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Maturation-associated alterations of the biochemical characteristics of the black truffle *Tuber melanosporum* Vitt.

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Abstract

Tuber melanosporum is a mycorrhizal ascomycetous fungus with subterranean fruiting bodies. With the aim of characterizing its state of maturity, we have undertaken a comparative analysis of the biochemical composition of tree stages. The various components were determined by the usual chromatographic and spectrometric techniques. The results showed that the factors characterizing the mature truffle are a relatively high level of carbohydrates and melanin (30% and 15% by dry weight, respectively) and the presence of rhamnose, calcium and iron. These biochemical markers could be used as indicators of the degree of ascocarp development and the attainment of maturity.

Keywords: *Tuber melanosporum*; Truffle; Maturation; Ascocarp; Biochemical characteristics

1. Introduction

Truffles are a myeorrhizal ascomycetous fungi with subterranean fruiting bodies. They belong to the family of *Tuberaceae* and the *Tuber* kind. Two species of this kind are the most appreciated of all truffles: black Perigord truffle (*Tuber melanosporum* Vitt.) and precious white truffle (*Tuber magnatum* Pico).

Few previous chemical analyses of *Tuber* species and other fungi, in relation to melanin, are to be found in the literature. The first biochemical analyses in the genus *Tuber* can be credited to Chatin, 1892. Although succinct, these analyses show that the truffle is rich in nitrogen-containing compounds and in mineral salts, in particular potassium and phosphorus, that it contains very little lipid (7–9% of total dry weight) and that its water content, over 75%, is lower than that found in most higher mushrooms. More recent biochemical analyses (Andreotti & Casoli, 1968; Bokhary & Parvez, 1993; Coli, Coli, Granetti, & Damiani, 1988; Manmozzi-Torini, 1970) provided similar results: (1) the main amino acids were aspartic acid, glutamic acid, lysine, tyrosine, alanine, valine and leucine; (2) the major monomers constituting the polysaccharides were glucose, galactose, mannose and xylose; (3) the fatty acids were represented by linoleic (C18:2), palmitic (C16:0) and oleic acids (C18:1). Large quantitative differences, probably due to the heterogeneity of the samples analysed (age, origin, type of soil, etc.) were reported by various authors.

For *T. melanosporum* Vitt., the development of the ascocarp is paralleled by an overall decrease in lipids (Sancholle, Weete, Kulifaj, & Montant, 1988), a constant ratio of ergosterol/brassieasterol during the development process (Harki, Talou, & Dargent, 1996; Weete, Kulifaj, Montant, Nes, & Sancholle, 1985), a qualitative variation of the volatile compounds responsible for its fragrance (Daiz, Ibanez, Senorans, & Reglero, 2003; Talou, Delmas, & Gaset, 1989) and an increase in the melanin content. In a previous study, we showed that the pathways involved in

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the synthesis of this melanin were different from those of dihydrophenylalanine (DOPA) melanin (Harki, Klaebe, Talou, & Dargent, 1997).

Owing to its gastronomic qualities and man's inability to control the development of its mycelium, this fungus has attracted bath economic and scientific interest. In France, its harvest has been in constant decline since the beginning of the century whereas demand has never stopped increasing. This deficit between supply and demand, along with the total absence of any legislation concerning the quality control of the fungus, are at the origin of numerous cases of fraud, consisting of artificial blackening, with cassel brown extract or various salts of iron and enhancing the aroma of white truffles which have a much lower value (i.e., *Tuber aestivum*, *T. indicum*). Although the Asiatic species (*T. indicum*, *T. himalayense* and *T. sinense*) have ascocarps with morphological characteristics very similar to those of *T. melanosporum*, they are of inferior flavour, and there is the risk of their being commercialized as *T. melanosporum*.

The morphological and cytological state of the ascocarps and their weight and ripeness, led to the definition of six stages of maturation in the biological cycle of the black truffle (*Tuber melanosporum*) (Montant, Kulifaj, & Gleyze, 1983; Kulifaj, 1984). In the present study, we only investigated the sub-adult and adult stages of development, i.e. those of commercial interest and thus susceptible to fraud (stages IV, V and VI).

Stage IV corresponds to fruiting bodies weighing 20–30 g. The colour of the skin is still reddish in patches and the flesh is white.

Stage V covers the fungi collected using dogs at the start of the season from the end of September to November. The ascocarps, weighing 30–40 g, have certain aromatic qualities and the colour of the flesh varies from white to grey. Formation of the ascospores is not quite complete with a few still developing just under the scales.

Finally, stage VI includes the fruiting bodies harvested during the months of January to March. They are fully mature and weigh over 50 g. The organoleptic qualities have reached their maximum. Cytologically, they are seen to be entirely composed of a fully melanised 'spore bag'. Ascosporogenesis is complete, the sterile part has reached minimum size and the air-veins are very compressed.

In this study, we approached the biochemical analysis of three well-defined stages of development of *T. melanosporum*, with the aim of characterizing its state of maturity.

2. Materials and methods

2.1. Fungus material

The truffles studied (stages IV, V and VI) all came from the same producing area near Lalbenque in the department of Lot (France). One kg of fresh truffles for each stage, was used in this study. They were freeze-dried and stored in a freezer at −25 °C prior to processing. Thirty, 25 and 15 truffles were used in stages IV, V and VI, respectively. Their size was 30 ± 5 g for stage IV, 40 ± 5 g for stage V and 60 ± 5 g for sage VI.

2.2. Statistics

Six samples, for each, stage were analysed individually and the results are shown as means ± SD ($n = 6$). A one-way analysis of variance was used in order to evaluate the effects of maturation on the chemical composition of the three studied training courses. Post hoc multiple comparisons analyses were performed by the Fisher's test. P values less than 0.05 were considered significant.

2.3. Biochemical techniques

2.3.1. Analysis and assay of the carbohydrates and uronic acids

For each analysis, 5 mg of ground lyophilized fruiting bodies were hydrolyzed in H_2SO_4 (Harris & Taber, 1973). The hydrolysates were recovered by filtration and neutralized with *N*-methyldioctylamine (Hough, Lones, & Wusteman, 1972). Identifications and assay of the various neutral and amino sugars were performed by HPLC on an analytical ion-exchange column (Carbo Pac PA1, 4×250 mm, Dionex V.K. Ltd., Camberley, UK). 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-1 Dionex) injected 0.3 M sodium hydroxide just before the pulsed ammeter detector in order to minimise base-line drift and ensure a high enough pH for accurate detection. The measurement electrode was subjected to a succession of three potentials: $E_1 = 0.05$ mV ($t_1 = 300$ ms), $E_2 = 0.60$ mV ($t_2 = 150$ ms), $E_3 = -0.80$ mV ($t_3 = 300$ ms). The uronic acids were assayed by the technique of Blumenkrantz and Asboe-Hansen (1973).

2.3.2. Protein assay

For each analysis, 5 mg of ground lyophilized fruiting bodies were suspended in 5 ml of 1% NaOH. After standing overnight at room temperature, the suspension was placed in a boiling water bath for 1 h. The dissolved proteins were then assayed by the method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin as standard.

2.3.3. Analysis and assay of the free amino acids

One hundred mg of ground lyophilized fruiting bodies were extracted successively with 20 ml 80% ethanol, 20 ml 60% ethanol and 20 ml distilled water. The resulting pooled supernatants were passed through a Dowex 50 resin column (50–100 mesh) in the H^+ form. The cationic components were eluted with 50 ml 2 M NH_4OH. The eluate, containing the amino acids, was evaporated to dryness and washed several times in distilled water. The various

amino acids were identified and assayed by HPLC on an Aminoquat version of the Hewlett-Packard 1090 chromatograph. After modification of the primary amino acids with orthophthalaldehyde (OPA) and the secondary amino acids with 9-fluorylmethoxycarbonyl (FMOC), the complexes were separated on a C18 column. A diode array spectrophotometer detected the OPA derivatives at 338 nm and the FMOC derivatives at 262 nm.

2.3.4. Extraction, fractionation and assay of the lipids

The lipids were extracted using the method of Bligh and Dyer (1959), modified by Weete, Sancholle, and Montant (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 phospholipids were separated in chloroform/methanol/water/acetic acid (65/25/4/1) and also stained with rhodamine B. The proportions of the various neutral lipids and phospholipids were determined after elution of the spots with chloroform/methanol (2/1) containing 0.5% acetic acid. After 2 h of stirring, the residual silica was eliminated by centrifugation and the supernatant evaporated to dryness. The level of polar lipids in the resulting residue was determined by assaying the level of phosphorus. The various classes of fatty acids were analysed by gas-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 ionisation detector. The analyses were performed at a constant column temperature of 185 °C with the injector at 200 °C and the detector at 220 °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.

2.3.5. Identification and assay of mineral elements

For each analysis, 1 g of lyophilised fruiting bodies was calcinated in the oven at 550 °C for 3 h. The mineral elements were identified and assayed by energy dispersion spectrometry. The position of the peak identified the clement, and the area under the peak-proportional to the X-ray intensity emitted by the element – gave its quantity.

2.3.6. Sterols and melanins

The sterol composition of *T. melanosporum* was examined by medium pressure liquid and high-performance liquid chromatography, nuclear magnetic resonance spectroscopy, and mass spectrometry. Ergosterol (ergosta-5, 7, 22-trienol) and brassicasterol (ergosta-5, 22-dienol) were identified as the major components. Their quantities and their relative concentrations were determined during the maturation of the fungus (Harki et al., 1997).

The melanins of *T. melanosporum* were extracted with KOH under nitrogen and purified with chloroform. Their physical and chemical properties were determined, including their elemental composition and ultraviolet (UV) and infrared (IR) spectra (Harki et al., 1996).

3. Results

The chemical compositions of the three stages of *T. melanosporum* ascocarps are shown in Table 1. in the immature truffles (stage IV), the major constituent is protein, which makes up 27.6% of the dry weight. Carbohydrates and total lipids are present in about the same proportions, i.e. 8% each. The mineral salts are more abundant than the melanins, which only represent 3.1%. Finally, the sterols reach 1.2%.

Maturation of the ascocarp brings about statistically significant differences in the proportions of the various constituents. Except for the means of mineral salts and lipids, all stage V means differ from stage VI means at $P < 0.05$ (Table 1). Although the proteins and the lipids were not greatly affected, there was a strong increase in carbohydrates and melanins – about 282% and 390%, respectively. The level of sterols was halved.

The various monomers constituting the polysaccharides (Table 2) were, in all three stages, glucose, which remained the major sugar, and mannose. Rhamnose was detected in the ripe fruiting bodies. Uronic acids and glucosamine were also present. The proportions of glucose, mannose and glucosamine increased with the age of the fungus, peaking at stage VI. Uronic acids did not present any significant differences between the stage IV and stage VI, but the mean doubled at stage V.

Concerning the proteins, the nature and the proportions of the various free amino acids released during the development of the fungus are listed in Table 3. Except for lysine, only detected in stage IV, the variations observed were quantitative: during maturation, in particular between stages IV and VI, a significant decrease was seen in the proportions of a certain number of amino acids, namely γ-amino butyric acid (−77%), cysteine (−52%) and glutamic acid (−54%), but very significant increase, were seen, however, in the proportions of cystine (increased by 61 times), isoleucine (+444%), serine (+338%), alanine (+250%) and glutamine (+212%).

(Table 4) shows that, during maturation, the glycolipids (GL) and the fatty (FA) increased by 31% and 13%, respectively, whereas the level of phospholipids (PL) fell by

Table 1
Total composition of the three maturation stages of the black Perigord truffle in % dry weight

	Stage IV	Stage V	Stage VI
Proteins	27.6 ± 2.3^{a}	31.7 ± 1.9^{a}	29.7 ± 1.1
Carbohydrates	8.0 ± 1.7^{a}	11.0 ± 2.0^{a}	30.6 ± 2.1
Lipids	7.8 ± 0.9^{a}	5.0 ± 0.7	5.4 ± 0.7
Sterols	1.2 ± 0.2^{a}	0.9 ± 0.2^{a}	0.6 ± 0.1
Melanin	3.1 ± 0.5^{a}	8.3 ± 0.7^{a}	15.2 ± 0.9
Mineral salts	5.0 ± 1.2^{a}	7.3 ± 0.9	7.4 ± 0.8

[a] Different from stage VI means at $P < 0.005$.

Table 2
Levels of various sugars and sugar derivatives at the three maturation stages of the black Perigord truffle

	Stage IV		Stage V		Stage VI	
	μg/mg[a]	%	μg/mg[a]	%	μg/mg[a]	%
Neutral monosaccharides	55.0 ± 2.1[A]	68.9	73.4 ± 2.3[A]	66.9	248 ± 2.7	80.9
Glucose	47.1 ± 1.8[A]	59.0	64.2 ± 1.2[A]	58.5	210 ± 1.9	68.9
Mannose	7.9 ± 0.8[A]	9.9	9.2 ± 1.4[A]	8.4	27.5 ± 1.2	9.8
Rhamuonose	–	–	–	–	10.0 ± 1.0	3.2
Amino-monosaccharides						
Glucosamine	15.7 ± 1.8[A]	19.7	18.3 ± 2.1[A]	16.7	50.0 ± 3.3	16.3
Uronic acids	9.1 ± 1.2	11.4	18.0 ± 3.0[A]	16.4	8.6 ± 1.7	2.8

[A] Different from stage VI means at $P < 0.05$: a, dry weight; %, total sugars.

Table 3
Levels of free amino acids at the three *Tuber* maturation stages

Amino acids	Stage IV		Stage V		Stage VI	
	μmol/g[a]	%	μmol/g[a]	%	μmol/g[a]	%
Glutamic acid	15.4	4.96	18.3	7.48	7.02	1.35
Proline	6.60	2.12	5.05	2.06	13.5	2.59
α-Amino butyric acid	8.66	2.78	9.45	3.85	7.78	1.50
γ-Amino butyric acid	14.7	4.71	3.17	1.29	3.36	0.64
Lysine	0.17	0.05	–	–	–	–
Onithine	3.55	1.14	7.44	3.03	2.66	0.51
Arginine	5.73	1.84	20.0	8.16	2.04	0.39
Histidine	4.66	1.49	5.51	2.25	4.91	0.94
Serine	16.7	5.35	14.0	5.70	56.4	10.8
Glycine	15.7	5.05	5.82	2.37	23.9	4.60
Ethanolamine	10.1	3.25	5.30	2.16	7.68	1.48
Methionine	0.21	0.07	0.47	0.19	0.56	0.11
Cystine	0.47	0.15	6.11	2.49	28.8	5.53
Cysteine	51.1	18.3	56.6	23.1	27.4	5.27
Aspartic acid	3.72	1.19	27.2	11.1	2.40	0.46
α-Aminoadipic acid	1.24	0.40	1.55	0.63	2.63	0.50
Threonine	9.48	3.04	1.40	0.57	9.88	1.90
Alanine	84.2	27.0	7.42	3.02	209	40.1
Valine	12.0	3.86	7.78	3.17	22.8	4.36
Leucine	0.40	0.13	0.58	0.24	1.77	0.34
Isoleucine	5.24	1.68	3.24	1.34	23.3	4.47
Phenylalanine	1.98	0.63	0.68	0.28	2.00	0.38
Tyrosine	1.08	0.35	0.27	0.11	1.93	0.37
Asparagine	8.97	2.88	7.22	2.95	6.78	1.30
Glutamine	23.5	7.53	30.8	12.6	49.8	9.56
Total	312	100	245	100	520	100

a, Dry weight; %, total amino acids. Standard errors: ±0.05 μmol/g[a].

41.4%. The glycerides were composed essentially of triglycerides (TG) (68–63%) and diglycerides (DG) (29–35%), monoglycerides being present only in small quantities (2–3%). The FA were mainly present in the non-esterified form (NEFA) (95%) in the three samples studied, esterified fatty acids (EFA) representing only about 5%.

The different classes of phospholipids detected and their proportions are shown in Table 4. In the three stages analysed, phosphatidylcholine (PC) was the major PL. With the exception of lysophosphatidylethanolamine (LPE), which was present at relatively high levels in mature truffles, the proportions of the other PL (phosphatidylethanolamine (PE), phosphatidylinisitol (PI), phosphatidic acid (PA) and lysophosphatidylcholine (LPC)), did not present any significant differences among the three stages.

In mature truffles (Table 5), the major fatty acids were linoleic acid (C18:2) (55.9%), oleic acid (C18:1) (23.1%) and palmitic acid (C16:0) (11.7%). The saturated myristic acid (C14:0) and stearic acid (C18:0) and the unsaturated linolenic acid (C18:3) were also detected but in much smaller quantities (less than 10% in all). In comparison with immature truffles, it was observed that, during maturation, the percentages of oleic acid and linolenic acid increased by 6- and 3-fold, respectively. The amount of stearic acid was halved and the other FA showed random variations (Table 5).

Table 4
Levels of total lipids at the three *Tuber*, maturation stages

Stages	FA	GL	PL	FA (%)		GL (%)			PL (%)				
				NEFA	EFA	TG	DG	MG	PC	PE	PI	LPE	PA
IV	38.2 ± 1.2^{a} (0)	28.7 ± 0.9^{a} (0)	33.1 ± 1.0^{a} (0)	94.8 ± 1.5	5.2 ± 1.5	68.4 ± 2.2^{a}	29.1 ± 1.2^{a}	2.5 ± 1.4	56.2 ± 1.6^{a}	20.0 ± 2.2^{a}	16.3 ± 1.5	3.6 ± 1.2^{a}	3.1 ± 1.4
V	39.4 ± 1.5^{a} (+3.1)	35.1 ± 1.1 (+22.3)	25.5 ± 1.2^{a} (−22.9)	95.6 ± 1.4	4.4 ± 1.4	63.1 ± 1.7	35.0 ± 1.3	1.9 ± 1.2	57.0 ± 2.3^{a}	15.8 ± 1.6	17.1 ± 1.4	5.9 ± 1.2^{a}	1.4 ± 2.1
VI	42.6 ± 1.3 (+11.5)	38.0 ± 1.4 (+32.4)	9.4 ± 1.0 (−41.4)	96.2 ± 1.6	3.8 ± 1.6	62.9 ± 1.4	35.4 ± 1.1	1.7 ± 1.3	51.7 ± 2.0	14.4 ± 1.8	14.3 ± 1.7	15.7 ± 1.3	2.3 ± 1.2

FA, fatty acid; GL, glycolipids; PL, phospholipids; NEFA, non-esterified fatty acids; EFA, esterified fatty acids; TG; triglycerides; DG, diglycerides; MG, monoglycerides; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; LPE, lysophosphatidylethanolamine; PA, phosphatidic acid; LPC, lysophosphatidylcholine.
[a] Different from stage VI means at $P < 0.05$. Values in parentheses indicate percent variation from stage IV.

The levels of the mineral elements identified are listed in Table 6. Whatever the stage of maturation, potassium was the major mineral, representing about 60% of the total mineral present. Maturation of the ascocarp brought about both quantitative and qualitative modifications. Silicon and magnesium decreased and phosphorus and sulfur increased slightly with the age of the truffles. Calcium and iron were absent in immature truffles and appeared at relatively high levels in the mature fungi.

4. Discussion

The chemical composition of the black Perigord truffle (*T. melanosporum*), is, with the exception of the melanins and mannitol, similar to that reported for other fungi round in the literature (Bokhary & Parvez, 1993; Brennan, GritIin, Lösel, & Tyrell, 1974; Holtz & Schisler, 1971). 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). The most noteworthy aspect of the chemical composition of *T. melanosporum* described here is the absence of mannitol, which has been known as a major mushroom carbohydrate since 1947 (MaConnell & Esselen, 1947).

In comparison with other species belonging to the genus *Tuber* in particular, *T. magnatum* Pico (precious white truffle of Alba or Acqualagna) (Coli et al., 1988), *T. melanosporum* showed only differences in the quantity of melanin. However, these two species (*T. melanosporum* and *T. magnatum*), which are the most appreciated of all truffles, are rich in proteins, potassium, phosphorus, and certain trace components, namely the sulphur-containing amino acids and certain fatty acids, such as linoleic acid. These are of great importance for the aroma and flavour of these species (Daiz et al., 2003; Talou et al., 1989).

The stages of development of *T. melanosporum* were defined cytologically by Montant et al. (1983) and structurally by Parguey-Leduc, Montant, and Kulifaj (1985, 1987). The present biochemical study provides further depth to this classification. Our chemical balance sheet, determined for the three later stages of maturation, shows that the mature black Perigord truffle (stage VI) presents a higher level of carbohydrate (30.6% of the total dry weight), contains rhamnose, is rich in free amino acids, such as alanine (40.1% of all free amino acids), serine (10.8%) and glutamine (9.6%), has a high percentage of lysophosphatidylethanolamine, a low level of sterols, contains calcium and iron, and above all has a black coloration due to the presence of melanins which represent up to 15% of the dry weight. These chemical changes can be correlated with the physiological development and the structural modifications occurring in the ascocarp. For example, the variations observed in the carbohydrate composition of the three stages studied can be explained by the fact that, in immature truffles, mycelial structures are abundant whereas, in the mature truffles, spore structures, rich in carbohydrate, are preponderant. The possible functions of

Table 5
Levels of fatty acids at the three *Tuber* maturation stages

	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	DU
Stage IV	2.6 ± 0.4[a]	16.0 ± 0.5[a]	6.2 ± 0.7[a]	3.8 ± 0.6[a]	70.5 ± 0.4[a]	0.9 ± 0.3[a]	1.48
Stage V	6.3 ± 0.8[a]	18.9 ± 0.3[a]	4.7 ± 0.6[a]	20.6 ± 0.5[a]	47.9 ± 0.8[a]	1.6 ± 0.4[a]	1.21
Stage VI	3.6 ± 0.2	11.7 ± 0.4	3.3 ± 0.8	23.1 ± 0.4	55.9 ± 1.0	2.4 ± 0.4	1.42

Note. Values are expressed as a percent of total fatty acids.
DU = degree of unsaturation = $\Sigma 1 \times$ (% monoenes)/100 + $\Sigma 2 \times$ (% dienes)/100 + $\Sigma 3 \times$ (% trienes)/100.
[a] Different from stage VI means at $P < 0.05$.

Table 6
Levels of minerals at the three *Tuber* maturation stages, expressed in % dry weight

	StageIV		StageV		StageVI	
	Atom (%)	Weight (%)	Atom (%)	Weight (%)	Atom (%)	Weight (%)
Mg	4.23 ± 0.45[a]	0.21	3.40 ± 0.62[a]	0.25	1.78 ± 0.29	0.13
Si	2.86 ± 0.27[a]	0.10	1.36 ± 0.44[a]	1.10	0.83 ± 0.60	0.06
P	18.7 ± 0.31[a]	0.94	22.6 ± 0.36	1.64	22.8 ± 0.72	1.68
S	11.1 ± 0.52	0.56	11.8 ± 0.57	0.86	12.4 ± 0.53	0.91
K	63 ± 0.48[a]	3.17	60.9 ± 0.66[a]	4.43	58.7 ± 0.48	4.33
Ca	–		–		2.30 ± 0.29	0.17
Fe	–		–		1.22 ± 0.30	0.09

[a] Treated means differ from stage VI means at $P < 0.05$.

these large stores of free carbohydrate have been explored by a number of workers and it appears that they are endogenous carbon reserves for the spores. Such a role has been proposed for mannitol and trehalose during germination of sclerotia of *Sclerotinia sclerotiorum* (Le Tourneau, 1966). Moreover, the levels of free amino acids at stages V and VI show that some amino acids (alanine, serine and glutamine) reach higher values. These results can be compared with the analyses of volatile components made by Talou et al. (1989) and Daiz et al. (2003). Indeed, the degradation of these amino acids can be directly related to the formation of certain alcohols and aldehydes, such as acetaldehyde, 2-methylpropanal, 2-methyl-1-propanol and 2-methyl-2-butanol, which contribute to the fragrance of the black truffle.

In a recent study of the biosynthetic pathways leading to the formation of melanin in mature *T. melanosporum*, it was shown that cysteine and tyrosine act as precursors (Harki, 1996). This could offer an explanation for the variations of these two amino acids between immature and mature truffles.

Finally, from the study of the lipid composition (total fatty acids and sterols) (Harki et al., 1996), we were able to follow the maturation of the *Tuber* ascocarp by an original approach, complementary to the morphological and physiological studies. Owing to their structural and organisational role, lipids, or more precisely the variations of the lipid content, provide a signature of the alterations occurring in the different cell types that make up the ascocarp.

In conclusion, these novel chemical findings are correlated with the physiological development and the structural modifications of the ascocarp. They do not all present differences that are sufficiently marked to be used to evaluate the state of maturation of *T. melanosporum*.

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