Fourier transform infrared spectroscopic study of *truffles*

Dezhang Zhao [a], Gang Liu[1,a] , Dingshan Song [b], Jian-hong Liu [c], Yilan Zhou [d], Jiaming Ou [a], Shizhong Sun [c]

[a] Department of Physics, Yunnan Normal University, Kunming, 650092, China;
[b] Kunming Institute of Edible Fungi, Kunming, 650223, China;
[c] Center of Analysis and Testing, Yunnan Normal University, Kunming, 650092, China;
[d] Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650204, China

ABSTRACT

Truffles are rare wild growing edible mushrooms belonging to Ascomycetes. In this paper, Fourier transform infrared (FTIR) spectroscopy was used to obtain vibrational spectra of truffles. The results show that the mushrooms exhibit characteristic spectra. The two strongest absorption bands appear at about 1077cm^{-1} and 1040 cm^{-1}, which were described as C-O stretching in carbohydrate. The vibrational spectra indicate that the main compositions of the truffles are polysaccharide and protein. According to the characteristics bands and absorption ratios of spectra, different species of truffles can be discriminated. It is also found the great changes between moldy and healthy truffles, which the major differences are observed in the bands of protein. In addition, FTIR spectral differences are observed between the same species of truffles from different producing areas. It is showed that the FTIR spectroscopic method is valuable tool for rapid and nondestructive analysis of truffles prior to any extraction method used.

Keywords: Truffles, Mushroom, Fourier transform infrared spectroscopy, discrimination

1. INTRODUCTION

Recently, there has been increase of general interest in fungi because of the possible medical applications of their polysaccharide constituents, some of which are reported to have immunomodulatory and antitumor properties [1-4]. Generally chemical extraction methods were used to analyze chemical constituents of fungi at present [5-7]. Since an extraction method can change the chemical composition of a substance, especially a delicate one such as fungal *thallus*, it is necessary to know more about the studied matter in advance in order to choose the chemical procedure properly. Vibrational spectroscopy is exactly an effective means to fulfill these requirements. Fourier transform infrared spectroscopy (FTIR) is a powerful technique that can provide information on molecular structure. Both qualitative and quantitative information can be obtained using FTIR spectroscopy. A number of organic compounds and functional groups can be identified by their wavenumber of bands, and the absorption intensity can be used for the calculation of their relative concentration [8]. FT-IR spectroscopy has been successfully used to identify and study bacteria in clinical medicine with lots of papers [9-14], while only several reports about macrofungi [15-17].

Truffles are rare wild growing edible mushrooms belonging to the Tuberaceae of Ascomycetes [18]. Because of narrow geographical distribution, their output is limited and their commercial value is very high [19]. Polysaccharide of Tuber sinica (PTS) may have antitumor purpose through promoting the immune function of the host [20], so, truffles still have potential pharmic value.

[1] Corresponding Author:gliu66@163.com

ICO20: Biomedical Optics, edited by Gert von Bally, Qingming Luo, Proc. of SPIE Vol. 6026, 60260H, (2006) · 0277-786X/06/$15 · doi: 10.1117/12.667133

In this paper, FTIR spectroscopy was utilized to obtain the vibrational spectra of untreated fruiting bodies of five truffles (T.magnatum, T.indicum, T.indicum (KM), T.indicum (CX) and T. excavatum) growing in the mountains of Yunnan province, Southwest of China. The goal of this study is to develop a rapid, nondestructive method to supply valuable information about chemical constituents of the intact truffles and to distinguish different species of the same genus.

2. EXPERIMENTAL

2.1. FTIR spectroscopy

Infrared spectra were measured on a BIO-RAD FTS-40 spectrometer with MCT detector. Sixteen scans were accumulated with a resolution of 4 cm^{-1} in the range 4000-400 cm^{-1}.

2.2. Materials

The samples were identified and provided by Kunming Institute of Edible Fungi. The chemical used for comparative purposes (protein, starch, chitosan and β-glucan) are purchased from Sigma-Aldrich. The fruiting bodies of truffles were cleaned and left to dry in air. For transmission infrared spectroscopy a small piece of a dried fruiting body from section plane is milled with KBr into powder and pressed into a pellet. Then the FTIR spectra are measured.

3. RESULTS AND DISCUSSION

3.1 The spectral features of truffles

The FTIR spectra of five truffles (T.magnatum, T.indicum, T.indicum (KM), T.indicum (CX) and T. excavatum) are shown in Figure 1. The spectra of all five truffles exhibit characteristic features in three regions. The first region, between 4000 and 1800 cm^{-1}, presents a prominent broad band centered at about 3390 cm^{-1}, corresponding to the absorption due to the stretching mode of hydroxyl bond [15, 21, 22]. The two sharp bands at about 2925 and 2855 cm^{-1} are assigned to out-of-phase CH_2 stretching and in-phase CH_3 stretching of fatty acids from the cell wall, respectively[14, 21].

The second region, between1800 and 1500 cm^{-1}, is composed of the vibrational mode of carbonyl and the C=C double bond. There are two major bands at about 1646 and 1560 cm^{-1} respectively. The first band arises from the amide I band of protein. The second one is assigned to the amide II band of protein [22]. The band at about 1742 cm^{-1}, corresponding to the carbonyl stretching vibration of alkyl-esters [21] indicates that the truffles contain oil.

The third region, between 1500 and 750 cm^{-1}, is associated with the vibration of protein, lipid and carbohydrate. The sugars of natural products absorb in this region. There are following several main observed absorption bands: 1400, 1378, 1316, 1246, 1155, 1077, 1042, 890 and 816 cm^{-1}. In this region, the two strongest absorption bands of truffles appear at about 1077 and 1042 cm^{-1}, which were described as C-O stretching in carbohydrate [15, 21-27]. The vibrational spectra indicate that the main compositions of the truffles are polysaccharide and protein.

The bands, between 950 and 750 cm^{-1}, are very weak, but they are useful for the identification of anomeric configuration of polysaccharides. The band at about 890 cm^{-1} corresponds to β-glycosides, whereas the band at 860-810 cm^{-1} corresponds to α-glycosides [15, 21-26]. The reference substances β-glucan and chitosan (Fig 2. a, b), whose glycosidic linkage are β- type, show a band at about 889 cm^{-1} and 897 cm^{-1} respectively. In the case of starch (Fig 2. c), whose glycosidic linkage is α- type, the characteristic band is at 858 cm^{-1}. In the spectra truffles, there are two weak bands at about 890 and 816 cm^{-1}. The results indicate that both α- and β-glycosidic linkage exist in truffles.

3.2. Spectral discrimination of different species Truffles

According to the characteristics bands and absorbance ratios of spectra, different truffles can be discriminated. The obvious differences between T.indicum (CX) (Fig 1.e) and other truffles are observed in the region of C-H stretching vibration mode. T.indicum (CX) shows the strongest band at 2925 $cm^{-1}$and strong bands at 2854, 1645 and 1077cm^{-1}. While other truffles show the strongest band at about 1077 and 1042 cm^{-1} and the band at about 2924 cm^{-1} is a medium absorbance band. Furthermore there is an obvious band at 3010 cm^{-1} in T.indicum (CX), while others do not like this. These spectral differences can be used to discriminate T.indicum (CX) from other truffles.

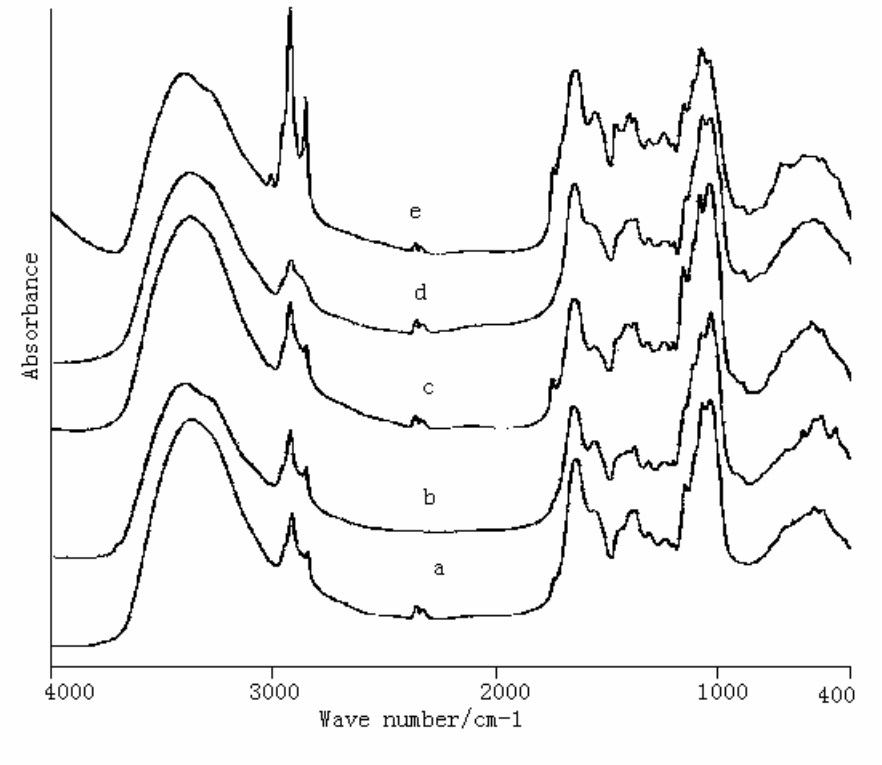

Figure 1. FTIR spectra of Truffles. a, T.magnatum; b, T.excavatum; c, T.indicum; d, T.indicum(KM); e, T.indicum(CX)

The spectra of T.magnatum (Fig 1 a) and T.indicum (KM) (Fig 1 d) are very similar. They appear strongest bands at about 1077, 1043 cm^{-1} and 1077,1040 cm^{-1}, and strong bands at about 1646, 3385cm^{-1} and 1654, 3388 cm^{-1} respectively. The most remarkable difference between the spectra of T.magnatum and T.indicum (KM) is that the band at about 890 cm^{-1} is not obvious in T.magnatum. In addition, the absorption ratio of 1077 and 1042 cm^{-1} of T.magnatum is 0.993, while the A_{1077}/A_{1040} of T.indicum (KM) is 1.007. These evidences can be used to distinguish T.magnatum from T.indicum (KM). On the other hand, the absorption intensity of two bands at 1077 and 1042 cm^{-1} of T.magnatum and T.indicum (KM) are very close, while the absorption intensity of the corresponding bands of other truffles has large difference. The spectrum of T.indicum (KM) shows two weaker peaks with equivalent intensity at 816 and 888 cm^{-1} while the band at about 816 cm^{-1} of other truffles is not apparent. There has only a weak band at 895 cm^{-1} in T.indicum (CX) (Fig 1.e), a weak band at 914 cm^{-1} in T.excavatum (Fig 1.b) and a weak band at 891 cm^{-1} in T.indicum (Fig 1.c).

The spectra of T.excavatum (Fig 1 b) and T.indicum (Fig 1 c) show the strongest band at 1035 and 1037 cm^{-1}, and strong bands at 1076, 1652, 3397 cm^{-1} and 1079, 1650, 3397 cm^{-1} respectively. The absorption ratio of 1076 and 1035 cm^{-1} of T.excavatum and the ratio A_{1079}/A_{1037} of T.indicum are 0.924 and 0.973 respectively; both of them are less than 1. On the other hand, their differences are very apparent. T.excavatum has a visible peak at 1111 cm^{-1}, while T.indicum has not the corresponding band. The band at 1155 cm^{-1} was not observed in the spectrum of T.excavatum (Fig 1 b), but very apparent in the spectrum of T.indicum. In addition, the spectrum of T.indicum has an obvious band at 1746 cm^{-1}, while the spectrum of T.excavatum does not show the band.

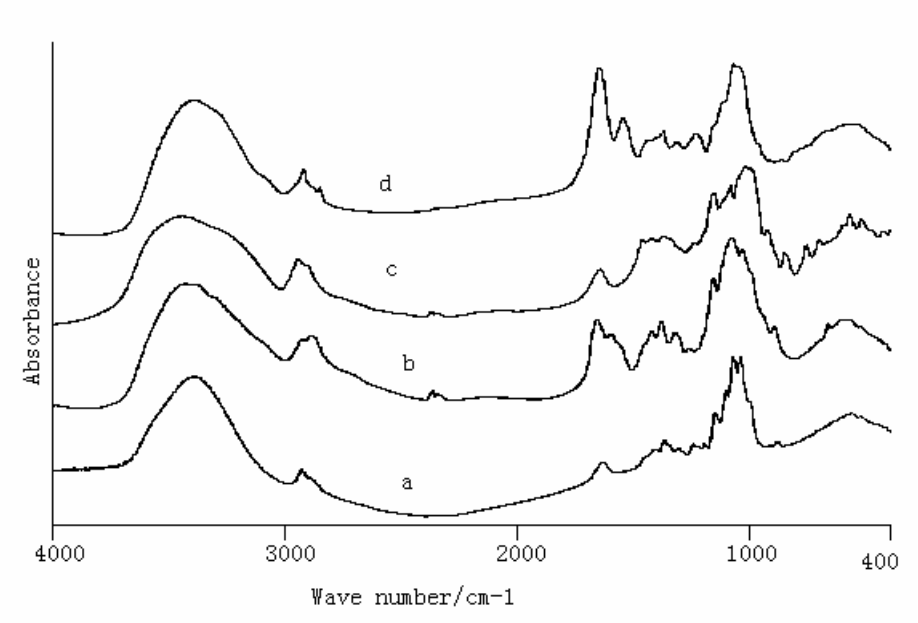

Figure 2. FTIR spectra of reference substances. a, β-glucan; b, chitosan; c, starch; d, protein

In conclusion, the different spices of truffles can be discriminated by FTIR spectroscopy, according to their spectral feature and absorption ratios. Furthermore, the same species truffles with different producing areas can be discriminated by FTIR spectroscopy. The intensity ratios of A_{2926}/A_{1041}, A_{2926}/A_{3388}, A_{2926}/A_{1579} and A_{1264}/A_{1318} are 0.555, 0.670, 0.828 and 0.981 respectively for T.indicum (KM); and the corresponding ratios of T.indicum (CX) and T.indicum are 0.976, 1.004, 1.183, 1.012 and 0.710, 0.775, 1.181, 0.994 respectively. It was found that the ratio of T.indicum (KM) is the minimum. The ratio of T.indicum is the maximum.

3.3. Healthy and Mouldy Truffles

Figure 3 shows the FTIR spectra of healthy and moldy truffles. The significant differences between moldy and healthy truffles are mainly observed in the bands of protein. The band center at about 3380 cm^{-1} of mold truffles becomes broader than healthy truffles. The ratios of A_{1652}/A_{1035} and A_{1557}/A_{1035} of T.excavatum are decreased from 0.668 and 0.543 for the healthy to 0.505 and 0.420 for the moldy. And the ratios of A_{1645}/A_{1077}, A_{1555}/A_{1077} and A_{1247}/A_{1077} of T.indicum (CX) are decreased from 0.960, 0.883 and 0.841 respectively for the healthy to 0.811, 0.721 and 0.703 respectively for the moldy. The bands at about 1645, 1555 and 1247 cm^{-1} are mainly assigned to the amide I, amide II and amide III of protein [14, 22, 23]. The changes indicates that the protein content of the moldy truffles become less compared with the healthy truffles.

4. CONCLUSION

In conclusion, FTIR spectra give information about chemical constituents of the intact truffles, which indicate that the main compositions of the truffles are polysaccharide and protein. The observed differences in the spectra of truffles in 1800-750 cm^{-1} can be utilized for discriminating different species of truffles. In addition, some vibrational spectral differences are observed between the same species of truffles from different producing areas. It is also found the great changes between moldy and healthy truffles, which the major differences are observed in the bands of protein. This study illustrated that the FTIR spectroscopic method is a valuable tool for rapid and nondestructive analysis of truffles.

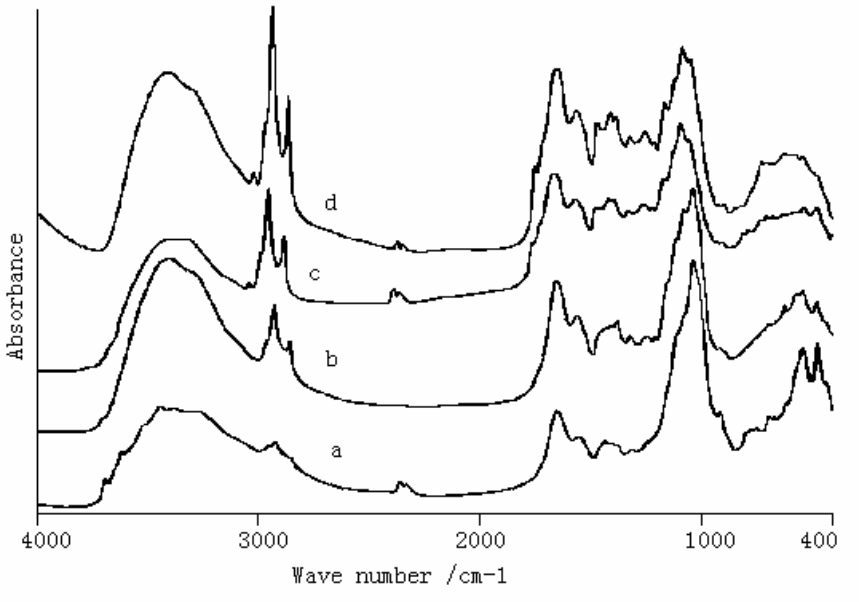

Figure 3. FTIR spectra of the healthy Truffles with mold. a, moldy T.excavatum; b, healthy T.excavatum; c, moldy T.indicum(CX); d, healthy T.indicum(CX)

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China, grant No.30360068. We give thanks to Professor Qin Dong of Yunnan University for the help in measuring the spectra.

REFERENCES

1. X.Bao, C.Liu, J.Fang and X.Li, "Structural and immunological studies of a major polysaccharide from spores of Ganoderma lucidum (Fr.) Karst," *Carbohydr Res*. **332**(1), pp.67-74, 2001.
2. B.J.Grube, E.T.Eng, Y.C.Kao, A.Kwon and S.Chen, "White button mushroom phytochemicals inhibit aromatase activity and breast cancer cell proliferation," *J.Nutr.* **131**(12), pp.3288-3293, 2001.
3. S.P.Li and L.G.An,"Advances in research on immune activities of polysaccharides from fungi," *Mycosystema.* **20**(4), pp.581-587, 2001.
4. C.Y.Guo,"The immunomodulatory of polysaccharides from fungi," *Edible Fungi of china*. **19**(3), pp. 6-7, 2000.

5. Y.B.Wang, P.Lü and C.K.Zhang,"Purification and properties of polysaccharide Ck_1-A from cultured mycelia of cordyceps kyushuensis," *Mycosystema*. **22**(3), pp.452-456, 2003.
6. T.Q.Chen, K.B.Li, J.Xu, P.G.Zhu and Y.Zheng,"Amino acids,fatty acids and element composition of the spores of log-cultivated ganoderma lucidum," *Mycosystema*. **16**(1), pp. 52-56, 1997.
7. H.X.Wang, T.B.Ng and S.W.Chiu,"A distinctive ribonuclease from fresh fruiting bodies of the medicinal mushroom Ganoderma lucidum," *Biochem. Biophys. Res.Commun*. **314**(2), pp.519-522, 2004.
8. J.G.WU, *Techniques and Applications of Modern Fourier Transform Infrared Spectroscopy* , Science and Technology Literature Press, Beijing, 1994.
9. D.Naumann, D.Helm and H,Labischchinski, "Microbiological characterizations by FT-IR spectroscopy," *Nature*. **351** (6321), pp81-82, 1991.
10. D.Naumann, "The ultra rapid differentiation and identification of pathogenic bacteria using FT-IR techniques," *SPIE Fourier* Comput. *Infrared Spectrosc*. **553**, pp268-269, 1985.
11. D,Naumann, V.Fijala, H,Labischchinski and P.Giesbrecht, "The rapid differentiation and identification of pathogenic bacteria using Fourier-transform infrared spectroscopic and multivariate statistical analysis," *J. Mol. Struct*.**174,** pp,165-170,1988.
12. P.D.Nichols, J.M.Henson and J.B.Guckert, "Fourier-transform infrared spectroscopic methods for microbial ecology: analysis of bacteria, bacteria-polymer mixtures and biofilms," *J. microbiol. Methods*. **4**, pp79-94, 1985.
13. C.Kirschner, K,Maquelin and P.Pina, "Classification and identification of enterococci: a comparative phenotypic, genotypic, and vibrational spectroscopic study," *J.Clin. Microbiol*. **39**(5), pp1763-1770, 2001.
14. M.S.Lin, M.A.Holy, H.A.Qadiri, D.H.Kang, A.G.Cavinato. Y.Q.Huang and B.A.Rasco, "Discrimination of intact and injured Listeria monocytogenes by Fourier Transform Infrared Spectroscopy and Principal Component Analysis," *J. Agric. Food Chem*. **52**, pp5769-5772, 2004.
15. V.M.Grošev, R.Božac and G.J.Puppels, "Vibrational spectroscopic characterization of wild growing mushroom and toadstools," *Spectrochemica Acta A*.**57**, pp. 2815-2829, 2001.
16. S.Q.Sun, X.Y.Liang and X.R.Yang, "A Rapid Method for Detecting Ganoderma Lucidum of Six Colors by FTIR Spectroscop," *Spectroscopy and Spectral Analysis*.**22** (2), pp226-228, 2002.
17. G.Liu, J.H.Liu, A.M.Yang, Q.Dong and D.S.Song, "Identification of Edible Mushrooms By Fourier Transform Infrared Spectroscopy," *Spectroscopy and Spectral Analysis*.**24** (8), pp941-945, 2004.
18. N.L.Huang, *Colored illustrations of macrofungi (mushrooms) of China*, Chinese Agricultural Press, pp.22, Beijing, 1998.
19. Y.L.Chen and M.X.Gong,"Truffles(Tuber spp.):biodiversity and geological distribution," *Edible Fungi of china*. **19**(5), pp. 6-7, 2000.

20. H.J.Hu, P.Z.Li, T.Lin, B.Q.Hang and Y.W.Guo,"Effects of polysaccharide of tuber sinica on tumor and immune system of mice," *Journal of China Pharmaceutical University*. **25**(5), pp.289-292, 1994.
21. C.S.Pappas, P.A.Tarantilis, P.C.Harizanis and M.G.Polissiou, "New method for pollen identification by FT-IR spectroscopy," Applied Spectroscopy. **57** (1)**,** pp23-27, 2003.
22. J.X.XIE, *Application of infrared spectroscopy in Organic Chemistry and Pharmic Chemistry*, Science Press, pp.69-310, Beijing, 2001.
23. A.Synytsya, J.Čopíková, P.Matějka and V.Machovič, "Fourier transform Raman and infrared spectroscopy of pectin," *Carbohydrate Polymers*. **54**, pp.97-106, 2003.
24. A.Gutiérrez, A.Prieto and A.T.Martínez, "Structural characterization of extracellular polysaccharides produced by fungi from the genus pleurotus," *Cabohydrate Research.* **281**, pp.143-154, 1996.
25. A.M.Barbosa, R.M.Steluti, R.F.H.Dekker, M.S.Cardoso and M.L.Corradi da Silva, "Structural characterization of Botryosphaeran: a (1→3; 1→6) –β-D-glucan produced by the ascomyceteous fungus, Botryosphaeria sp.," *Cabohydrate Research.* **338**, pp.1691-1698, 2003.
26. M.Kačuráková, P.Capek, V.Sasinková, N.Wellner and A.Ebringerová, "FT-IR study of plant cell wall model compounds: pectin polysaccharides and hemicelluloses," *Carbohydrate Polymers*. **43**, pp195-203, 2000.
27. C.C.Silva, C.G.A.Lima, A.G.Pinheiro, J.C.Goés, S.D.Figueiró and A.S.B.Sombra, "On the piezoelectricity of collagen-chitosan films," *Phys.Chem.Chem.Phys.* **3**, pp.4154-4157, 2001.