Application of FT Spectroscopy in the Study of Fungi

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The present study is devoted to the IR–FT characterization of some fungi species, to the identification of their main functional groups, as well as to emphasize the presence of some toxic substances in the structure of certain sporiferous plants.

Keywords: spectroscopy IR–FT, fungi, functional groups

As generally known, as a result of the catabolic pathways they have for a large variety of organic substances, the fungi make use of various types of nutrients. Theoretically, almost each substance present in the environment – of either organic or inorganic nature–may be accessible to fungi, whatever complex would its chemical structure be.

The mycellium produces extracellular enzymes capable of splitting the degradation-resisting molecules, such as the lignin, cellulose, various carbohydrates, oil products, pesticides etc., up to their reduction to non-toxic products. The fungi are known both for their nutritive value and for their major involvement in various biochemical processes [1–3].

That is why, special interest is manifested for an appropriate selection of some mycorrhizing species, known as playing an important part in the preservation of biodiversity, in mycoremediation processes as well as in the biological control of mycelium cultures with applications in permaculture.

Fourier transform infrared (FT IR) spectroscopy and Raman microscopy provide rapid and hight reproductible discrimination and have been applied to characterise many sinthetic and natural products.

They are automated and offer hight throughput analyses. Recently, FT IR has been used in combination with other methods for qualitative and quantitative studies on intraspecific diversity.

IR–FT is a rapid, non-destructive spectroscopic approach for whole-sample fingerprinting. This technique is based on the absorbtion of IR ligh directed onto a sample.

The amont of light absorbed depends on the molecules found within the sample. It measures dominantly vibrations of functional groups and high polar bonds. Therefore it gives a lot of information about the total biochemical composition of the sample regarding the molecule composition, structure and interactions [4, 5].

IR–FT spectrometers record the interaction of IR radiation with samples, measuring the frequencies at which the sample absorbs the radiation and the intensities of these absorptions.

IR–FT spectroscopy has the major advantages that it is non-destructive, reproductible and is very rapid both for a single sample and with respect to the automated high throughput of samples [6].

Experimental part

The mushroom samples, cleaned up of earth and any vegetal rests (leaves, roots), are put into paper bags and preserved at a temperature of 4°C, for maximum 24 h. Under unsuitable conservation conditions (high temperature, high humidity), their protein content may be modified.

The fungi subjected to analysis have been collected in the Calimani Massif (from sterile dumps belonging to the former Calimani mining area), while the reference samples come from the neighbouring zones of the dumps.

In the laboratory, the samples were dried in glass Petri boxes, at room temperature. Parts of the sporiferous body were jarred in KBr, after which the IR spectra were recorded over the 4000-400 range.

Results and discussion

Besides proteins, the fungi also contain other nitrogenous organic substances, such as free amino-acids, amines, amides, etc.

The concentration of nitrogenous organic substances present in fungi depends on the species, on the stage of their development as well as on the amount of nitrogenous substances from the soil.

The proteins, the albumines and other nitrogenous substances represent between 3 and 5% of the organic substances present in fungi.

The proteic component represents half of the total nitrogen amount, its concentration varying as a function of species, development stage and amount of nitrogenous substances present in soil.

In the composition of fungi, the amount of proteins varies between 0.8–3.5 g% fresh matter and 19.0–39.0 % dry matter. The content of proteins is high in the lamellae and in the cap's cuticle, *i.e.* of 41% *vs.* only 33% in the foot.

The proteins include in their structure the following essential amino-acids: leucine, isoleucine, treonine, alanine, glycine, arginine, valine, methionine, phenyl-alanine, histidine, glutamic and aspargic acid, proline, serine, lysine and triptophane.

Proteins' determination establishes the extent to which chemical pollution affects the normal composition of the fungi, as well as the manner in which proteins are involved.

The retention of pollutants is present in both soil and air. The fungi, either edible or toxic, may accumulate chemical pollutants, which might increase the toxic risk when they are consumed [7]. The maximum value of pollutant agent admitted as a polluting chemical substance from the soil should not exceed the maximum concentration accepted in water, air, plants, as a result of their transfer from the soil towards these media [8].

The pollutants identified in the samples of edible fungi may have toxic effects on the human organism [9–12].

The nitrites (either as such or resulted from nitrogen oxides' combination with water) have a direct

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methemoglobinizing action, a hemolytic action, as well as a vasohypotomic and hypotensive action. More than that, they may generate N-nitro compounds (nitrozamines) known as cancerigenous for both humans and animals.

Consequently, determination of the nitrite content in fungi is aimed at estimating the capacity of certain species of accumulating the nitrogen oxides present in water and soil, either as such or as nitrites [13].

The nitrates, resulting from the mineralization of the organic substances from both water and soil, belong to the mineral oxidating substances obtained from the reaction of nitrogen dioxide with water. They manifest a characteristic methemoglobinizing toxic action, preceded by hemolysis of hematies, an action induced by the nitrites resulted from nitrates reduction.

The fungi growing on a soil rich in organic substances are capable of accumulating nitrates and nitrites, thus playing an important part in the recovery of polluted soils.

The sulphites result from the combination of sulphur dioxide with water. Once entering the organism, they induce metabolic acidosis and reduction of blood's alkaline reserve.

Sulphur dioxide is a cellular toxic substance causing disorders of the intermediary metabolic processes through enzymes' inhibition and depriving the organism of vitamins B and C. In high doses, it depresses hematopoiesis and may cause methemoglobinemy–*i.e.* hemoglobin (Fe^{2+}) is transformed into methemoglobin (Fe^{3+}), which is incapable of transporting the oxygen.

Determination of fungi' sulphite content follows the estimation of their capacity of accumulating the sulphites present in water and soil [14].

Metals. From a nutritional perspective, the metals present in fungi may be classified into 2 categories, namely: metals with physiological role (Ca, Mg, K, Na, Fe) and metals with a toxic potential (Cd, Zn, Pb, Ni, Mn, Al, Cr, Cu). For both categories of metals, any increase of their concentrations in food, over certain values, may exert noxious effects upon consumers. The seriousness extent of the toxic effect depends on the nature, amount and chemical form in which the metal occurs in some aliments, as well as on the weight of the contaminated nutrient in the structure of the menu, on the organism's resistance or on the synergetic or antagonic effect of other chemical contaminants.

The fungi are known for their high ability of accumulating metals from the environment, which is a function of their species, harvesting place, environment conditions, and development stage [15].

The mineral salts occur as phosphates, carbonates, bicarbonates, sulphates, nitrates, silicates, etc. The phosphate ion, PO_4^{3-}, evidences a relatively intense band between 1100–950 cm^{-1}, while the NO_3^- group shows vibration bands between 141410–1340 and 860–800 cm^{-1} [16,17].

The bands characteristic to the sulphate ion, S, occur between 1130-1080 and 680-610 cm^{-1}, while those characteristic to the carbonate ion – between 1450-1410 and 880-800 cm^{-1}. The $O=U=O]^{2+}$ uranyl groups show a characteristic band around 920 cm^{-1}.

Determination of fungi' metal content is useful in the evaluation of the way in which they might contribute to the recovery of polluted soils, as well as of the risk to which some people consuming chemically contaminated fungi are exposed too [18].

Lead (Pb) and its anorganic compounds evidence hemato-, neuro-, vascular- and nephro-toxicity. The metal interferes haem's biosynthesis, which is accompanied by inhibition of hemoglobin's synthesis, altering (through a direct toxic action), the erythrocytary membrane, thus reducing erythrocytes' lifetrine.

The lead is accumulated at the SNC level, causing saturnine encephalopathy while, at peripheral .levels, induces myelin degeneration, which is accompanied by the installation of motory nevrite. Its toxic action is manifested at the level of vessels, too. Also, it provokes lesions at the level of the renal duct.

Manganese (Mn) causes degenerative alterations of the nervous cells, mainly in the gray matter, which are accompanied by neurological manifestation of the Parkinson- type.

Copper (Cu) exerts its toxicity directly upon the tissues in which it gets accumulated, mainly in the liver, as well as by haemolytical and methemoglobinizing effects.

Zinc (Zn) manifests its toxic action at the level of the central nervous system, of the cardiovascular system and of the muscles.

Chromium (Cr) has an irritating, allergic, emetizing dual metho-action.

Iron (Fe) is toxic only in very high doses (kg body), when it causes gastro-intestinal disorders at the level of the central nervous, cardiovascular and hepato-renal systems.

The nickel (Ni) has a toxic action upon the central nervous system and the myocardium, its toxic mechanism being attributed to the inhibition of certain phosphatases.

Cadmium (Cd) is a cellular toxic, its main toxic action being of the tyolopriving type (*i.e.*, it blacks the enzymes' SH groups) which also alters tissular breathing and several intermediary metabolisms.

Natrium (Na) brings about disequilibria of the hydro-mineral metabolism and water's retention in the organism.

Potassium (K) manifests toxic action at the level of the cardiovascular system, perturbing the hydro-mineral metabolism. Several species of fungi have been harvested and analyzed, their FT spectra being plotted in figure 1.

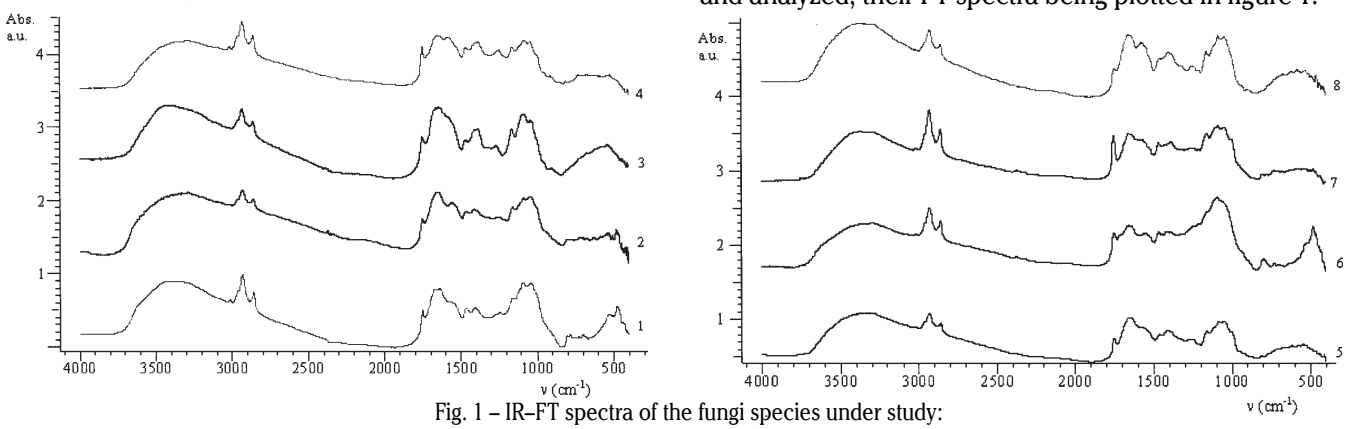

Fig. 1 – IR–FT spectra of the fungi species under study:
1. Clitopilus prunulus; 2. Pleurotus eryngii; 3. Macrolepiota rhacodes; 4. Suillus luteus; 5. Lyophyllum connatum; 6. Lactarius volemus; 7. Paxillus involutus; 8. Lacaria laccata

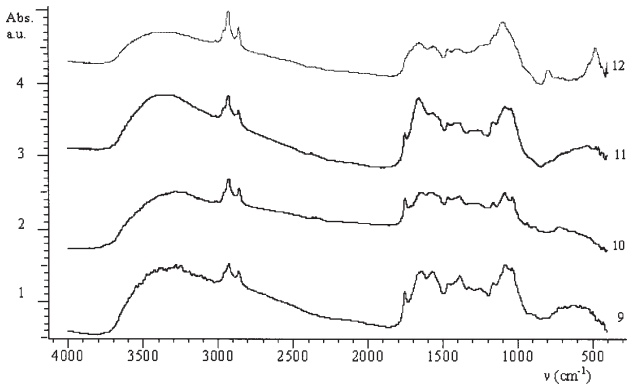

Fig. 1 – IR–FT spectra of the fungi species under study: 9. Hebeloma subsaponaceum; 10. Cortinarius uliginosus; 11. Gyromitra infula; 12. Hypholoma fasciculare

The characteristic bands, occurring in the IR domain, may be assigned as follows [19,20]:

-the wide central band at 3400 cm^{-1} is attributed to the water molecules present in the fungi;

-the bands from the 2800–3000 cm^{-1} domain belong to the symmetrical and asymmetrical valence vibrations of the –CH and $–CH_2$ groups, mainly from the aliphatic compounds;

-between 3700–3300 cm^{-1} there occur the valence vibrations corresponding to the O–H groups from alcohols, phenols, acids, etc.;

-the 3550–3100 cm^{-1} domain is characteristic to the valence vibrations specific to the N–H links from primary and secondary amines, amides etc.;

-over the 1800–1500 cm^{-1} domain there appear absorption bands attributable to the valence vibrations corresponding to the C=O, C=N, C=C, –NHCO–, aryl–NH– , double links or to the aromatic groups;

-the other bands from the 1500–600 cm^{-1} domain belong to the valence and deformation vibrations of the –C–C–, –CH–NH–, –C–O links. In this domain , there also appear valence vibrations corresponding to some inorganic ions, such as SO_4^{2-}, NO_3^-, CO_3^{2-};

-over the 700–400 domain there appear valence and deformation vibrations corresponding to some anorganic compounds.

The wide band occurring between 3700–3000 cm^{-1}, belonging to the water molecules, usually covers the other bands present in this domain, such as those of the valence vibrations OH (3700–3300 cm^{-1}) or of the symmetrical and asymmetrical N-H vibrations from amides (3500–3100 cm^{-1}). The bands appearing in the '*fingerprint*' domain (1800–600 cm^{-1}) are characteristic to a certain substance [20].

This domain may be employed for the identification of some compounds, present in various natural or synthetic mixtures.

The presence of several compounds in a single sample makes difficult the assignment of each band within this interval, so that, from the '*fingerprint*' domain, it is only the bands characteristic to certain compounds that are employed. The intense band situated around 1650 cm^{-1} is assigned to the C=O vibrations from the structure of proteins –melanine and aminoacids, especially – which evidence, too, another relatively intense band around 1550 cm^{-1}, assigned to the secondary amines.

Over the 1650–1850 cm^{-1} domain, a series of bands of lower intensity may be also observed, assigned to the deformation vibrations of the N–H links from amides. The intense absorption band occurring around 1080 cm^{-1} corresponds to the valence vibration corresponding tot the C–O bond [21].

Proteins' IR spectra show the most distinctive bands, characteristic to the amidic groups present around 1650 cm^{-1} for the C=O groups, and around 1550 cm^{-1}, respectively, corresponding to the C–N valence vibrations and to the N–H deformation bands.

The valence vibration of the C-N bond appears around 1330 cm^{-1}, bending bands appear at 750 and 600, for N–H and, respectively C=O. In spite of all these consideration, it is still difficult to make spectroscopic distinctions among chitin, protein mixtures and other compounds present in fungi. Some difficulties of this type may be overcome by comparing the spectra obtained both before and after samples' treatment with hot alkaline solutions in which chitin is much less soluble than the proteins [4].

The spectra of the cellulosic compounds evidence bands around 1430, 1100 and 990 cm^{-1} much more simply to be solved. The most important difference observed between the spectrum of of yeast glucan and celluloses that of cell refers to the relatively intense band occurring around 2925 cm^{-1}.

The spectrum of glucomannan is resemblable to that of cellulose, except for the presence of additional bands near 870 and 850 cm^{-1}, while galactans may be characterised by a band near 768 cm^{-1}. The bands appearing around 3265, 3105, 1655, 1620 and 1550 cm^{-1} are characteristic to chitin [22].

In the FT spectra of the mushroom species under study, some of the previously presented bands may be observed. Usually, the bands characteristic to the various mushroom compounds, evidencing similar functional groups, may be very close or even superposed. Evidencing (fig. 2) of each of this band may be realised by either deconvolution or derivation of the IR spectrum [23].

The same procedures may be applied for the identification of some toxic compounds present in some fungi.

In this way, the characteristic bands of the 2800-3000 cm^{-1} domain may be better evidenced, as well as those of the *fingerprint* (compare spectra 1 and 2 of fig. 2).

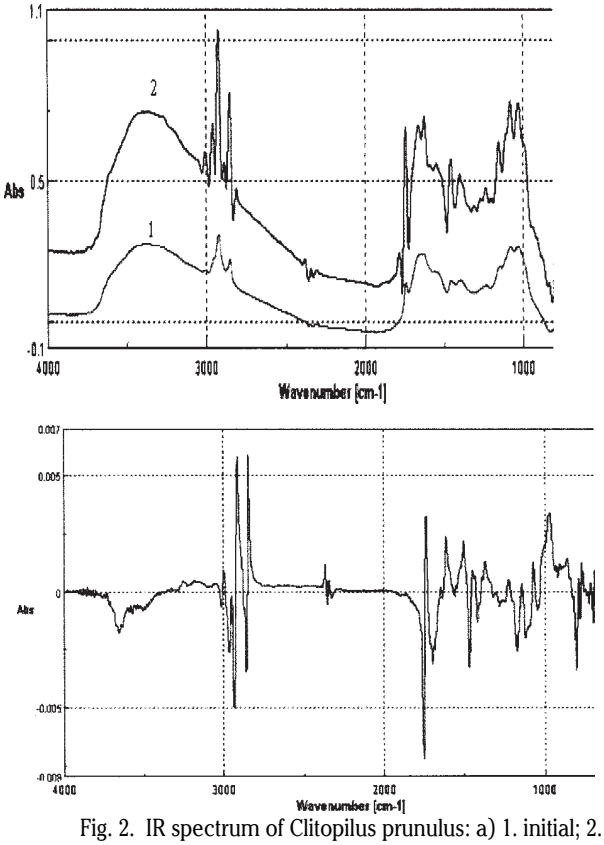

Fig. 2. IR spectrum of Clitopilus prunulus: a) 1. initial; 2. deconvolution 70%; b) first-order derivation

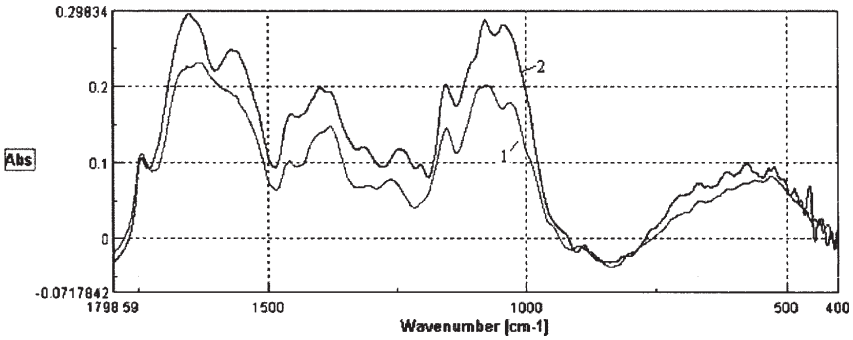

Fig.3 . IR spectra for: 1) Macrolepiota rhacodes (edible); 2) Gyromitra infula (toxique)

In the comparative IR spectra of some edible and toxic fungi, several differences may be observed in the *fingerprint* domains. Thus (fig. 3) plots comparatively the IR spectrum (1) for *Macrolepiota rhacodes* (an edible mushroom) with that (2) of *Gyromitra infula* (a toxic species).

In the IR spectrum of *Gyromitra infula*, the band corresponding to the C-O link appears at 1652 and not 1630 cm^{-1} – which is the case of *Macrolepiota rhacodes.*

The second band, situated at 1569, is very intense in the *Gyromitra infula*. species, appearing instead only as a shoulder in *Macrolepiota rhacodes*. More than that, *Gyromitra infula* evidences two absorption bands at 1310 and 1204 cm^{-1}, which are absent in *Macrolepiota rhacodes*, to be possibly assigned to some valence vibrations of the –NO_2 or –SO_2 groups, or to some phenolic C–O groups.

Other modifications may be also noticed in the positions of the vibration bands belonging to the C–O groups from the aliphatic compounds. In the case of *Gyromitra infula*, they appear at 10 while, in *Macrolepiota rhacodes*, they are present at 1076 and, respectively, 1034 cm^{-1}.

Conclusions

IR–FT spectroscopy is capable of evidencing the position and intensity of various bands, corresponding to some chemical groups present in different mushroom compounds.

Also, this technique provides useful observations on the accumulation of certain categories of toxic substances in various mushroom species.

Utilization of some bands from the '*fingerprint*' domain permits, too, correct estimations on the content of organic and inorganic substances of various mushroom categories, as well as on some species' capacity of accumulating various types of pollutants.

The results obtained evidence the extremely flexible ability of fungi of accumulating most diverse pollutants.

An important aspect, to be approached and elucidated in subsequent studies, refers to the correlations to be established between the concentration of some toxic substances and fungi' component parts.

At present, the risk of severe acute intoxications provoked by polluted edible fungi is still under debate, although it cannot be neglected.

References

1. PARIKH, S. J., CHOROVER, J., Geomicrobiology Journal, 2005, 22, 207
2. BERRARDO, N., PISACANE, V., BATTILANI, P., SCANDOLARA, A., PIETRI, A., MAROCCO, A., J. Agric. Food Chem, 2005, 53, 8128
3. NAUMANN, A., NAVARRO-GONZALEZ, M., PEDDIREDDI, S., KUES, U., POLLE, A., Fungal Genetics and Biology, **42**, 2005, p. 829
4. MICHELL, A. J., SCURFIELD, G., Arch. Biochem. Biophys. **120**, 1967, 628
5. PIERCE, J. A., RAST, D. M., Phytochemistry, **39**, 1995, p. 49
6. GORDON, S. H., JONES, R. W., MCCLELLAND, J. F., WICKLOW, D. T., GREENE, R. V., J. Agric. Food. Chem, 1999, **47**, 5267
7. BERNAŒ, E, JAWORSKA, G., LISIEWSKA, Z., 2006, Acta Sci. Pol., Technol. Aliment., **5**, nr. 1: 5
8. GIACOMONI, L., 1999, Les champignons. Intoxications, pollutions, responsabilites, Billes Productions, Malakoff: 17.
9. COTRĂU, M., POPA, L., STAN T., PREDA, N., KINCSES–AJTAY, M. 1991, Toxicologie, Editura Didactică şi Pedagogică, Bucureşti: 125
10. POP, Ad., NICOARA, A., 1996, Studia Univ. Babeş-Bolyai, Biologia, **XLI**(1-2): 93
11. POP, Ad., 1997, Studii şi cercetări (Şt. Naturii), Bistriţa, **3**: 235
12. VOICU, V. A., 1997, Toxicologie clinică, Editura Albatros, Bucureşti: 45
13. MIRCEA, C., BUTNARU, C., TĂNASE C., AGOROAEI L., BUTNARU E., PROCA M., 2004, Revista Medico-Chirurgicală a Societăţii de Medici şi Naturalişti din Iaşi, **108**, nr. 3, p. 708
14. AGOROAEI, L., MIRCEA, C., TĂNASE, C., BUTNARU, C., BUTNARU, E., PROCA, M., 2004, Mycological Studies, Ed. Presa Universitară Clujeană: 5–11
15. BUTNARU, E., MIRCEA, C., BUTNARU, C., AGOROAEI, L., TĂNASE, C., PROCA, M., 2003, Revista Medico-Chirurgicală a Societăţii de Medici şi Naturalişti din Iaşi, **107**, ne. 3: 358
16. PAIM, S., LINHARES, L. F., MANGRICH, A. S., MARTIN, J. P., Biol Fertil Soil, **10**, 1990, p. 72
17. RUSSEL, J. D., JONES, D., VAUGHAN, D., FRASER, A. R., Geodema, **24**, 1980, p. 207
18. SVOBODA, L., HAVLIÈKOVÁ, B., KALAÈ, P., 2006, Food Chemistry, **96**: 580
19. PASCU, M., RUSU, V., VASILE, C., Aplicaţii ale spectroscopiei IR în medicină şi farmacie, Tehnopress, Iaşi, 2003
20. STUART, B., Infrared spectroscopy: Fundamentals and applications, Wiley, Chichester, UK, 2004
21. BORLESCU, C., GIURGINCA, M., DEMETRESCU, I., Rev. Chim. (Bucureşti), **57**, nr. 11, 2006, p.1086
22. SENESI, N., MIANO, T. M., MARTIN, J. P., Biol Fertil Soil, 5, 1987, p. 120
23. STUART, B., Biological Applications of Infrared Spectroscopy, ACOL series, Wiley, Chichester, UK, 1997

Manuscript received: 12.07.2007