mushrooms from northwest Spain. *Food Chem.* **2001**, *75*, 417-422.

(32) Butinar, B.; Bučar-Miklavčič, M.; Valenčič, V.; Raspor, P. Stereospecific analysis of triacylglycerols as a useful means to evaluate genuineness of pumpkin seed oils: lesson from virgin olive oil Analyses. *J. Agric. Food Chem.* **2010**, *58*, 5227-5234.

Table 1. Fatty acids composition (%) in some selected studies using the species herein studied. The results are presented, except when non-avaliable, as mean±SD.

Order	Family	Species	Palmitic acid	Oleic acid	Linoleic acid	References
Agaricales	*Agaricaceae*	*Agaricus silvaticus*	11.7±0.1	6.67±0.01	74.78±0.01	(*3*)
Agaricales	*Agaricaceae*	*Agaricus silvicola*	10.0±0.2	3.5±0.2	76.5±0.2	(*3*)
Agaricales	*Amanitaceae*	*Amanita caesarea*	12.4±0.4	54±1	26±2	(*10*)
Tricholomatales	*Tricholomataceae*	*Armillaria mellea*	11.0±0.1	47.7±0.4	27.7±0.3	(*11*)
Boletales	*Boletaceae*	*Boletus edulis*	10.0±0.3	40±2	44±2	(*3*)
			9.6±0.2	42.1±0.2	41.3±0.1	(*8*)
			9.8	36.1	42.2	(*27*)
			21.6	31.1	33.8	(*28*)
Boletales	*Boletaceae*	*Boletus erythropus*	21±1	15±1	49±1	(*6*)
			11.20	18.00	63.00	(*27*)
Boletales	*Boletaceae*	*Boletus fragrans*	14.9±0.1	20±1	57±1	(*6*)
Boletales	*Boletaceae*	*Boletus impolitus*	16.8±0.4	14±1	61±1	(*9*)
Boletales	*Boletaceae*	*Boletus reticulatus*	11.0±0.1	47.2±0.1	32.83±0.01	(*8*)
Lycoperdales	*Lycoperdaceae*	*Bovista aestivalis*	21±2	12.6±0.1	42±4	(*9*)
Lycoperdales	*Lycoperdaceae*	*Bovista nigrescens*	17.4±0.1	21.0±0.2	38.3±0.2	(*9*)
Tricholomatales	*Tricholomataceae*	*Calocybe gambosa*	15±1	18±1	58±1	(*3*)
			13.6±0.5	33±1	43.9±0.3	(*11*)

Agaricales	*Agaricaceae*	*Chlorophyllum rhacodes*	16.4±0.3	5.7±0.1	72.6±0.5	(*9*)
Clavariales	*Clavariadelphaceae*	*Clavariadelphus pistillaris*	17±1	49.1±0.2	25±1	(*9*)
Cortinariales	*Cortinareaceae*	*Cortinarius violaceus*	14.02±0.04	15±1	66±1	(*10*)
Polyporales	*Fistulinaceae*	*Fistulina hepatica*	10±1	31.5±0.1	52±1	(*7*)
Tricholomatales	*Hydnangeaceae*	*Laccaria amethystina*	6.9±0.4	14±1	74.4±0.2	(*29*)
Tricholomatales	*Tricholomataceae*	*Lepista nuda*	11.8±0.1	29.53±0.04	51.5±0.1	(*5*)
Agaricales	*Agaricaceae*	*Leucoagaricus leucothites*	12.2±0.2	6.3±0.4	75±1	(*9*)
Agaricales	*Tricholomataceae*	*Leucopaxillus giganteus*	13.5±0.1	21.1±0.5	46.2±0.5	(*4*)
Lycoperdales	*Lycoperdaceae*	*Lycoperdon molle*	13.7±0.2	8.6±0.1	64.2±0.4	(*5*)
Lycoperdales	*Lycoperdaceae*	*Lycoperdon umbrinum*	19.9±0.1	22.8±0.3	29.4±0.1	(*9*)
Agaricales	*Lepiotaceae*	*Macrolepiota procera*	4.6	17.2	47.0	(*28*)
Clavariales	*Ramariaceae*	*Ramaria aurea*	7.32±0.04	56.9±0.5	25.6±0.2	(*9*)
Clavariales	*Ramariaceae*	*Ramaria botrytis*	9.91±0.03	43.9±0.1	38.3±0.1	(*5*)
Russulales	*Russulaceae*	*Russula cyanoxantha*	13.0±0.2	28±1	44±1	(*6*)
			17.20	26.00	47.40	(*30*)
Telephorales	*Bankeraceae*	*Sarcodon imbricatus*	11.14±0.05	45.1±0.2	35.4±0.4	(*4*)
Tricholomatales	*Tricholomataceae*	*Tricholoma imbricatum*	7.4±0.2	51.5±0.4	33.0±0.1	(*7*)
Tricholomatales	*Tricholomataceae*	*Tricholoma portentosum*	5.60±0.01	58.4±0.1	30.9±0.1	(*4*)
			7.6	58.0	27.9	(*31*)

Table 2. Triacylglycerol composition (%).The results are presented as mean±SD.[a]

		LLnLn	LLLn	LLL	OLL	PLL	OOL	POL	OOO	POO	PPO
Species	*Agaricus silvaticus*	9.4±0.3 e	nd	29±1 f	12±1 ij	17±1 d	12±1 no	4.3±0.2 lmn	11.7±0.4 k	nd	4.2±0.2 de
	Agaricus silvicola	1.2±0.1 jkl	0.4±0.1 gh	47±1 b	8±1 no	26±1 b	9±1 p	0.5±0.1 qr	2.8±0.3 no	1.7±0.3 i	3.2±0.3 efg
	Amanita caesarea	nd	nd	3.2±0.1 qr	5.0±0.2 q	1.6±0.1 mno	23±1 gh	5.5±0.5 kl	24±1 f	33±1 a	4.3±0.2 de
	Armillaria mellea	0.18±0.02 no	0.19±0.02 ghi	3.5±0.2 q	12±1 hi	2.4±0.3 jklmn	25±1 g	9±1 ef	30±1 e	16±1 d	1.3±0.1 ijkl
	Boletus edulis	nd	nd	9.2±0.3 lm	11.0±0.3 ijk	3.1±0.1 jkl	21±1 ijk	22±1 b	15.2±0.5 ij	nd	18±1 a
	Boletus erythropus	nd	nd	19.2±0.2 j	9.4±0.3 lm	7.3±0.3 h	32±2 d	9.5±0.2 ef	5.8±0.3 m	9±1 f	7±1 c
	Boletus fragrans	1.2±0.3 jkl	2.0±0.2 e	34±1 d	11±1 jkl	8.8±0.4 g	16±1 m	8±1 fgh	4.9±0.3 m	6.8±0.5 g	8±1 c
	Boletus impolitus	1.7±0.2 ij	0.41±0.04 gh	24±1 h	5.5±0.4 pq	5.9±0.5 i	15.8±0.5 m	7.0±0.3 ij	24.8±0.4 f	13.6±0.3 e	1.2±0.1 jkl
	Boletus reticulatus	1.3±0.2 jkl	1.6±0.1 e	6.9±0.5 no	8.8±0.4 mn	0.13±0.01 p	39.4±0.5 b	2.5±0.3 op	36±1 c	nd	4.0±0.3 de
	Bovista aestivalis	10.8±0.4 d	4.4±0.2 b	13.6±0.3 k	18±1 def	2.3±0.2 jklmn	19.3±0.5 kl	4.2±0.3 mn	20±1 h	nd	8±1 c
	Bovista nigrescens	12.0±0.4 c	3.6±0.3 c	19±1 j	16.6±0.4 fg	2.0±0.3 klmn	18.2±0.5 l	5.2±0.2 kl	15.9±0.5 i	nd	7.7±0.3 c
	Calocybe gambosa	nd	nd	32±1 e	17±1 efg	6.0±0.4 i	19±1 l	4.8±0.3 klm	13.7±0.4 j	6.2±0.4 g	2.2±0.3 ghij
	Cantharellus cibarius	0.8±0.1 klmn	0.28±0.05 ghi	8±1 mno	6.8±0.5 op	3.4±0.3 j	20±1 jkl	5.1±0.4 klm	40±1 b	13.4±0.5 e	2.6±0.4 fgh
	Chlorophyllum rhacodes	1.39±0.02 jk	0.06±0.01 hi	31±1 ef	19±1 d	35±1 a	11±1 op	1.4±0.1 pq	0.25±0.02 q	0.23±0.03 jk	0.36±0.02 l
	Clavariadelphus pistillaris	1.6±0.2 j	nd	5.0±0.2 pq	18.3±0.4 de	7.8±0.3 gh	34±1 cd	28.9±0.4 a	2.0±0.2 op	1.6±0.3 ij	1.1±0.2 jkl
	Cortinarius violaceus	0.6±0.2 mn	1.1±0.2 f	25.0±0.4 gh	22±1 c	21±1 c	10±1 op	11±1 d	5.1±0.4 m	nd	4.7±0.2 d
	Fistulina hepatica	22±1 a	nd	43±1 c	13±1 hi	2.2±0.2 klmn	6.7±0.3 q	6.0±0.4 jk	4.2±0.2 mn	nd	3.5±0.2 def
	Laccaria amethystina	7.2±0.2 f	nd	26±1 g	10.3±0.3 jklm	3.1±0.2 jk	15.8±0.4 m	8.4±0.5 fg	14.2±0.5 j	9.9±0.4 f	0.5±0.1 l
	Lepista nuda	4.7±0.2 g	1.0±0.1 f	22.2±0.5 i	27±1 b	5.0±0.3 i	22±1 hi	10±1 de	5.1±0.2 m	nd	2.5±0.2 fghi
	Leucoagaricus leucothites	nd	nd	57±1 a	10±1 klm	14.6±0.5 e	11±1 op	3.5±0.3 no	1.7±0.2 opq	nd	2.2±0.1 ghij

	Leucopaxillus giganteus	0.4±0.1 no	nd	30±1 ef	36±1 a	11±1 f	14±1 mn	7.1±0.1 hij	0.42±0.04 pq	0.21±0.03 jk	nd
	Lycoperdon molle	2.3±0.2 hi	0.39±0.02 ghi	30±1 f	3.1±0.3 r	1.6±0.2 mno	1.2±0.1 r	nd	60±1 a	1.1±0.2 ijk	0.8±0.2 kl
	Lycoperdon umbrinum	13.8±0.5 b	27±1 a	34±1 d	4.9±0.5 q	5.1±0.4 i	3.2±0.2 r	1.2±0.2 qr	9.3±0.3 l	nd	1.9±0.3 hijk
	Macrolepiota procera	0.9±0.1 klm	nd	1.6±0.3 r	8.7±0.4 mn	2.6±0.2 jklm	22±1 hi	8±1 ghi	9±1 l	28±1 b	19±1 a
	Ramaria aurea	1.7±0.1 j	nd	6.0±0.2 op	11.1±0.4 ijkl	1.3±0.1 nop	29±1 e	3.9±0.1 mn	39.7±0.3 b	6.9±0.5 g	0.4±0.1 l
	Ramaria botrytis	1.2±0.2 jkl	0.5±0.1 g	7.9±0.1 mn	13±1 h	1.8±0.2 lmn	25±1 fg	4.6±0.2 lmn	33±1 d	10±1 f	3.0±0.4 efgh
	Russula cyanoxantha	4.3±0.3 g	1.6±0.1 e	13±1 k	10.1±0.4 jklm	5.0±0.5 i	22±1 hij	8.9±0.5 fg	22±1 g	nd	14±1 b
	Sarcodon imbricatus	2.4±0.2 h	2.7±0.2 d	1.5±0.2 r	7.8±0.4 no	1.8±0.3 lmn	42±1 a	13±1 c	4.2±0.4 mn	24.5±0.5 c	nd
	Tricholoma imbricatum	0.4±0.1 mno	nd	9.8±0.2 l	15.8±0.5 g	1.4±0.1 mno	27±1 f	7.8±0.4 ghi	29±1 e	9±1 f	0.4±0.1 l
	Tricholoma portentosum	0.19±0.05 no	1.7±0.2 e	4.0±0.4 q	11±1 ij	0.4±0.1 op	36±1 c	3.6±0.5 no	37±1 c	4.3±0.2 h	2.3±0.4 ghij
Homocedasticity[1]	*P*-value	0.004	<0.001	0.004	0.428	0.001	0.176	0.048	0.022	<0.001	<0.001
One-way ANOVA[2]	*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Homoscedasticity among cultivars was tested by means of the Levene test

[2]$P<0.05$ meaning that the mean value of the evaluated parameter of at least one cultivar differs from the others (in this case multiple comparison tests were performed).

[a]Means within a column with different letters differ significantly ($p < 0.05$). The results were evaluated either using the multiple comparison Tukey's HSD or Tamhane's T2 tests, depending on the fulfilment or not of the homoscedasticity requirement.

Table 3. LDA parameters considering different grouping variables.

Grouping variable	Number of functions (Wilks' Λ test)	Correctly classified groups	
		Original grouped cases	Cross-validated grouped cases
Order	$p < 0.001$	75.8	64.2
Family	$p < 0.001$	95.8	93.3
Genus	$p < 0.001$	99.2	99.2
Species	$p < 0.001$	100.0	100.0

Figure 1. Individual chromatogram of TAG profile in **(A)** *Lycoperdon* molle and **(B)** *Ramaria aurea*. 1- LLnLn; 2-LLLn; 3- LLL; 4- OLL; 5- PLL; 6- OOL; 7- POL; 8- OOO; 9- POO; 10- PPO.

Figure 2. Biplot of objects and component loadings using genus as labeling variable. Aga- *Agaricus*; Ama- *Amanita*; Arm- *Armillaria*; Bol- *Boletus*; Bov- *Bovista*; Cal- *Calocybe*; Can- *Camtharellus*; Chl- *Chlorophyllum*; Cla- *Clavariadelphus*; Cor- *Cortinarius*; Fis- *Fistulina*; Lac- *Laccaria*; Lep- *Lepista*; Leur- *Leucoagaricus*; Leux- *Leucopaxillus*; Lyc- *Lycoperdon*; Mac- *Macrolepiota*; Ram- *Ramaria*; Rus- *Russula*; Sar- *Sarcodon*; Tric- *Tricholoma*.

Figure 2. Canonical analysis of mushroom genera based on triacylglycerols profiles.

Figure 1 (A).

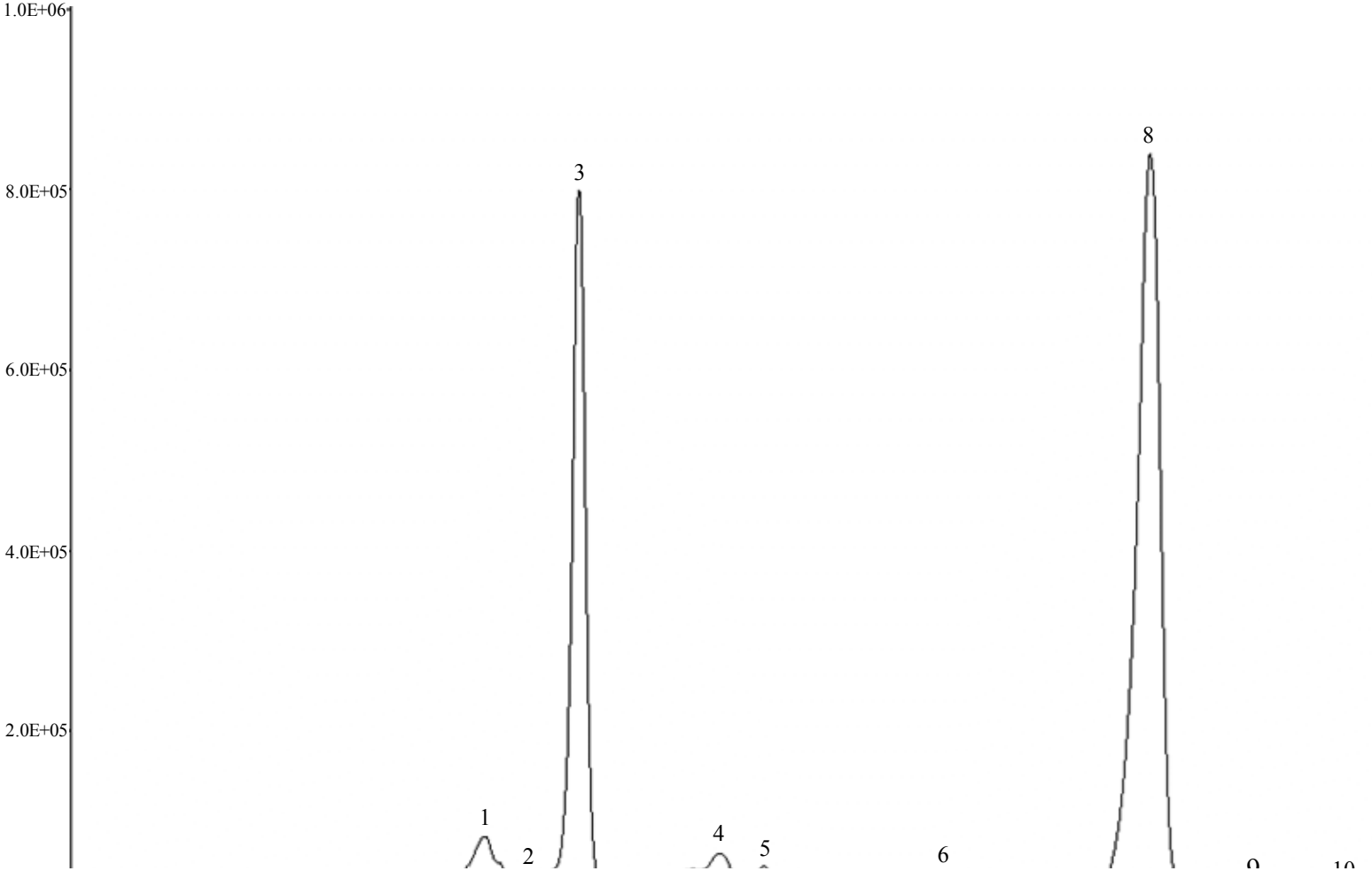
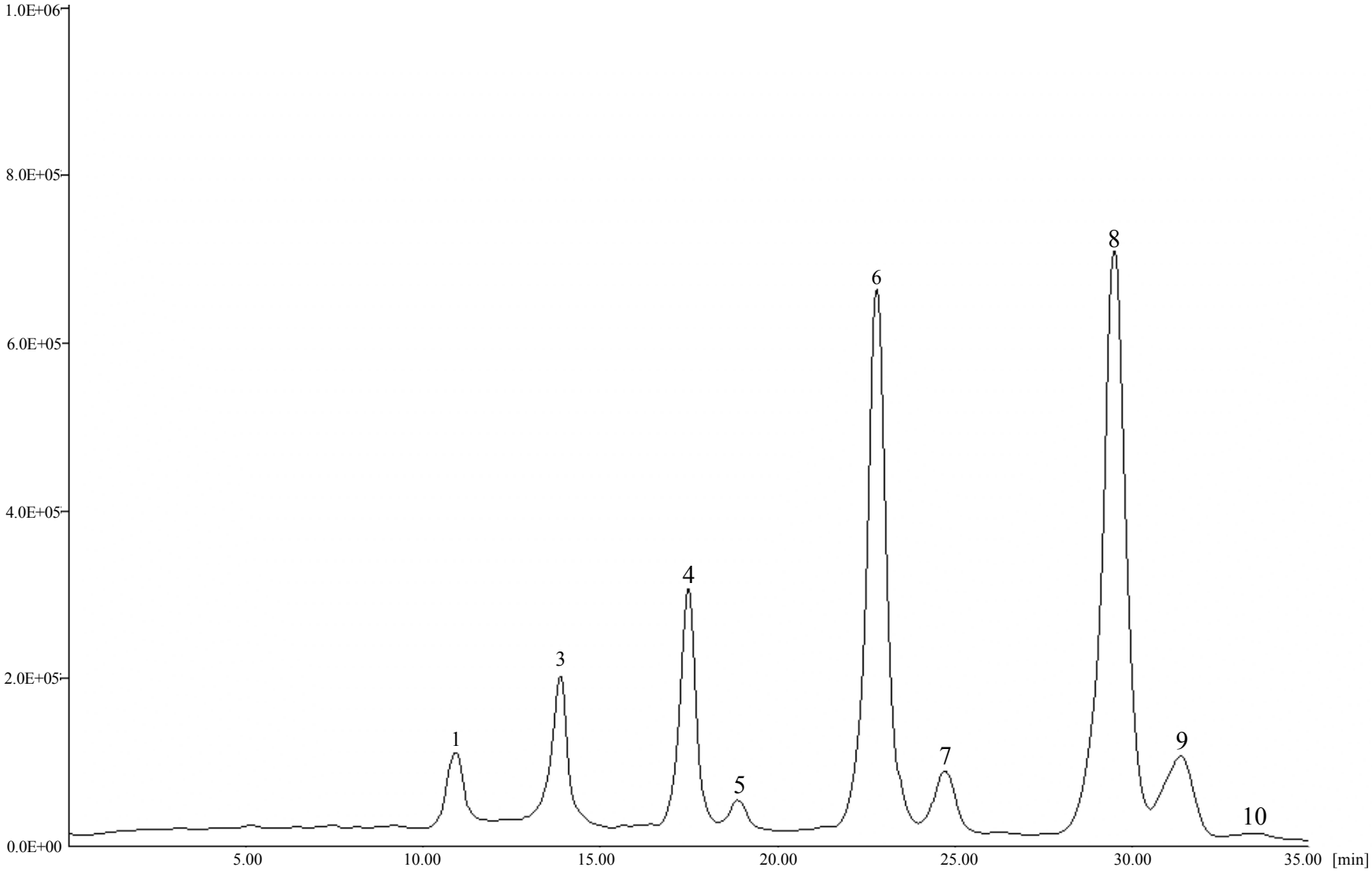


Figure 1 (B).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6.0E+05
4.0E+05
2.0E+05
0.0E+00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
[min]
1
3
4
5
6
7
8
9
10

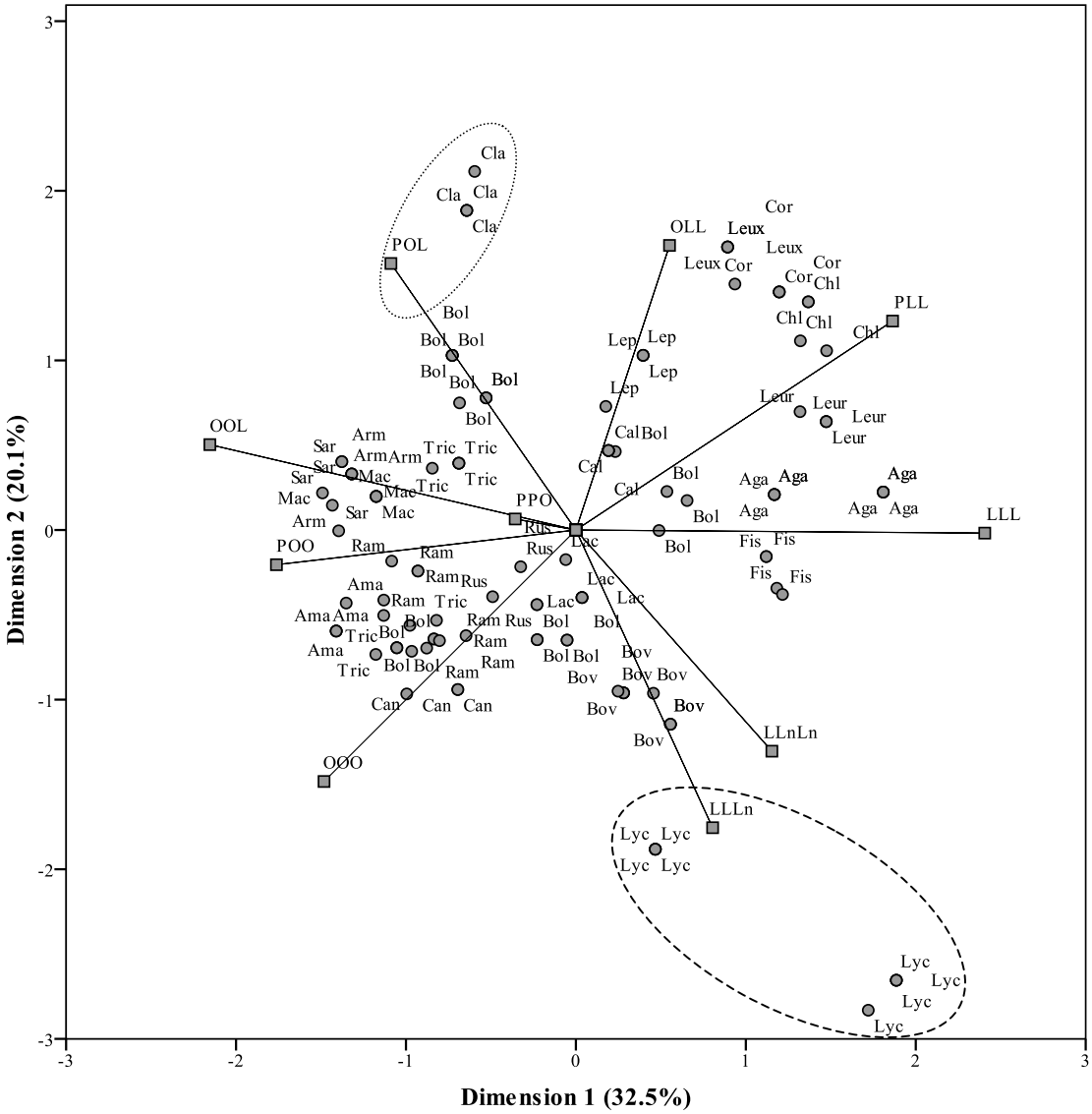
Dimension 2 (20.1%)
Dimension 1 (32.5%)
POL
OLL
PLL
LLL
OOL
PPO
POO
OOO
LLnLn
LLLn
Cla
Cor
Leux
Chl
Bol
Lep
Leur
Cal
Aga
Fis
Sar
Arm
Tric
Mac
Ram
Rus
Lac
Ama
Bov
Can
Lyc

Figure 2.

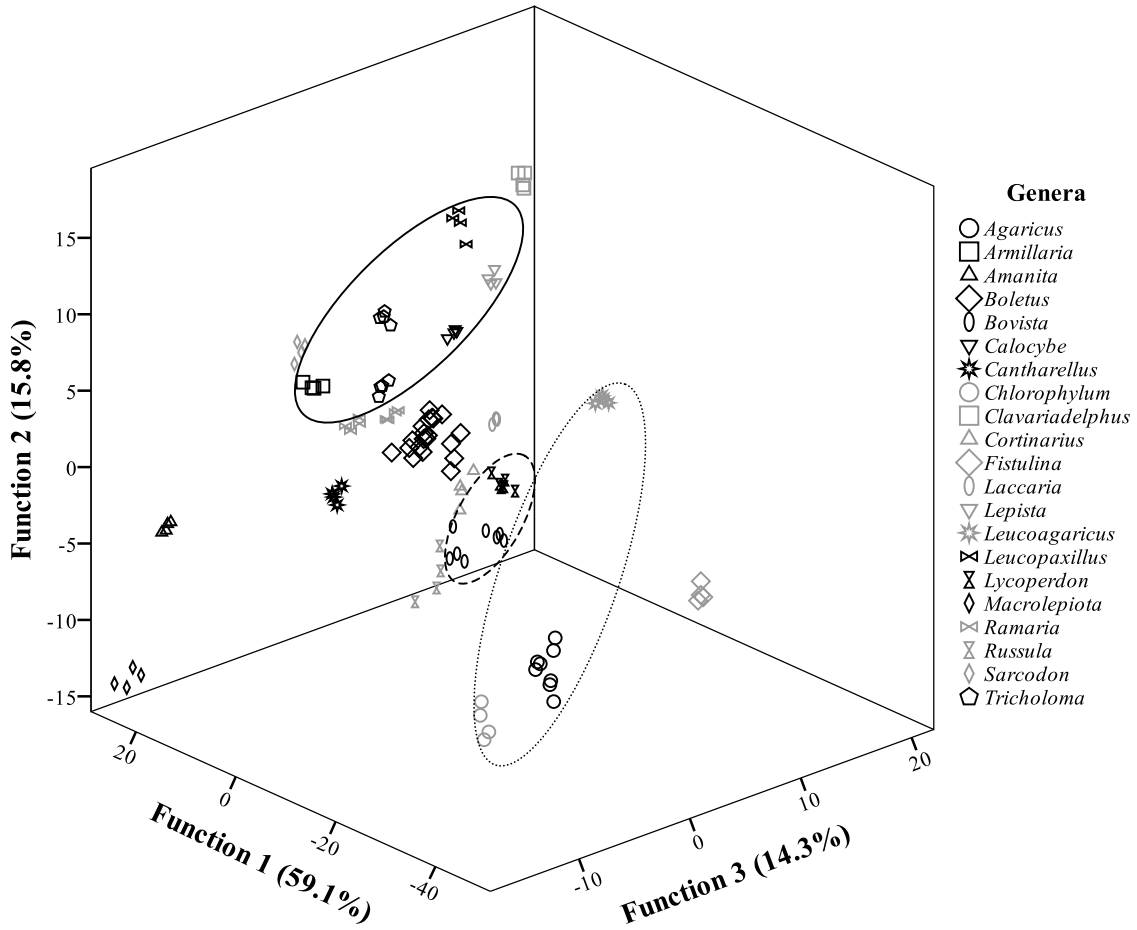
Genera
Agaricus
Armillaria
Amanita
Boletus
Bovista
Calocybe
Cantharellus
Chlorophylum
Clavariadelphus
Cortinarius
Fistulina
Laccaria
Lepista
Leucoagaricus
Leucopaxillus
Lycoperdon
Macrolepiota
Ramaria
Russula
Sarcodon
Tricholoma
Function 2 (15.8%)
15
10
5
0
-5
-10
-15
Function 1 (59.1%)
20
0
-20
-40
Function 3 (14.3%)
-10
0
10
20

Figure 3.

TOC Graphic

Triacylglycerol Profile as a Chemical Fingerprint of Mushroom Species: Evaluation by Principal Component and Linear Discriminant Analyses

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