Chromatographic investigation of sugars and lipids from *Tuber oligospermum* and *Terfezia arenaria*

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Abstract

Tuber oligospermum and *Terfezia arenaria* are two most important truffle species among all the edible mushrooms in Morocco. They are a popular food and they have a high economic value. Their sugars and lipids content were evaluated by the usual chromatographic and spectrometric techniques. Their sugar contents are estimated to 26.2 and 24.7% respectively. The various monomers constituting the polysaccharides were, in all two simples, glucose, which remained the major sugar, mannose and rhamnose. Uronic acids and glucosamine were also detected. The lipids accounts are 5.1 and 4.4% at *Tuber oligospermum* and *Terfezia arenaria* respectively. The rate of free fatty acids is constant at 26 to 27% in both species studied, while the rates of phospholipids (PL) and glycolipids (GL) are reversed: 38.4 and 35.4 in the same order in *T. oligospermum* and 34.5 and 38.1 in *T. arenaria.* Finally, the acid linoleic (C18: 2) is a majority of fatty acids in both species with 54% for *Tuber oligospermum* and 51% for *T. arenaria*.

Key words: *Tuber oligospermum*, *Terfezia arenaria*, Moroccan truffles, Biochemical characterization.

Introduction

In Morocco, several species of edible mushrooms grow in the forests of humid and subhumid regions in the Rif and Atlas. Among the hundreds of wild forest mushrooms Moroccan, many are edible. The others are either without interest or suspects or poisonous (Harki and Hammoudi, 2008).

Edible species most important are: *Tuber oligospermum, Terfezia arenaria, Tricholoma caligatum, Boletus mamorensis, Cantharellus cibarius, vaginata Amanita, Pleurotus ostreatus, Agaricus bisporus, Suillus* spp. and *Morchella vulgaris* (Harki and Hammoudi, 2008).

Tuber oligospermum and *Terfezia arenaria* are mushrooms Ascomycetous, under class of Discomycetous pertaining to the order of Tuberales. This order is represented in Morocco by some species belonging to the kinds: *Tuber*, *Terfezia*, *Delastria*, *Picoa* and *Tirmania* (Khabar et al., 2001). These fungus are underground mushrooms which grow in symbiosis with roots of certain trees like the oak (*Quercus* spp.), the hazel tree (*Corylus* spp.), the pine (*Pinus* spp.) (Reyna, 2000) or numerous herbaceous plants, mainly of the genus *Helianthemum* (Khabar et al., 2001) with which they exchange metabolites, mineral salts and ions (Read, 1995; France et al., 1983; Bonfante and Perotto, 1992; Miranda et al., 1997). The tubers develop with a depth from 5 to 20 cm; what makes their gathering (the calvage) always random. Their research and their harvest

are done primarily using an animal like the dog, the pig, the fly with truffles (genus *Suilla*) or thanks to the mark cracking of the ground crust under the pressure of the mushroom (Harki, 1996). Some species of the genus *Tuber* are known internationally for their gastronomically qualities and their economic importance (i.e. black Perigord truffle (*Tuber melanosporum* Vitt.) (economic value more than €1000 kg^{-1}) and the white truffle of Italy (*Tuber magnatum* Pico.). In Morocco, eight truffle species are listed (Khabar et al., 2001). They are commonly called "Terfass". Two of them are particularly appreciated and very required (*Tuber oligospermum* and *Terfezia arenaria*). They are the subject of an important trade at the edge of the roads and on the central markets of certain areas (economic value more than €50 kg^{-1}). Few scientific research tasks were devoted to Moroccan truffles. The studies carried out are of a nature taxonomic and floristic (Chatin, 1891a; 1891b; Malençon 1973) or cytological and ultrastructural (Khabar, 1988; Khabar et al., 1994).

In this study, we approached the quality and quantity of sugars and lipids of two species one of the most important economic points of view i.e. *Tuber oligospermum* and *Terfezia arenaria* in order to try to establish a chemical method for the identification of Moroccan truffles.

Materials and methods

Fungus material

The truffles studied are collected in the forest of Mamora between Rabat and Kénitra (Morocco) in March 2011. They were freeze-dried and stored in a freezer at -25°C until processing. Twenty truffles were used for etch species. Their size was 30 ± 5g.

Biochemical techniques

Analysis and assay of the carbohydrates and uronic acids

For each analysis, 5 mg of ground lyophilized fruiting bodies were hydrolyzed in H_2S0_4 (Harris and Taber, 1973). The hydrolysates were recovered by filtration and neutralized with N-methyldioctylamine (Hough et al., 1972). Identifications and assay of the various neutral and amino sugars were performed by HPLC on an analytical ion-exchange column (Carbo Pac PAl, 4 x 250 mm, Dionex V.K. Ltd., Camberly, V.K.). Elution was carried out with an isocratic gradient of 0.014 M NaOH for 15 min followed by 25 min with ultra-pure de-ionized water (18 MW) which had been filtered and degassed on a 0.2 µm nitro-cellulose filter. At the column outlet, a single-piston pump (DQP-l Dionex) injected 0.3 M sodium hydroxide just before the pulsed ammeter detector in order to minimize base-line drift and ensure a high enough pH for accurate detection. The measurement electrode was subjected to a succession of three potentials: El = 0.05 mV (t1 = 300 ms), E2 = 0.60 mV (t2 = 150 ms), E3 = -0.80 mV (t3 = 300 ms). The uronic acids were assayed by the technique of Blumenkrantz and Asboe-Hansen (1973).

Extraction, fractionation and assay of the lipids

The lipids were extracted using the method of Bligh and Dyer (1959) modified by Weete et al., (1983). The total phospholipids were assayed using the method of Bartlett reported by Kates (1975). The neutral lipids were separated by thin layer chromatography in hexane / diethyl ether / acetic acid (78/20/4) and stained with 0.01 % rhodamine B in 95% ethanol. The level of polar lipids in the resulting residue

was determined by assaying the level of phosphorus. The various classes of fatty acids were analyzed by gag-phase chromatography using the method of McGee and Allen (1974) modified by Gerhardt and Gehrke (1977) on an Intersmat IGC 121 DSL equipped with a flame ionization detector. The analyses were performed at a constant column temperature of 1850 C with the injector at 2000 C and the detector at 2200 C. The amounts of fatty acid were determined by comparison to a known internal standard, heptadecanoic acid (C17: 0) which was otherwise absent from our biological material.

Statistical analysis

Six samples, for each, stage were analyzed individually and the results are shown as means ± SD (n = 6).

Results and discussion

The proportions of lipids and polysaccharides obtained for the two truffle species studied are given in Table 1. The polysaccharides represent, in dry matter of *Tuber oligospermum*, 27.4% and 28.2 % in *Terfezia arenaria* while lipids make up only 5.1 and 4.4% respectively.

Table 1. Total composition of the two truffle studies

	Tuber oligospermum	*Terfezia arenaria*
Carbohydrates	27.4 ± 1.3	28.2 ± 1.9
Lipids	5.1 ± 0.6	4.4 ± 0.9

Truffle in % dry weight.

Table 2 shows the various monomers constituting the polysaccharides for two species. Glucose is remained the major sugar followed by mannose and Rhamnose. Uronic acids and glucosamine were also detected. The proportions of glucose, mannose, glucosamine and Uronic acids did not present any significant differences between *Tuber oligospermum* and *Terfezia arenaria* but rhamnose most doubled at *Terfezia arenaria* (Figures 1 and 2).

For the lipids (Table 3), we noted that, the fatty acids (FA) proportion is stable at 26 - 27 in two simples, but the glycolipids (GL) and phospholipids proportions (PL) were inversed between 35 and 38 %.

In *T. oligospermum* (Table 4), the major fatty acids were linoleic acid (C18:2) (65.5%), oleic acid (C18:1) (10.5%), stearic acid (C18:0) (10.2%) and palmitic acid (C16:0) (9.3%). In *T. arenaria*, the major fatty acids were linoleic acid (C18:2) (51.3%), oleic acid (C18:1) (20.2%), and palmitic acid (C16:0) (14.6%). The saturated mystiric acid (C14:0) and the unsaturated linolenic acid (CI8:3) were also detected but in much smaller quantities less than 10% in two species (Figures 3 and 4).

The chemical composition of the two Moroccan truffles species studies (*Tuber oligospermum* and *Terfezia arenaria*), is similar to that reported for other fungi found in the literature (Brennan et al., 1974; Holtz and Schisler, 1971; Bokhary and Parvez, 1993; Harki et al., 2006). Indeed, fungi in general are rich in proteins, carbohydrates and mineral salts. However, they are poor in lipids and sterols (e.g. ergosterol and bracecasterol) (Harki et al., 1997). The most noteworthy aspect of the chemical composition of truffles described there

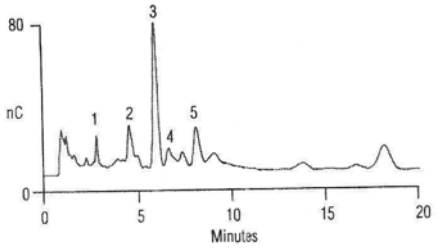

Figure 1. Neutral and amino sugars of *T. oligospermum* performed by HPLC. (1): rhamnose 3.2%, (2): mannose 12.9%, (3): glucose 63.1%, (4): galactose internal standard and (5): glucosamine 16.2%.

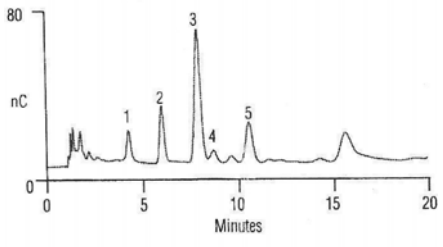

Figure 2. Neutral and amino sugars of *T. arenaria* performed by HPLC. (1): rhamnose 5.2%, (2): mannose 15.8%, (3): glucose 59/1%, (4): galactose internal standard and (5): glucosamine 16.5%.

is the absence of mannitol, which has been known as a major mushroom carbohydrate since 1947 (McConnell and Esselen, 1947).

In comparison with other species belonging to the genus *Tuber*, in particular *Tuber melanosporum*, showed only differences in the proportions of each component by dry weight (Harki et al., 2006).

Our chemical balance sheet, determined for the two truffle studies, shows that the: (i) *Tuber oligospermum* presents a level of carbohydrate 27.4 % of the total dry weight, has a high percentage of phospholipids (38% of total lipids) and linoleic acid (C18:2) (65.5%). (ii) *Terfezia arenaria* shows some similar results as *Tuber oligospermum* but it is distinguished by a higher level of carbohydrate (28.2%) and Glycolipids (GL) (38.4%).

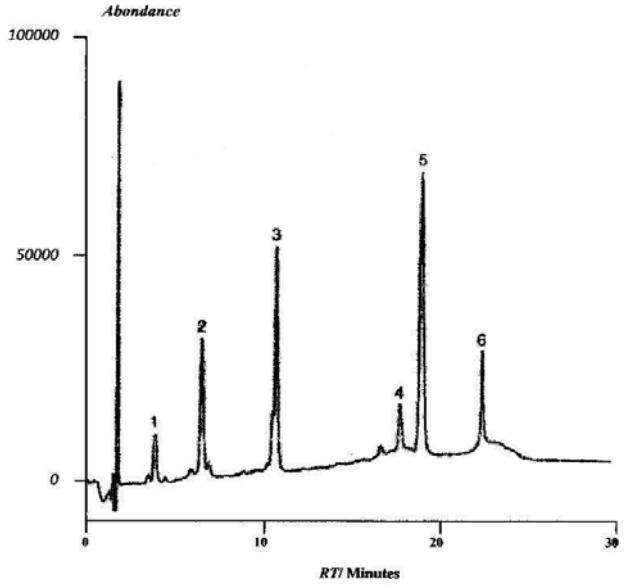

Figure 3. Fatty acids of *T. oligospermum* analyzed by GC. (1): C:14:0, (2): C:16:0, (3): C18:0, (4): C18:1, (5): C18:2 and (6): C18:3.

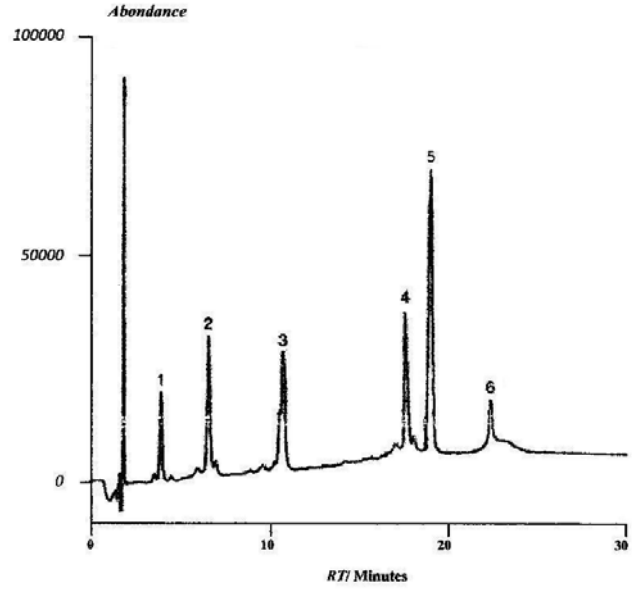

Figure 4. Fatty acids of T. arenaria analyzed by GC. (1): C:14:0, (2): C:16:0, (3): C18:0, (4): C18:1, (5): C18:2 and (6): C18:3.

Table 2. Levels of various sugars and sugar derivatives in the two truffle studies

	Tuber oligospermum		*Terfezia arenaria*	
	µg/mg[a]	%	*µg/mg*[a]	%
Neutral monosaccharides	*217.3 ± 1.8*	*79.2*	*224.1 ± 2.2*	*80.1*
♦ Glucose	173.1 ± 2.0	63.1	165.6 ± 1.7	59.1
♦ Mannose	35.4 ± 0.7	12.9	44.4 ± 0.6	15.8
♦ Rhamnose	8.8 ± 0.4	3.2	14.1 ± 07	5.2
Amino-monosaccharides				
♦ Glucosamine	44.4 ± 0.9	16.2	46.2 ± 1.7	16.5
Uronic acids	*10.5 ± 0.8*	*4.6*	*9.9 ± 0.4*	*3.4*

a, dry weight. %, Total sugars.

Table 3. Levels of total lipids in the two truffle studies.

	FA	GL	PL
Tuber oligospermum	26.2± 0.2	35.4± 1.3	38.4±1.2
Terfezia arenaria	27.4 ±0.5	38.1± 0.9	35.0±1.0

FA: Fatty acid; GL: Glycolipids; PL: Phospholipids

Conclusion

Systematic of the fungi is mainly based on the observation of morphological characteristics a specific species. When the morphological characteristics are ineffective to differentiate between two species, physiological and biochemical techniques could be used. However, these methods are laborious, time consuming and sometimes inadequate. So, molecular methods and chemical methods have been developed in order to obtain more information on the taxonomy of a species.

Thus, the analytical results obtained can be used in combination with various features such as morphological, physiological and ecological for a right classification and correct identification of different species of truffles Moroccan. However, it is necessary to standardize the methods and conduct chemical characterization of all species of truffles harvested in Morocco.

Table 4. Levels of fatty acids in the two *truffle studies*.

	C14 : 0	C16 : 0	C18 : 0	C18 : 1	C18 : 2	C18 : 3	D.U
Tuber oligospermum	1.6 ± 0.5	9.3 ± 0.2	10.2 ± 0.5	10.5 ± 0.8	65.5± 0.4	2.9 ± 0.6	1.05
Terfezia arenaria	3.6 ± 0.5	14.6 ± 0.2	8.7 ± 0.4	20.2 ± 0.5	51.3 ± 0.6	1.6± 0.4	1.40

Note: Values are expressed as a percent of total fatty acids.
D.U. = degree of unsaturation = Σ1 x (% monoenes)/IOO + Σ2 x (%

References

Blumenkrantz N., Asboe-Hansen G. (1973). New method for quantitative determination of uronic acids. Anal. Biochem. **54**: 484-489.

Bligh E.G., Dyer W.S (1959). A rapid method for total lipid extraction and purification. Can. J. Biochem. Biophys. **37**: 911-917.

Bokhary H.A., Parvez S. (1993). Chemical composition of desert truffles

Terfezia claveryi. J. Food Comp. Analysis. **6 (3)**: 285-293.

Bonfante P., Perotto S. (1992). Plants and endomycorrhizal fungi : the cellular and molecular basis of their interaction. In: Molecular Signals in Plant Microbe Communications (Verma, D.P.S., Ed.). CRC Press, Boca Raton, FL. pp. 445-470.

Brennan P. J., GritIin P.F.S., Lösel D.M., Tyrell D. (1974). The lipids of fungi. Prog. Chem. Fas. Gth. Lip. **14**: 49-89.

Chatin A. (1891a). Terfas ou truffes d'Afrique et d'Arabie, genres *Terfezia* et Tirmania. Bull. Soc. Bot. France. **38** : 59-64.

Chatin A. (1891b). Contribution à l'histoire botanique de la truffe : Kamé de damas (*Terfezia* clavertyi). Bull. Soc. Bot. France. **38** : 332-335.

France R.C., Reid C.P.P. (1983). Interactions of nitrogen and carbon in the physiology of ectomycorrhizae. Can. J. Bot. 61: 964-984.

Gerhardt K.O., Gehrke C.W. (1977). Rapid micro determination of fatty acids in biological materials by gas-liquid chromatography. J. Chromatogr. **143** : 335-344.

Harki E., Hammoudi A. (2008). Les champignons comestibles du Maroc: données et état actuel. Revue AFN Maroc **2-3**: 89-99.

Harki E., Bouya D., Dargent R. (2006). Maturation-associated alterations of the biochemical characteristics of the *Tuber melanosporum* Vitt. Food Chem. **99**: 394-400.

Harki E., Klaebe A., Talou T., Dargent R. (1997). Identification and quantification of *T. melanosporum* Vitt. sterols. Steroids. **61**: 609-612.

Harki E. (1996). Caractérisation structurale et biochimique de la truffe noire du Périgord *(T. melanosporum* vitt.). Etude des mélanines. Thesis, L'Institut National Polytechnique de Toulouse, France, 212 p.

Harris J.L., Taber W.A. (1973). Compositional studies on the cell walls of the synnema and vegetative hyphae of Ceratocystis ulmi. Can. J. Bot. **51**: 1147-1153.

Holtz R.B., Schisler L.C. (1971). Lipid metabolism of Agaricus bisporus (Lange) Sing. 1. Analysis of sporophore and mycelial lipids. Lipids. **6**: 176-180.

Hough L., Lones J.V.S. & Wusteman P.J (1972), On the automated analysis of neutral monosaccharides in glycoproteins and polysaccharides. Carbohydr. Res. **21**: 9-17.

Kates M. (1975). Techniques of lipidology: analysis and identification of lipids. North-Holland Publishing Co., Amsterdam.

Khabar L. (1988). Le genre *Terezia* Tul. (Terfass) de la forêt de la Mamora (région de salé): étude systématique, écologique, morphologique, cytologique et ultrastructurale. Thesis, Rabat University, Morocco, 178 p.

Khabar L., Najim L., Janex-Favre M.C., Parguey-Leduc A. (1994). L'ascocarpe de Terfezia leonis (Discomycètes, Tubérales). Cryptog. Mycol. **15 (3)**: 187-207.

Khabar L., Najim L., Janex-Favre M.C., Parguey-Leduc A. (2001). Contribution à l'étude de la flore mycologique du Maroc : Les truffes marocaines (Discomycètes). Bull. Soc. Mycol. Fr. **117 (3)**: 213-229.

Malençon G. (1973). Champignons hypogés du nord d'Afrique. I. Ascomycètes. Persoonia. **7(2)**: 261-288.

MaConnell J.E.W., Esselen W.B. (1947). Carbohydrates in cultivated mushrooms. J. Food Research. **12**: 118-121.

McGee J., Allen K.G. (1974). Preparation of methylesters from the saponifiable fatty acids in small biological specimens for gas-liquid chromatographie analysis. J. Chromatogr. **10**: 35-42.

Miranda M., Zarivi O., Bonfigli A., Amicarelli F., Aimola P., Ragnelli A.M., Pacioni G. (1997). Melanogenesis, tyrosinase expression, and reproductive differentiation in black and white truffles (Ascomycotina). Pigment Cell. Res. **10**: 46-53.

Read D.J. (1995). Ectomycorrhizae in the ecosystem. In: Biotechnology of Ectomycorrhizae: Molecular Approaches (Stocchi, V., Bonfante, P. and Nuti, M., Eds.). Plenum Press, New York. pp. 1-23.

Reyna S. (2000). Truficultura y Selvicultura Trufera, Mundi-Prensa, Madrid.

Weete J.D., Sancholle M., Montant C. (1983). Effects of triazoles on fungi. II. Lipid composition on Taphrina deformans. Biochem. Biophysical Acta. **752**: 19–29.