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Correlation between Fatty Acid Content and Aromatic Compound Release in Fresh Blewit (*Lepista nuda*)

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Identification, quantification, and location of fatty acids in the newly cultivated fresh mushroom, *Lepista nuda*, as well as three aromatic effluents (1-octen-3-ol, 1-octen-3-one, and 3-octanone) from mushroom homogenate, were established. The highest amounts of fatty acids as well as the three aromatic compounds formed and released were encountered in the gills rather than in the pileus or the stipe. Among these components, 1-octen-3-ol ("mushroom alcohol") was the major aromatic compound trapped, and linoleic acid was the major fatty acid extracted, whatever the morphological tissue considered. Observed results indicate that the gills are the major compartment for the metabolic interconversion of fatty acids into aromatic compounds.

INTRODUCTION

The blewit (*Lepista nuda)* is a wild edible mushroom (recently) cultivated at the Mushroom Research Station of Villenave d'Ornon (INRA-France) and which presents numerous organoleptic qualities including a delicate flavor and good postharvest conservation, which are important economic aspects influencing sales and prices (Guinberteau et al., 1989). Its strong and subtle flavor, its texture, and its violet coloration present some originality which could justify widespread cultivation to diversify the production of edible mushrooms in France (Guinberteau et al., 1989, 1991; Audouin et al., 1989).

The present investigation is one of numerous studies about the flavor of edible mushrooms (Cronin and Ward, 1971; Pyysalo, 1976; Maga, 1981) and aims to provide a better knowledge of the location [pileus (P), gills (G), and stipe (S)] of aromatic compound release in fresh *L. nuda*. A correlation was also made between these released compounds and extractable unsaturated fatty acids, which are their direct precursors (Tressl et al., 1982; Grosch and Wurzenberger, 1984; Chen and Wu, 1984; Mau et al., 1992).

MATERIALS AND METHODS

Mushroom Homogenates. Mushrooms of a new hybrid strain of *L. nuda,* named Ferland (LR28 X E40), were harvested the evening before analysis and stored at 4 °C during the night and transport. Sporophores of 40 mm diameter were selected and divided into S, P, and G. Five grams of each fresh tissue was then homogenized in 5 mL of water using an Ultra-turrax (9500 rpm) in the presence of antioxidants (butylated hydroxytoluene, *N*-propyl gallate).

Released Volatile Compound and Fatty Acid Analysis. The method used in this study to evaluate the aromatic mushroom intensity consists of trapping the sample aromatic effluent from tissue homogenate during a fixed time, carried by a gas vector (helium); we obtained quantities of released aromatic compounds in a short time. These measures allow us to determine and characterize the olfactory quality of *L. nuda.* The volatile compounds were extracted from 1 mL of each homogenate in a purge and trap system (Tekmar LSC 2000) by helium sweeping for 3 min, trapped on Tenax, desorbed at 180 °C, and condensed at −100 °C before being pulsed at 240 °C into a capillary column chromatograph (Varian) for separation. The GC analysis parameters were as follows: column, PTE-5 (pretested environmental SPB-5), 30 m × 0.32 mm i.d.; film thickness, 0.25 μm; carrier gas, helium. The oven temperature program was as follows: 40 °C for 5 min, ramped from 40 to 84 °C (2 °C/min), 84 °C for 8 min, ramped from 84 to 134 °C (5 °C/min) and from 134 to 204 °C (10 °C/min). After separation, volatile compounds were ionized in a mass spectrometer (Finnigan ITS 40 ion trap). Identification of substances was performed by comparison of their mass spectra with those of the NIST MS library or our own library spectra. Quantifications were made using a reference standard solution of the three commercial C_8 compounds considered (1-octen-3-ol, 1-octen-3-one, and 3-octanone) and α-pinene, at the equal concentration of 1 ppm (1 mg/L, w/v). α-Pinene was also added to the watery homogenate as an internal standard. For each volatile compound, a response coefficient was calculated as the ratio between the correspondant peak area [arbitrary unit (au)] in the chromatogram and the weight (in nanograms) of the injected analyte. A relative coefficient was also established between α-pinene and the three aromatic constituents to ensure that no deviations of the detector response could occur due to uncontrolled modifications of the ion trap sensitivity. Measurements were finally expressed in nanograms of C_8 compounds formed, released, and detected from 1 gram of fresh tissue in the homogenate after helium sweeping.

Lipids were extracted from 1 mL of the same homogenate according to the method of Folch et al. (1957). Fatty acid methyl esters were made by esterification of the lipidic fraction for 1 h at 100 °C in benzene and BF₃−methanol. Methyl esters were extracted and chromatographed on a Shimadzu GC 14A chromatograph equipped with a hydrogen flame ionization detector (FID). Separations were effected with a 25 m long × 0.32 mm i.d. column coated with 0.25 μm Carbowax 20M. The

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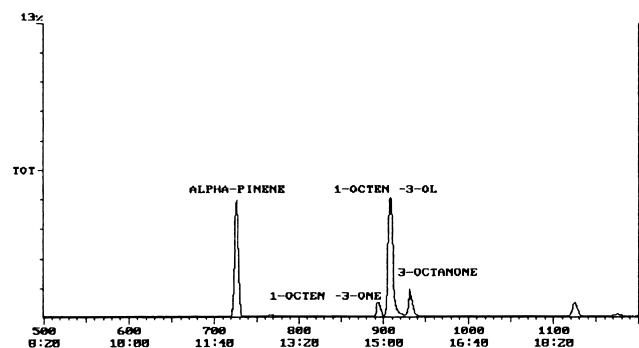† Université Bordeaux I.
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Figure 1. Chromatogram of the three aromatic compounds considered from blewit.

Table 1. Quantities (Nanograms) of the Three Released and Trapped Volatile Compounds per Gram of Fresh Mushroom Stipe (S), Pileus (P), and Gills (G)[a]

volatile compound	P	S	G
1-octen-3-ol	89.7[b] ± 10.2	29.4[c] ± 3.8	299.0[a] ± 36.0
1-octen-3-one	31.0[b] ± 4.5	2.6[c] ± 0.4	14.5[a] ± 1.7
3-octanone	17.5[b] ± 1.9	15.3[b] ± 3.0	86.8[a] ± 12.3

[a] Values represent means obtained from seven extractions. Values with the same superscript letter within a row are not significantly different at the level of 0.05.

column was programmed at 180 °C for 20 min and then from 180 to 200 °C at 7.5 °C/min and 20 min at 200 °C. On injection, the stream of helium and the injected sample's volume were split (1:100). Detector and injector temperatures were 240 and 250 °C, respectively. The peaks were identified by comparison to the retention times of known compounds. An internal standard solution (an equal weight of $C_{10}-C_{24}$ chain length commercial fatty acids and margaric acid as internal standard) was used to determine the linearity of peak area with concentration and to quantify each fatty acid (micrograms per gram of fresh tissue).

The experimental data were subjected to statistical analysis (Student "*t*" test) to determine the significant differences between averages at the level of $p = 0.05$.

RESULTS

The three following aromatic compounds, 1-octen-3-ol, 3-octanone, and 1-octen-3-one, were chosen because quantitatively they belong to the major volatile compounds released from mushrooms and they are generally considered as the primary flavor source for most mushroom species (Maga, 1981). A chromatogram example is shown in Figure 1, and the results are summarized in Table 1. Results show that whatever the morphological tissue considered (S, P, or G), 1-octen-3-ol is the major constituent followed by 3-octanone and 1-octen-3-one. Calculated percentages of each component compared with the total of the three compounds show that 1-octen-3-ol represents 62–75% of these components whatever the morphological tissue considered. Moreover, these results show that the gills seem to be the most aromatic tissue of *L. nuda* since the amounts of the three released C_8 compounds predominate from the gill homogenate.

On the other hand, eight fatty acids (C14:0 to C20:4) were identified and quantified (Table 2). The major fatty acid is an unsaturated one, linoleic acid (C18:2), which is recovered in the majority of all morphological tissues considered (64% in G, 58% in P, and 63% in S); oleic acid (C18:1) was also present in large amounts (15.5% in G, 5.5% in P, and 6.5% in S). Therefore, these two unsaturated C_{18} fatty acids account for at least 64–80% of the total fatty acid content measured. The third

important fatty acid was palmitic acid (C16:0): 15.7% in G, 23.0% in P, and 19.8% in S. Moreover, we observed that for all of the fatty acids determined, G tissue shows the higher content versus P and S. Indeed, if we considered the three larger fatty acid concentrations (C16:0, C18:1, and C18:2), we observed very significant differences among the gills and the other morphological tissues: 386 μg/g for C16:0 for G versus 134 μg/g in P and 142 μg/g in S; 382 μg/g for C18:1 for G versus 32 μg/g in P and 46 μg/g in S; 1574 μg/g for C18:2 for G versus 341 μg/g in P and 453 μg/g in S.

DISCUSSION

Our results show that 1-octen-3-ol is the major effluent compared with 3-octanone and 1-octen-3-one in the blewit. These results are in agreement with those reported by Mau et al. (1992) in *Agaricus bisporus*. Mushrooms were divided into three parts: G, S, and P. Each compartment proportion (percent of weight) was established from 12 samples to take into account mushroom heterogeneity. G represents about 14%, P 38%, and S 48% of the whole mushroom. Considering these proportions, results of Table 1 show under our experimental conditions the following: for 1-octen-3-ol, 90.0 ng released from 1 g of whole fresh mushroom; for 1-octen-3-one, 15.1 ng; and for 3-octanone, 26.2 ng. These results confirmed the dominant role of 1-octen-3-ol in the subtle aroma of the whole mushroom. Few studies have reported the volatile compounds formed and released from this mushroom homogenate. Audouin et al. (1989) have studied aromatic compounds in dried wild *L. nuda* and have emphasized the dominant role played by 1-octen-3-ol in the flavor of this mushroom. In the same way, many authors have shown the importance of C_8 compounds (1-octen-3-ol, octanol, 3-octanol, 1-octen-3-one, 2-octenol, and 3-octanone) in the flavor of different varieties of mushrooms: *A. bisporus* (Cronin and Ward, 1974; Pyysalo, 1976), *Coprinus comatus* (Dijkstra and Wiken, 1976), and *Marasmius oreades* (Vidal et al., 1986).

Table 1 also shows that the gills seem to have a predominant aromatic intensity in the blewit, rather than the pileus or the stipe. Bernhard and Simone (1959) have noted significant olfactory differences in aroma intensity when the stipes and gills on one hand, and the caps and gills on the other hand, were compared in *A. bisporus*. Furthermore, they found more aroma in the gills. As shown by Maga (1981) in a sensory study, it was interesting in this work to calculate the ratio (R) corresponding to the composition in weight of 1-octen-3-ol to 1-octen-3-one, which are, respectively, associated with desirable and undesirable aromas. These ratios provide estimations of the oxidation activity in each compartment of the fresh *L. nuda*. Indeed, as previously reported by Grosch and Wurzenberger (1984), 1-octen-3-ol seems to be primarily converted to 1-octen-3-one by an oxidation pathway, although the reduction of 1-octen-3-one to 1-octen-3-ol could also be possible (Chen and Wu, 1984). We found R_G, R_S, and R_P approximately equal to 21, 11, and 3, respectively. Therefore, 1-octen-3-ol release is about 21 times higher than 1-octen-3-one release in gills; this is in agreement with the comparable study of Bernhard and Simone (1959) in *A. bisporus* and in relation to an increased metabolism in gills as reported by Hammond and Nichols (1975) or Burton et al. (1994) in *Agaricus*.

The fatty acid compositions in *L. nuda* were also studied because of the well-known metabolic relation

Table 2. Fatty Acid Concentration (Parts per Million) in Fresh Mushroom Stipe (S), Pileus (P), and Gills (G)[a]

tissue	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:4
G	15.18[a] ± 3.60	386.20[a] ± 49.10	78.20[a] ± 10.80	382.10[a] ± 108.40	1573.80[a] ± 190.30	5.40[a] ± 1.00	4.72[a] ± 1.20	11.99[a] ± 4.40
P	13.20[b] ± 2.80	134.40[b] ± 25.20	41.50[b] ± 9.80	32.20[b] ± 7.10	341.10[b] ± 45.30	4.00[a] ± 0.20	5.60[b] ± 2.10	12.40[b] ± 2.40
S	14.17[c] ± 4.50	142.40[b] ± 29.80	50.50[c] ± 8.10	46.60[c] ± 6.80	453.40[c] ± 80.40	4.40[a] ± 0.30	4.20[c] ± 1.50	3.30[c] ± 0.45

[a] Values represent means obtained from 10 extractions. Values with the same superscript letter within a column are not significantly different at the level of 0.05.
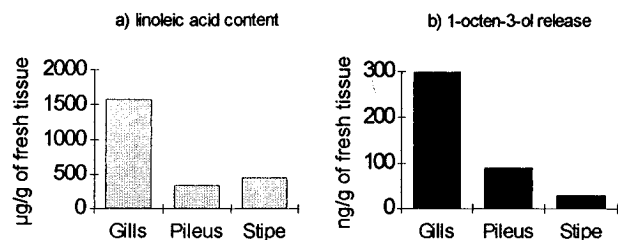

Figure 2. Correlation between linoleic acid concentrations (a) and 1-octen-3-ol releases (b) in gills compared to the other tissues.

between the linoleic acid (C18:2) and 1-octen-3-ol. Results given in Table 2 are close to those obtained for *A. bisporus* by Hughes (1962), Holtz and Schisler (1971), Byrne and Brennan (1975), Hira et al. (1990), or Abdullah et al. (1994). Considering the proportion of each morphological tissue in the whole mushroom as already cited, quantitative results show that fatty acids represent in total about 900 μg/g of the whole fresh mushroom. This value is in total agreement with different published results (Hughes, 1962; Delmas, 1978) in which the lipidic fraction represents 0.4−0.7% of the fresh mushroom weight and contains about 33% of fatty acids. Concerning the different morphological tissues of *L. nuda* (Table 2), gills have very significantly higher palmitic, oleic, and linoleic acid content compared with pileus or stipe, which have fatty acid concentrations similar to each other. The high levels of these three fatty acids in gills correspond to an increased metabolism activity in this tissue, as already reported (Hammond and Nichols, 1975; Burton et al., 1994; Mau et al., 1993).

The major observation of this study is that, whatever the morphological tissue, the major volatile aromatic component released from fresh *L. nuda* homogenate was 1-octen-3-ol, and the major fatty acid extracted from the same homogenate was linoleic acid (C18:2). It is well-known that 1-octen-3-ol is mainly formed from the enzymatic breakdown of linoleic acid (Varoquaux and Avisse, 1975; de Lumen et al., 1978; Tressl et al., 1982; Wurzenberger and Grosch, 1982; Grosch and Wurzenberger, 1984; Mau et al., 1992) and more precisely when the fruiting body tissue is damaged. It is of great interest to note the correlation between 1-octen-3-ol release and its main precursor content in fresh *L. nuda* when the two measurements are from the same homogenate of mushroom. The highest concentrations of these two compounds are obtained from gills. Emphasizing this point, it has already been reported that linoleic acid addition before mushroom blending leads to an increase of 1-octen-3-ol by an enzymatic conversion pathway (Mau et al., 1992). Therefore, it seems logical to observe a high level of C18:2 in a compartment from which the highest level of this alcohol has been trapped (Figure 2). In the gills, the ratio ρ between 1-octen-3-ol released and linoleic acid content is $\rho_G = 1.9 \times 10^{-4}$, when it is only 1.5×10^{-4} for the whole mushroom. So, if we consider that the measured amounts of 1-octen-3-ol released were representative of these amounts

formed in each tissue, these ratios point out the relative importance of the gills in the aromatic activity of the whole *L. nuda*. Mau et al. (1993) have recently reported a similar observation about 1-octen-3-ol extractions from the gills and the whole mushroom in *A. bisporus*.

CONCLUSIONS

Results of this research allow us to establish and compare the location of released volatile compounds and, on the other hand, the location of fatty acid content in fresh *L. nuda*. This comparison shows an obvious correlation between 1-octen-3-ol and linoleic acid.

Although gills are shown in this study as the most "aromatic" tissue of the blewit, it is interesting to note that when the different proportions of each compartment in the whole mushroom are taken into account, the cap (pileus with gills) releases the largest quantities of volatile compounds (more than 90%). Therefore, from an economic point of view, the cap represents the most interesting part of the blewit compared to the stipe for aroma quality.

The results obtained in this particular mushroom are similar to those found in some studies performed on *A. bisporus*. Lipoxygenase and hydroperoxide lyase activities in each compartment of *L. nuda* need to be studied for they may account for the differences observed in the levels of 1-octen-3-ol released among the different morphological tissues. Furthermore, studies at different stages of development and at different postharvest times on this mushroom should be made to provide more information on the effect of maturity and on conservation of flavor, which is an important quality parameter for the widespread consumption of these edible mushrooms.

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