Fatty Acid Content and Some Flavor Compound Release in Two Strains of *Agaricus bisporus*, According to Three Stages of Development

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The fatty acid concentrations and the flavor compound release levels of two different strains *Agaricus bisporus* were studied at different stages of development. Two strains (hybrid white and brown) and three developmental stages (button, medium, and flat) were considered. Results showed that linoleic acid and 1-octen-3-ol are the major fatty acid and flavor compound, respectively, whatever the strain or the developmental stage. Moreover, higher linoleic acid concentrations were found at the button stage in the hybrid white strain and at the medium stage in the brown strain. In contrast, greater amounts of 1-octen-3-ol were encountered at the medium stage in the two strains. The flavor quality ratio, *r*, defined as the ratio of the amount of 1-octen-3-ol released and detected to the linoleic acid content, was greater at the medium stage, whatever the strain, and in all developmental stages for the Br one.

Keywords: *Agaricus bisporus; fatty acids; flavor; strain; development*

INTRODUCTION

Lipid composition and metabolism of *Agaricus bisporus* mycelium or sporophore have already been investigated extensively. A preliminary study was reported by Hughes (1962), who identified 10 fatty acids, among which linoleic acid (18:2) varied from 63% to 74% with strain. It was shown later that *A. bisporus* included mainly C_8 to C_{24} fatty acids (Holtz and Schisler, 1971; Byrne and Brennan, 1975; Weete et al., 1985; Mau et al., 1991; Stancher et al., 1992).

Lipids play an important role in the mycelial growth increasing the mushroom production (Wardle and Schisler, 1969). The chief unsaturated fatty acid of mushroom lipids, linoleic acid, is the precursor of the "mushroom alcohol" (1-octen-3-ol) (Tressl et al., 1982; Wurzenberger and Grosch, 1982; Grosch and Wurzenberger, 1984; Mau et al., 1992). This alcohol, together with the two associated C_8 ketones (1-octen-3-one, 3-octanone), constitute the main volatiles and are considered the major contributors to the characteristic mushroom flavor (Cronin and Ward, 1971; Pyysalo, 1976; Maga, 1981).

In this work, we present a simultaneous comparison of the changes in the fatty acid content and the three flavor component release, at three developmental stages of two commercial strains of *A. bisporus*: an hybrid white (AMYCEL 2100) and a brown strain (LION C9).

MATERIALS AND METHODS

Mushrooms. Cultivated commercial hybrid white (HW) strain (AMYCEL 2100) and brown (Br) strain (LION C9) of *A. bisporus* were used. All mushrooms were grown on traditional horse manure compost in natural quarries at a local production center (Champidor Society, 24650 Chancelade, France) and harvested on first flush (first break of the crop cycle) 1 h before analysis.

For each strain, mushrooms were harvested at three stages of development. The first (A) was the button stage which consisted of closed caps about 20 mm in diameter. The medium stage (B) was represented by caps of about 35 mm in diameter with closed veils. The third stage (C) had open caps of about 70 mm in diameter (flat mushroom). These three stages were designated 2, 3, and 7, respectively, in the classification of Hammond and Nichols (1975) or 1−2, 3−4, and 5−6−7, respectively, in the classification of Mau et al. (1993). Each of these six mushroom batches was analyzed in seven to eight replicates for fatty acids and aroma extractions.

Fatty Acids and Aroma Analysis. For each extraction, 20 g of fresh cap was rapidly cut into small cubes of 5 × 5 mm, then blended with 20 mL of water by using an ultra-turrax homogenizer (9500 rpm) in the presence of an antioxidant, the *n*-propyl gallate at 0.05%. Lipids were extracted according to the method of Folch et al. (1957), in the presence of an antioxidant, the butylated hydroxytoluene, at 0.016%. Fatty acids methyl esters were obtained by transesterification of the lipid fraction during 1 h at 100 °C in benzene and BF_3−methanol (BF_3:CH_3OH, 14%). Margaric acid (17:0) was added in the homogenate as an internal standard. Methyl esters were analyzed on a Shimadzu GC 14A chromatograph equipped with an hydrogen flame ionization detector (FID). Separations were realized with a 25 m × 0.32 mm i.d. column coated with 0.25 μm of carbowax 20M. The oven was programmed at 180 °C for 20 min, then ramped from 180 to 200 °C at 7.5 °C/min and held at 200 °C for 20 min. The injector has a 1/100 sample splitter, and detector and injector temperatures were 240 and 250 °C, respectively.

A standard mixture of equal weights of C_{10} to C_{24} fatty acids was used to determine peak response factor. Antioxidants, fatty acids, and BF_3 came from SIGMA-Chimie, Saint Quentin Fallavier, France.

For released aroma analysis, 1 mL of fresh cap homogenate was put into a flask connected to a purge and trap system (Tekmar LSC 2000). Volatile com-

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Table 1. Fatty Acid Concentrations in Mushroom Caps[a]

	strain HW			strain Br		
fatty acid	A	B	C	A	B	C
14:0	0.9 ± 0.2^{a1}	1.6 ± 0.2^{b1}	traces^{c1}	0.6 ± 0.1^{ac2}	1.8 ± 0.4^{b1}	1.7 ± 0.4^{bc2}
16:0	20.2 ± 3.4^{a1}	22.3 ± 3.0^{a1}	11.6 ± 1.5^{b1}	11.1 ± 1.0^{a2}	26.5 ± 3.9^{b2}	16.9 ± 2.1^{c2}
18:0	8.2 ± 1.5^{a1}	11.7 ± 1.7^{b1}	5.2 ± 1.2^{c1}	7.0 ± 1.5^{a1}	9.9 ± 1.8^{b2}	10.3 ± 1.4^{b2}
18:1	8.4 ± 1.5^{a1}	9.1 ± 2.2^{a1}	3.2 ± 1.0^{b1}	5.9 ± 0.9^{a2}	7.1 ± 1.4^{b2}	8.5 ± 0.7^{c2}
18:2	102.9 ± 14.7^{a1}	85.3 ± 9.5^{b1}	79.6 ± 13.4^{b1}	51.2 ± 3.9^{a2}	108.9 ± 11.1^{b2}	78.9 ± 3.5^{c1}
20:0	2.2 ± 0.4^{a1}	2.5 ± 0.2^{b1}	1.8 ± 0.4^{c1}	1.6 ± 0.3^{a2}	2.6 ± 0.2^{b1}	1.9 ± 0.1^{c1}

[a] Values are expressed as micrograms per milligram of protein, for hybrid white (HW) and brown (Br) strains, according to three developmental stages (A, button; B, medium; C, flat). Values represent means obtained from eight extractions. Values with a different superscript letter within a row and a strain are significantly different at the level of 0.05. Values with a different superscript number within a row and a developmental stage are significantly different at the level of 0.05, between HW and Br strains.

pounds were carried by helium (40 mL/min) at 20 °C, for 3 min, trapped on Tenax, desorbed at 180 °C, then concentrated by cooling to −100 °C and pulsed at 240 °C into a capillary column chromatograph for separation.

Volatile flavor components were separated and identified with a Varian gas chromatograph equipped with a mass spectrometry detector. The GC 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; oven temperature program, 40 °C for 5 min, ramped from 40 to 84 °C at 2 °C/min, 84 °C for 8 min, ramped from 84 to 134 °C at 5 °C/min and from 134 to 204 °C at 10 °C/min. After separation, volatile compounds were ionized in a Finnigan ITS 40 ion trap with the following parameters: 220 °C; ionization energy, 70 eV.

The 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, from Lancaster Synthesis Ltd, Bischeim, France). For each volatile compound, a response coefficient was calculated as the ratio between the compound peak area (arbitrary unit, a.u.) in the chromatogram and the weight (in nanograms) of the injected analyte.

Measurements were finally expressed in nanograms of C_8 compounds released and detected, after helium sweeping for 3 min, per milligram of protein, and in micrograms of fatty acids per milligram of protein. The protein concentration was assayed by the method of Bradford (1976), with bovine serum albumin (SIGMA) as a standard.

The experimental data were subjected to statistical analysis (Student's t test) to determine the significant differences between averages at the level of $p = 0.05$.

RESULTS AND DISCUSSION

The measurements of fatty acid concentrations from fresh *A. bisporus* caps (Table 1) show the presence of saturated as well as unsaturated fatty acids. Among these, palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids account for more than 96% of the extract, whatever the strain and the developmental stage. Linoleic acid is the major one, varying from 66% to 79% of the total fatty acids. These results are in agreement with previous studies on *Agaricus* spp (Hughes, 1962; Holtz and Schisler, 1971; Hira et al., 1990; Mau et al., 1991; Stancher et al., 1992; Abdullah et al., 1994).

Quantitatively, we found about 1.5 mg of fatty acids extracted per 1 g (0.15%) of fresh mushroom cap, which is a value in agreement with those found by several authors (Hughes, 1962; Byrne and Brennan, 1975; Delmas, 1978; Abou-Heilah et al., 1987) who have shown that the lipid fraction in *Agaricus* spp, represents about 0.4%−0.7% of fresh mushroom weight and contains about 33% of fatty acids.

During mushroom development, we observed a general and significant decrease of fatty acid concentrations between A and C stages for HW strain. This decrease was already significant (Figure 1a) at stage B for linoleic acid (85.3 μg/mg of protein versus 102.9 μg/mg of protein at stage A). A similar change was recently mentioned for linoleic acid between closed and opened caps of *A. bisporus* (Hira et al., 1990). On the contrary, concentrations of fatty acids in Br strain were found greater at stage C than at stage A, with a maximum at stage B for palmitic and linoleic acids.

At full maturity (stage C), all fatty acid concentrations were found higher for the Br strain than for the HW one. These strain differences in the evolution of fatty acid content during development were thought to be in relation with their lipid metabolic activities. Indeed, changes in metabolism, as large increases in intracellular protease activities, during sporophore development in *A. bisporus*, were already described (Wood, 1979). Many other studies have shown changes during development in some enzymatic activities involved in carbohydrates and nitrogen metabolism (Hammond, 1985; Wells et al., 1987; Burton et al., 1994). The results obtained (Table 1) seem to indicate that there are also changes in lipid metabolism during development of fruiting bodies and depending on the strain.

Linoleic acid is recognized to be the precursor of the 1-octen-3-ol (Varoquaux and Avisse, 1975; De Lumen et al., 1978; Tressl et al., 1982; Wurtzenberger and Grosch, 1982; Grosch and Wurtzenberger, 1984; Mau et al., 1992), which was identified in many mushroom species, as the major volatile constituent of aroma being a good parameter for mushroom flavor quality (Cronin and Ward, 1971; Pyysalo, 1976; Dijkstra and Wiken, 1976; Vidal et al., 1986).

Thus, to correlate the observations concerning the evolution of fatty acid content with those of flavor released constituents of *A. bisporus*, samples were analyzed to evaluate the relative importance of the 1-octen-3-ol among the main volatile components and to follow its evolution during development, as it could be defined by sensory analysis or consumer perception.

Results (Table 2) show that, in HW or Br strain, the released amounts of these three flavor compounds are found to be greater at the second stage of development (B) and are significantly smaller at stage C. In Br strain, the release of flavor compounds is higher than in the HW one, whatever the developmental stage,

Table 2. Amounts of the Three Volatile Compounds for HW and Br Strains, According to Three Developmental Stages (A, Button; B, Medium; C, Flat)[a]

	strain HW			strain Br		
volatile compd	A	B	C	A	B	C
1-octen-3-ol	112.0 ± 13.4	452.8 ± 38.5	25.7 ± 3.5	113.6 ± 8.8	732.2 ± 105.4	58.6 ± 7.1
1-octen-3-one	5.2 ± 0.7	14.0 ± 2.1	2.7 ± 0.3	6.0 ± 1.3	57.6 ± 4.0	5.1 ± 0.6
3-octanone	15.5 ± 1.5	97.9 ± 10.0	7.6 ± 1.2	13.1 ± 2.0	35.6 ± 3.3	8.5 ± 1.0

[a] Values are expressed as nanograms of released and detected components, for 3 min of purge, per milligram of protein. Values represent means obtained from seven different mushroom samples. For each flavor compound, results are significantly different between hybrid white and brown strains ($P \leq 0.05$), within a stage of development (except for 1-octen-3-ol and 1-octen-3-one, at stage A), and results are significantly different between developmental stages ($P \leq 0.05$), within a strain.

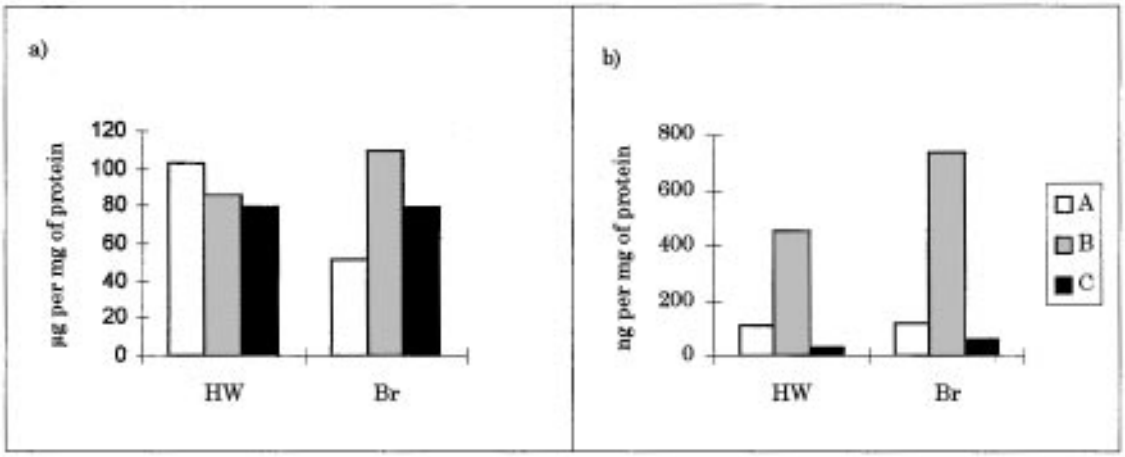

Figure 1. Correlation between linoleic acid content (a) and 1-octen-3-ol release (b) between developmental stages (A, B, or C), for HW and Br strains.

except for 3-octanone, which predominates in HW strain at stages A and B.

Results also show that, whatever the strain and the developmental stage, 1-octen-3-ol is the major effluent compared with 3-octanone and 1-octen-3-one, as already reported (Mau et al., 1992). Moreover, the highest 1-octen-3-ol amounts were trapped for 3 min and detected at the stage B (Figure 1b), whatever the strain considered. However, in the Br strain, the amount of 1-octen-3-ol is more important than in HW strain (732.2 ng/mg of protein versus 452.8 ng/mg of protein, respectively). The values are smaller than those generally reported in literature (Mau et al., 1993), explained by our experimental procedure described above, which is very different from a classical full solvent extraction (helium sweeping during a short and fixed time). One of the oxidation derivatives of 1-octen-3-ol, the 1-octen-3-one, associated with an "undesirable aroma" (Maga, 1981), presents the smallest quantities at the last developmental stage, especially for the HW strain. To evaluate this oxidation capacity, as proposed by Maga, we calculated the ratio (R) corresponding to the composition in weight of 1-octen-3-ol to 1-octen-3-one, at the three developmental stages and for the two strains. We found $R_A = 21.5$, $R_B = 32.3$, and $R_C = 9.5$ for the HW strain and $R_A = 18.9$, $R_B = 12.7$, and $R_C = 11.5$ for the Br one.

For the Br strain, we observed a slight decrease of this ratio during development, explained by higher variations of 1-octen-3-one quantities, than 1-octen-3-ol ones, as reported by Maga (1981) on white *A. bisporus*. We noticed a very different evolution of this ratio for the HW strain, which presents a maximum at the medium stage (32.3 versus 21.5 for R_A). But R_C is smaller whatever the strain, related with a significant decrease of 1-octen-3-ol release and a predictable increase of oxidation activity, by these aging mushrooms. 3-Octanone, which could be derived from 1-octen-3-ol by an isomerization, presents maximal quantities whatever the strain at the second stage of development, which seems to show the best metabolic activity.

On a metabolic point of view, it has already been reported by Mau et al. (1992) that linoleic acid addition before off-white *A. bisporus* blending leads to an increase of 1-octen-3-ol content, by lipoxygenase and hydroperoxide lyase activities. In the experimental conditions, we determined another ratio, r, between released and detected amounts of 1-octen-3-ol (μg/mg of protein) and linoleic acid content (μg/mg of protein) and found $r_{HW} = 1.1 \times 10^{-3}$, 5.5×10^{-3}, and 0.3×10^{-3} for HW strain. In contrast, we found $r_{Br} = 2.2 \times 10^{-3}$, 6.7×10^{-3}, and 0.7×10^{-3} at stages A, B, and C, respectively, for Br strain.

So, considering that changes of detected amounts of 1-octen-3-ol could be representative of an increased formation of this alcohol, and that changes of linoleic acid concentration were representative of an increase in cell membrane phospholipid incorporation or in metabolic degradation, these ratios could point out a higher flavor activity of the Br strain than the HW one, all along development. Results also seem to show a predominant enzymatic activity at stage B, for the two strains. This last observation is complementary to those of Mau et al. (1993), who found that immature (button stage) off-white *A. bisporus* had higher 1-octen-3-ol content, whatever the harvest flush. Indeed, the repartition in developmental stages (three stages rather than seven for Mau et al.), and especially our methodological approach of flavor compounds (headspace rather than the pentane extraction), are very specific and different from those of their study. Moreover, the traditional cultivation parameters (in natural limestone quarries rather than in cultivation houses) of the mushroom strains may be associated with a different

metabolic activity and, so, an olfactory quality of these mushrooms.

CONCLUSIONS

The main characteristic of this study is that analyses concerning flavor volatile release and fatty acid concentrations were performed simultaneously.

So, confirming several published results obtained separately and with different techniques, it has been shown that, whatever the strain, 1-octen-3-ol and linoleic acid are the major constituents of the three volatile released compounds and extracted fatty acids, respectively. This work points out an opposition at the stage B for which these two parameters are observed maximal for the Br strain, while 1-octen-3-ol release goes through a maximum and linoleic acid content through a minimum for the HW one. This indicates that these phenomena are strain-dependent.

The maximum flavor intensity is at the medium stage (B), rather than at the button stage (A). This result is all the more important, since our flavor compound analysis methodology includes technical conditions very close to sensory analysis or olfactory consumer perception.

Moreover, since the metabolic pathway between the linoleic acid and the 1-octen-3-ol has already been demonstrated, the ratio *r*, which could give an evaluation of the 1-octen-3-ol release evolution, compared to its precursor content, could be an interesting flavor indicator for each strain or developmental stage.

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