JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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pubs.acs.org/JAFC

Characterization of the Key Odorants in Pan-Fried White Mushrooms (*Agaricus bisporus* L.) by Means of Molecular Sensory Science: Comparison with the Raw Mushroom Tissue

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ABSTRACT: Application of aroma extract dilution analysis (AEDA) on the volatile fraction isolated from pan-fried white mushrooms (*Agaricus bisporus* L.) revealed 40 odor-active compounds in the flavor dilution (FD) factor range of 8−8192, among which the caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one showed the highest FD factor of 8192, followed by 2-propionyl-1-pyrroline (popcorn-like) and 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (seasoning-like). A total of 36 compounds are reported for the first time in processed mushrooms, and 25 odorants showing the highest FD factors were then quantitated by stable isotope dilution assays and their odor activity values (OAVs) were calculated as ratio of their concentrations to their odor thresholds. Among them, 3-methylbutanal (malty), 3-(methylthio)propanal (cooked potato), and 2-acetyl-1-pyrroline (popcorn-like) showed the highest OAVs (>100) in the pan-fried mushrooms, followed by 1-octen-3-one, 2-propionyl-1-pyrroline, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, phenylacetaldehyde, 2,3-diethyl-5-methylpyrazine, and 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one with OAVs >10. An aqueous aroma recombinate containing 13 odorants (OAV > 1) in their actual concentrations in the fried mushrooms showed a good similarity to the original aroma profile. The quantitation of the key odorants in raw mushrooms, identified with high FD factors during the AEDA, revealed that numerous odorants were quantitatively changed by the frying process, but in particular the concentrations of 2-phenylacetaldehyde and 3-methylbutanal were higher by factors of ∼40 and 6, respectively, compared to the amounts in the processed mushrooms. The data suggested an enzymatic formation of both Strecker aldehydes by the cut mushroom tissue. In total, 26 odorants were newly identified in raw mushrooms.

KEYWORDS: *white mushrooms, Agaricus bisporus, aroma, pan-frying, aroma extract dilution analysis, 2-acetyl-1-pyrroline, 2-propionyl-1-pyrroline, Ehrlich pathway*

INTRODUCTION

Due to their easy cultivation and superior aroma characteristics after thermal processing, white mushrooms (*Agaricus bisporus* (J. Lge.) Imbach) are the most popular edible fungi all over the world. They are widely used, for example, in soups and gravies and as pizza topping, but are also consumed raw, for example, in fresh salads. Because the unique "mushroom-like" odor of raw white mushrooms clearly changes after heat processing, for example, pan-frying or cooking, it is of special interest to clarify the compounds responsible for the overall changes in the mushroom aroma induced by processing. In particular, the unique aroma of pan-fried mushrooms is demanded to flavor processed food such as soups, gravies, and casseroles.

Over about 50 years numerous investigations on the volatile compounds of mushrooms, either raw or processed, have led to the identification of about 175 volatile compounds.[1] However, most of the studies on volatiles present in raw mushrooms were focused on compounds with eight carbon atoms, especially 1-octen-3-ol and 1-octen-3-one, because these two compounds exhibit a typical mushroom-like odor. Attempts to evaluate the impact of the entire set of volatiles on the overall mushroom aroma, are, however, scarcely available. Cronin and Ward[2] were among the first to investigate the volatiles of raw white mushrooms by using steam distillation for volatile isolation. By application of gas chromatography−olfactometry (GC-O), they were able to characterize 23 odor-active compounds, among them 1-octen-3-ol, 1-octen-3-one, 3-octanone, *n*-hexanol, furan-2-carbaldehyde, benzaldehyde, phenylacetaldehyde, α-terpineol, and phenylmethanol. Wasowicz[3] and Sulkowska and Kaminski[4] also applied GC-O and additionally detected 3-methylbutanol, but also confirmed 1-octen-3-one, 3-octanone, benzaldehyde, and phenylmethanol as odor-active compounds.

Fischer and Grosch[5] later reported on the four odor-active compounds 1-octen-3-one, 1-octen-3-ol, 3-octanone, and (*E*)-2-octen-1-ol by GC-O in a volatile fraction isolated by careful distillation of raw mushrooms at low temperature. By sniffing of serial dilutions in a procedure similar to aroma extract dilution analysis (AEDA), the highest (flavor) dilution (FD) factors of 50 and 40, respectively, were found for 1-octen-3-one and 1-octen-3-ol, and a somewhat lower FD factor of 10 was found for 3-octanone and (*E*)-2-octen-1-ol, thus corroborating 1-octen-3-one and 1-octen-3-ol as the key aroma compounds in raw mushrooms.

Compared to raw mushrooms, studies on the odorants of processed white mushrooms are rare, and the aroma of pan-fried mushrooms has not yet been investigated at all. Card and Avisse[6] isolated the volatiles from both raw and cooked mushrooms and identified hexanol, 3-octanol, 1-octen-3-ol, benzaldehyde, octanol, 1-octen-2-ol, phenylacetaldehyde, and phenylethanol in both extracts, but in particular the concentrations of 3-octanone and 1-octen-3-one were reported

Received: February 11, 2013
Revised: April 3, 2013
Accepted: April 4, 2013
Published: April 4, 2013

ACS Publications © 2013 American Chemical Society

dx.doi.org/10.1021/jf4006752 | J. Agric. Food Chem. 2013, 61, 3804−3813

to be higher in the cooked mushrooms. MacLeod and Panchasara[7] investigated the volatiles isolated by steam distillation from fresh cooked and from dried and then cooked white mushrooms. The authors reported 1-octen-3-ol and cyclo-octanol as the quantitatively dominating volatiles in the fresh cooked mushrooms, whereas benzaldehyde and hexanal showed the highest amounts in cooked dried mushrooms. In addition, the presence of 1-octen-3-one, benzaldehyde, benzyl alcohol, and furfural was confirmed in both mushroom extracts, whereas in the dried cooked mushrooms, additionally some pyrazines were detected. Misharina et al.[8] recently extracted the volatiles from mushrooms that were dried at 70−80 °C using steam distillation. They identified various heterocyclic compounds, carbonyl compounds, several pyrazines, and, among others, 3-(methylthio)propanal, 2-acetylpyrrole, and 2-acetylthiazole. Semiquantitative measurements done by FID peak area calculations revealed phenylacetaldehyde, benzaldehyde, and hexanal to be present in concentrations >1 mg/100 g of dried mushrooms. Quite high contents were also measured for undecanol and (*E*,*E*)-2,4-decadienal.

The literature review reveals that, besides 1-octen-3-one and 1-octen-3-ol, only a few other compounds were established as aroma compounds in mushrooms by methods combining analytical and sensory data, for example, a calculation of odor activity values (OAVs). Furthermore, in many cases steam distillation has been used to isolate the volatiles from raw mushrooms, which is, however, simulating a cooking process. Thus, the impact of single volatile compounds on the overall aroma of, in particular, pan-fried white mushrooms is yet unclear. Therefore, this study was aimed at identifying the key aroma compounds in pan-fried mushrooms by the molecular sensory science[9] concept, that is, GC-O, quantitation of odor-active compounds, calculation of OAVs, and recombination experiments. Then, the results were compared to the odorants present in the raw material to get some insight into odorant formation during frying.

MATERIALS AND METHODS

Mushrooms. White mushrooms were purchased from a local supermarket. They were organically grown on beds by Schmaus Champignons (Pöttmes, Germany).

Chemicals. Anhydrous Na_2SO_4, methylene chloride, and silica 60 were obtained from Merck, Darmstadt, Germany. Methylene chloride was freshly distilled before use.

Reference Odorants. The following reference odorants were obtained from the commercial sources given in parentheses: acetic acid, 2-acetyl-2-thiazoline, 2,3-butanedione, butanoic acid, δ-decalactone, γ-decalactone, 2,3-diethyl-5-methylpyrazine, (*E*,*E*)-2,4-decadienal, δ-dodecalactone, γ-dodecalactone, (*Z*)-6-dodeceno-γ-lactone, 3,5-dimethyl-2-ethylpyrazine, 2,5-dimethyl-3-ethylpyrazine, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 3-methylbutanal, 2-methylbutanoic acid, 3-methylbutanoic acid, 2-methylpropanal, 2-methylpropanoic acid, 3-(methylthio)propanal, (*E*,*E*)-2,4-nonadienal, (*E*,*Z*)-2,6-nonadienol, γ-nonalactone, δ-nonalactone, octanal, (*E*)-2-octenal, 1-octen-3-ol, pentanoic acid, phenylacetaldehyde, phenylacetic acid, 3-methylindole, 2-phenylethanol, 2,3,5-trimethylpyrazine, and γ-undecalactone (Sigma Aldrich Chemie, Taufkirchen, Germany); 1-octen-3-one (Alfa Aesar, Karlsruhe, Germany); (*R*)-δ-decenolactone (Wako Chemicals, Neuss, Germany); 4-hydroxy-3-methoxybenzaldehyde (Merck); 2-isobutyl-3-methoxypyrazine (Acros Organics BVBA, Geel, Belgium); and 4-methylquinazoline (Florida Center for Heterocyclic Compounds, Gainesville, FL, USA).

The following reference odorants were synthesized according to previous papers: 2-acetyl-1-pyrroline;[10] *trans*-4,5-epoxy-(*E*)-2-decenal;[11] 2-ethenyl-3-ethyl-5-methylpyrazine;[12] 3-methylnonane-2,4-dione;[13] (*Z*)-1,5-octadien-3-one;[14] and 2-propionyl-1-pyrroline.[15]

Isotopically Labeled Reference Compounds. The isotopically labeled standards, labeled witheither deuterium or carbon-13, were synthesized according to the literature cited: [$^2H_{2-5}$]-2-acetyl-1-pyrroline;[10] [$^2H_2$]-butanoic acid and [$^2H_{2-3}$]-δ-decalactone;[16] [$^2H_3$]-2,3-diethyl-5-methylpyrazine;[17] [$^2H_3$]- 3,5-dimethyl-2-ethylpyrazine and [$^2H_3$]- 2,5-dimethyl-3-ethylpyrazine;[17] [$^2H_2$]-γ-nonalactone and [$^2H_2$]-γ-dodecalactone;[18] [$^{13}C_2$]-3-hydroxy-4,5-dimethylfuran-2(5*H*)-one;[19] [$^{13}C_2$]-4-hydroxy-2,5-dimethylfuran-3(2*H*)-one;[20] [$^2H_3$]-3-(methylthio)propanal;[21] [$^2H_2$]-3-methylbutanal;[22] [$^2H_2$]-3-methylbutanoic acid;[23] [$^2H_3$]-3-methylnonane-2,4-dione;[24] [$^2H_4$]-1-octen-3-ol;[25] [$^2H_{2-4}$]-1-octen-3-one;[26] [$^{13}C_2$]-phenylacetaldehyde;[27] [$^2H_{2-4}$]-2-propionyl-1-pyrroline;[28] and [$^2H_2$]-γ-undecalactone.[18] [$^2H_3$]-Acetic acid and [$^{13}C_2$]-phenylacetic acid were obtained from Sigma-Aldrich.

Determination of the Concentrations of Isotopically Labeled Compounds. To determine the concentrations of the labeled compounds that were synthesized in a microscale range, mixtures of the compound with methyl octanoate as internal standard were determined by means of a Thermoquest Trace 2000 gas chromatograph with FID (Egelsbach, Germany). The concentration of the labeled compound was calculated from the ratio of peak areas and using a response factor obtained by analyzing different mixtures of the respective unlabeled compound with methyl octanoate.

Isolation of the Volatiles. Mushrooms were freed from soil with a brush and, after cropping, the material was cut into slices of ∼0.6 cm. To prepare pan-fried mushrooms, these slices were roasted in a pan at 140 °C for 6 min with permanent turnover. After cooling, the material was frozen in liquid nitrogen and then ground by means of a commercial blender upon addition of anhydrous sodium sulfate. For volatile isolation, either raw or pan-fried mushrooms (50 g wet weight) were ground in liquid nitrogen using a commercial blender, then mixed with anhydrous sodium sulfate, and twice extracted with methylene chloride (150 mL each) by vigorous stirring for 90 min. The mixture was filtered, and the residue was washed with methylene chloride (30 mL). The volatiles were isolated from the combined organic phases using the solvent-assisted flavor evaporation (SAFE) technique.[29] The distillate obtained was dried with anhydrous Na_2SO_4 and finally concentrated at 42 °C to 500 μL using a Vigreux column (50 cm × 1 cm i.d.) and a microdistillation apparatus.[30]

Fractionation of the Volatiles. For the identification experiments, a distillate from either raw or pan-fried mushrooms (500 g wet weight) was obtained as described above. To separate the acidic from the neutral and basic volatiles, the SAFE distillate was treated with aqueous sodium carbonate (0.5 mol/L, total volume = 300 mL) to obtain the neutral−basic fraction (NBF). The combined aqueous layers were then adjusted to pH 2 with hydrochloric acid, and the acidic volatiles (acidic fraction, AF) were extracted with methylene chloride (total volume = 300 mL). Both fractions were dried over anhydrous sodium sulfate, filtered, and concentrated to 500 μL.

For compound identification, the NBF was further fractionated by column chromatography on silica gel:[15] The volatiles, dissolved in ∼500 μL of *n*-pentane, were applied on a water-cooled glass column (30 cm × 1.8 cm) filled with a slurry of 30 g of purified silica 60 (7% water) in *n*-pentane and separated into six fractions using *n*-pentane/diethyl ether mixtures of increasing polarity (I, 100:0, 75 mL; II, 95:5, 80 mL; III, 90:10, 80 mL; IV, 75:25, 80 mL; V, 50:50, 80 mL; VI, 0:100, 110 mL) plus a seventh fraction using methylene chloride (100 mL). Each fraction was dried over anhydrous sodium sulfate, concentrated to 200 μL as described above, and analyzed by HRGC-O and HRGC-MS.

High-Resolution Gas Chromatography−Olfactometry (HRGC-O). This was performed using a Thermo Electron Trace GC Ultra gas chromatograph (Dreieich, Germany) and the following fused silica capillaries: DB-FFAP and DB-5 (both 30 m × 0.32 mm i.d.; 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA; Agilent Technologies, Santa Clara, CA, USA). The samples (1.0 μL) were applied by the cold on-column technique at 40 °C using helium as the carrier gas (flow rate = 1.7 mL/min). The oven temperature was programmed from 40 °C, held for 2 min, at 6 °C/min to 230 °C for

Table 1. Isotopically Labeled Standards, Selected Ions, and Accuracy of Calibration Lines Used for the Stable Isotope Dilution Assays

odorant	ion (m/z)	labeled standard	ion (m/z)	R^{2a}	C^b
acetic acid	61	[$^2H_3$]-acetic acid	64	0.9999	3
2-acetyl-1-pyrroline	112	[$^2H_{2-5}$]-2-acetyl-1-pyrroline	114−117[c]	0.9994	3
butanoic acid	89	[$^2H_2$]-butanoic acid	91	0.9999	3
δ-decalactone	171	[$^2H_{2-3}$]-δ-decalactone	173−174[c]	1	3
2,3-diethyl-5-methylpyrazine	151	[$^2H_3$]-2,3-diethyl-5-methylpyrazine	154	1	3
γ-dodecalactone	199	[$^2H_2$]-γ-dodecalactone	201	0.9999	3
(*Z*)-6-dodecene-γ-lactone	197	[$^2H_2$]-γ-dodecalactone	201	0.9998	3
3,5-dimethyl-2-ethylpyrazine	137	[$^2H_3$]-3,5-dimethyl-2-ethylpyrazine	140	1	4
2,5-dimethyl-3-ethylpyrazine	137	[$^2H_3$]-2,5-dimethyl-3-ethylpyrazine	140	0.9999	4
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	129	[$^{13}C_2$]-3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	131	1	4
4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	129	[$^{13}C_2$]-4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	131	0.9980	3
3-methylbutanal	87	[$^2H_2$]-3-methylbutanal	89	1	3
2- and 3-methylbutanoic acid	103	[$^2H_2$]-3-methylbutanoic acid	105	0.9998	3
3-methylnonane-2,4-dione	171	[$^2H_3$]-3-methylnonane-2,4-dione	174	0.9992	3
2-methylpropanoic acid	89	[$^2H_2$]-butanoic acid	91	0.9967	3
3-(methylthio)propanal	105	[$^2H_3$]-3-(methylthio)propanal	108	0.9996	3
γ-nonalactone	157	[$^2H_2$]-γ-nonalactone	159	0.9995	3
δ-nonalactone	157[d]	[$^2H_{2-3}$]-δ-decalactone	173−174[c]	1	3
1-octen-3-ol	111	[$^2H_4$]-1-octen-3-ol	115	0.9999	4
1-octen-3-one	127	[$^2H_{2-4}$]-1-octen-3-one	129−131[c]	1	3
phenylacetaldehyde	121	[$^{13}C_2$]-phenylacetaldehyde	123	0.9999	3
phenylacetic acid	137	[$^{13}C_2$]-phenylacetic acid	139	0.9999	4
2-propionyl-1-pyrroline	126	[$^2H_{3-4}$]-2-propionyl-1-pyrroline	129−130[c]	0.9907	5
γ-undecalactone	185	[$^2H_2$]-γ-undecalactone	187	0.9993	3

[a]Coefficient describing the accuracy of the calibration line. [b]Number of calibration points. [c]Internal standard was used as a mixture of isotopologues. [d]Quantitation was done using labeled δ-decalactone.

the DB-FFAP column or to 240 °C for the DB-5 column and held at the final temperature for 5 min. The flow of the carrier gas was split 1:1 at the end of the capillary column into an FID (250 °C) and a heated sniffing port (190 °C) using deactivated fused silica capillaries of the same length and a Y-shaped glass effluent splitter.

Aroma Extract Dilution Analysis. For the determination of FD factors, first, the original distillate was subjected to GC-O on the FFAP column to detect and evaluate the odors of all aroma-active areas. Then, the distillate was diluted stepwise 1:1 with solvent, and each dilution was analyzed in 1.0 μL aliquots by HRGC-O. To avoid overlooking odor-active compounds, the concentrated distillate was sensorially analyzed by at least three experienced panelists.

High-Resolution Gas Chromatography−Mass Spectrometry (HRGC-MS). For compound identification, mass spectra were acquired using a Hewlett-Packard gas chromatograph 5890 series II (Waldbronn, Germany) coupled to a Finnigan MAT 95 S sector field mass spectrometer (Bremen, Germany). Mass spectra in the electron impact (MS-EI) mode were generated at 70 eV, and chemical ionization (MS-CI) was performed at 115 eV using isobutane as the reactant gas.

Quantitation of volatiles by stable isotope dilution assays (SIDA) present in the higher micrograms per kilogram range was performed using a Varian CP 3800 gas chromatograph and a Varian Saturn 2000 ion trap mass spectrometer (Darmstadt, Germany). Two microliters of the solutions was injected on the DB-FFAP capillary column (30 m × 0.32 mm i.d.; 0.25 μm film thickness, J&W Scientific) by the cold on-column technique, and mass spectra of the separated compounds were recorded in the MS-CI mode using methanol as reagent gas (Table 1).

Two-Dimensional High-Resolution Gas Chromatography−Mass Spectrometry (TD-HRGC-MS). For compounds present in lower concentrations, a TD-HRGC-MS consisting of a Thermo Scientific Trace GC 2000 series (Dreieich, Germany) coupled to a Varian CP 3800 gas chromatograph and a Varian Saturn 2000 ion trap mass spectrometer was used.

After cold on-column injection, separation of the distillate in the first dimension was achieved on the DB-FFAP capillary column (30 m × 0.32 mm i.d.; 0.25 μm film thickness, J&W Scientific). The elution range containing the selected odorant and the internal standard was then transferred onto a cold trap by a moving capillary stream switching system (MCSS). After complete trapping, the analyte and the standard were transferred onto the second column, a DB-1701 (30 m × 0.25 mm i.d.; 0.25 μm film thickness, J&W Scientific) by heating the trap to 200 °C. Mass spectra were recorded in the CI mode using methanol as reagent gas (Table 1).

Calibration Curves. Mixtures of the respective labeled and unlabeled compound were prepared in five different mass ratios (1:5, 1:3, 1:1, 3:1, and 5:1) and analyzed by HRGC-MS to calculate the response factor (RF) for each component from the peak areas of the mass fragments (Table 1).

Quantitation of Odorants by Stable Isotope Dilution Assays. The labeled internal standards (0.1−70 μg), dissolved in methylene chloride, were added to the mushroom material, suspended in methylene chloride containing the respective target compounds in similar concentrations as determined in preliminary experiments. After 3 h of stirring, the mixture was filtered, and the volatile fraction was isolated by SAFE distillation.[29] The distillate was concentrated to 100−500 μL, depending on the amount of standard added, using the Vigreux column and the microdistillation apparatus described above.

Quantitation of acetic acid, butanoic acid, 2- and 3-methylbutanoic acid, and 2-methylpropanoic acid, which were present in the distillate in relatively high concentrations, was performed using one-dimensional HRGC-MS as described above. The remaining compounds were quantitated by TD-HRGC-MS.

Quantitation of 2- and 3-Methylbutanoic Acid. Both isomers could not be separated by GC-MS and, thus, the sum of both acids was first determined by SIDA. Then, to differentiate the respective amounts, the ratio of 2- and 3-methylbutanoic acid in the samples was determined by HRGC-MS by calculating the intensities of the fragments m/z 60 (3-methylbutanoic acid) and m/z 74 (2-methylbutanoic acid). For method standardization, defined mixtures of 2- and 3-methylbutanoic acid were analyzed under the same conditions and a calibration line was drawn plotting the intensity ratio

of m/z 60 over the sum of m/z 60 + m/z 74 against the percentage of 3-methylbutanoic acid in the mixture.[31]

Determination of Orthonasal Odor Thresholds. Orthonasal odor thresholds were determined using the triangular test with decreasing concentrations of aqueous odorant solutions against water as the control. Odorless Teflon vessels filled either with 20 mL of water or with the respective aqueous odorant solution were presented to a panel of 15−20 trained assessors, who were asked to identify the different sample in each row and describe the odor quality. Calculation of odor thresholds was performed as described before.[32]

Aroma Profile Analysis. Aroma profiles were determined by a trained panel consisting of 18−23 panelists, who participated in weekly sensory sessions to train their ability to recognize and describe different aroma qualities. The following reference compounds were used to define the aroma descriptors: 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (caramel), 2,3-diethyl-5-methylpyrazine (earthy), 3-methylbutanal (malty), 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (seasoning), 1-octen-3-one (mushroom), γ-nonalactone (coconut), 3-(methylthio)propanal (cooked potato), phenylacetaldehyde (flowery, honey), 2-acetyl-1-pyrroline (popcorn; roasty), and 3-methylbutanoic acid (sweaty). For aroma profile analysis, the intensities of the respective aroma qualities were ranked on a seven-point scale from 0 (not perceivable) over 0.5, 1.0, 1.5, ..., to 3.0 (strongly perceivable). The judgments of the panelists were averaged. Samples (20 g) were presented in Teflon vessels at room temperature.

Aroma Recombination Experiments. An aqueous aroma model was prepared using all quantitated aroma compounds with OAVs >1 in their actual concentrations as determined in the pan-fried mushrooms. The recombinate and the pan-fried mushrooms were each placed in closed glass vessels (20 g each) and presented to the sensory panel at room temperature. The overall aroma was evaluated on the basis of the same scale as used for aroma profile analysis. All evaluations were performed in triplicates.

RESULTS AND DISCUSSION

Identification of Odor-Active Compounds in Pan-fried Mushrooms. The volatile fraction of pan-fried white mushrooms was isolated immediately after frying by solvent extraction followed by SAFE distillation.[29] When the distillate was sniffed from a strip of filter paper, the typical roasty, earthy, caramel-like, and mushroom-like aroma of the pan-fried mushrooms could clearly be sensed by all 15 members of the sensory panel. To characterize the compounds responsible for the aroma impression, first, GC-O was applied, and a total of >50 odor-active regions could be detected in the original distillate. Sniffing of serial dilutions in the AEDA indicated compound **36**, exhibiting a caramel-like odor, with the highest FD factor of 8192, as well as compounds **10** and **42** showing a popcorn-like or seasoning-like odor quality, respectively, as the most intense aroma compounds among the entire set of 40 odorants showing FD factors between 8 and 8192 (Figure 1 and Table 2). A comparison of the odor qualities and retention indices with data in an in-house database suggested structures for the compounds detected in the FD chromatogram. Separation of the volatiles by column chromatography on silica gel followed by GC-O of the respective fraction to locate a target odorant finally afforded pure mass spectra of the respective compounds. Thus, compound **36** was identified as 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (caramel-like) (Figure 2), compound **10** as 2-propionyl-1-pyrroline (popcorn-like), and compound **42** as 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (seasoning-like). As a further odorant with a popcorn-like scent and a high FD factor 2-acetyl-1-pyrroline (**6**) was identified. In addition, δ-nonalactone (**37**, coconut-like), 2,3-diethyl-5-methylpyrazine (**17**, earthy), and 2- and 3-methylbutanoic acid (**26**, sweaty) are suggested as key contributors to the overall aroma (Table 2). However, surprisingly, the typical mushroom-like smelling compound 1-octen-3-one (**5**) was detected with only a low FD factor of 32 (Table 2), and 1-octen-3-ol was sensorially not detectable.

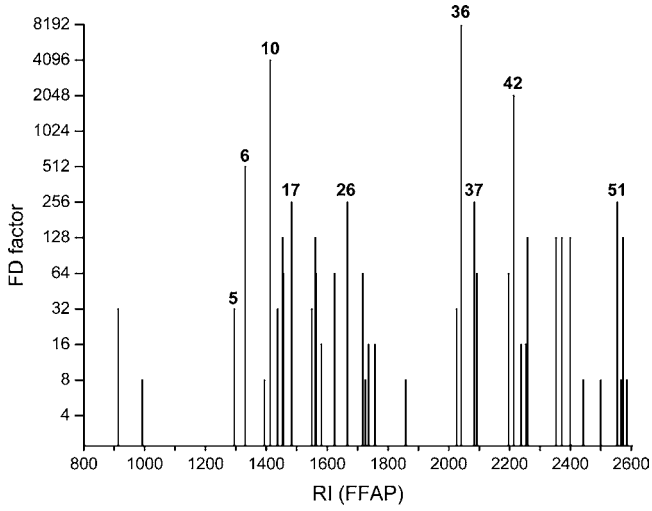

Figure 1. Flavor dilution (FD) chromatogram of the volatile fraction isolated from pan-fried mushrooms.

Quantitation of Selected Odorants in Pan-Fried Mushrooms. Dilution to odor threshold techniques like the AEDA are useful tools to screen the main aroma compounds in a complex set of food volatiles; however, taking into account the influence of the food matrix that is, the aroma release, these results should always be confirmed by application of the OAV concept,[30] that is, a quantitation of odor-active compounds and calculation of odor activity values followed by sensory evaluations. Therefore, 25 compounds were selected for quantitation by SIDA, because 23 of them had shown high FD factors during the AEDA (Table 2). Two additional compounds were included in the quantitative experiments for the following reasons: phenylacetaldehyde was chosen because it had been reported before as an odorant in cooked and dried mushrooms, respectively, and 1-octen-3-ol was included because, together with 1-octen-3-one, it is commonly regarded as a contributor to the typical aroma of mushrooms.[5,34]

The highest concentrations were measured for acetic acid (8.8 mg/kg) and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (1.2 mg/kg), followed by 3-methylbutanal, phenylacetic acid, 3-methylbutanoic acid, 2-methylpropanoic acid, 3-(methylthio)-propanal, and phenylacetaldehyde, which were present in concentrations above 100 μg/kg (Table 3). On the other hand, concentrations below 1 μg/kg were found for 2,3-diethyl-5-methylpyrazine, γ-undecalactone, 3-methylnonane-2,4-dione, 2-propionyl-1-pyrroline, δ-dedalactone, and δ-nonalactone.

Calculation of Odor Activity Values and Sensory Studies. Next, OAVs were calculated (ratio of concentration to odor threshold). The odor thresholds in water were either newly determined in this study or were taken from the literature.[32] The results revealed (Table 4) that among the 25 quantitated odorants, only 13 were present above their odor threshold. Among them, 3-methylbutanal showed the highest OAV of 885, followed by 3-(methylthio)propanal and 2-acetyl-1-pyrroline. Relatively high OAVs were also calculated for 1-octen-3-one, 2-propionyl-1-pyrroline, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, phenylacetaldehyde, 2,3-diethyl-5-methylpyrazine, and 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one. The data

dx.doi.org/10.1021/jf4006752 | J. Agric. Food Chem. 2013, 61, 3804−3813

Table 2. Most Odor-Active Compounds (FD ≥ 8) Identified in Pan-Fried Mushrooms (*Agaricus bisporus*)

no.	odorant[a]	odor quality[b]	retention index on FFAP	retention index on DB-5	FD[c]	previously identified[d]
2	3-methylbutanal	malty	913	661	32	7
3	2,3-butanedione	buttery	993	605	8	
5	1-octen-3-one	mushroom-like	1295	977	32	6, 7, 8
6	2-acetyl-1-pyrroline	popcorn-like	1331	916	512	
9	2,3,5-trimethylpyrazine	earthy	1394	1002	8	8
10	2-propionyl-1-pyrroline	popcorn-like	1413	1027	4096	
12	2,5-dimethyl-3-ethylpyrazine	earthy	1437	1079	32	
14	2,6-dimethyl-3-ethylpyrazine	earthy	1453	1084	128	
15	acetic acid	sour	1454	619	32	
16	3-(methylthio)propanal	cooked potato	1456	912	64	8
17	2,3-diethyl-5-methylpyrazine	earthy	1484	1156	256	
19	unknown	earthy	1550	nd[g]	32	
20	unknown	roasty, malty	1561	nd	128	
21	2-methylpropanoic acid	sweaty	1563	784	64	
22	2-ethenyl-3-ethyl-5-methylpyrazine[e]	earthy	1581	1174	16	
24	butanoic acid	sweaty	1624	806	64	
26	2- and 3-methylbutanoic acid	sweaty	1667	nd	256	
28	3-methylnonane-2,4-dione	hay-like, fishy	1717	1244	64	
29	unknown	earthy	1726	nd	8	
30	pentanoic acid	sweaty, fruity	1736	888	16	
31	2-acetyl-2-thiazoline	roasty	1758	1102	16	
33	2,3-dihydro-5-hydroxy-6-methyl-4*H*-pyran-4-one[f]	caramel-like	1859	nd	8	
35	γ-nonalactone	coconut-like	2026	1369	32	
36	4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	caramel-like	2041	1073	8192	
37	δ-nonalactone	coconut-like	2084	1397	256	
38	4-methylquinazoline	minty, rubber-like	2093	1340	64	
41	δ-decalactone	coconut-like	2197	1502	64	
42	3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	seasoning-like	2214	1113	2048	
43	(*R*)-δ-decenolactone	peach-like	2238	1483	16	
44	unknown	green, seasoning-like	2253	nd	16	
45	γ-undecalactone	peach-like	2259	1580	128	
46	unknown	herbaceous, flowery	2353	1480	128	
47	γ-dodecalactone	peach-like	2372	1687	128	
48	(*Z*)-6-dodecen-γ-lactone	peach-like	2399	1664	128	
49	δ-dodecalactone	peach-like, coconut-like	2442	1717	8	
50	3-methylindole	fecal	2500	1388	8	
51	unknown	fruity, coconut-like	2554	nd	256	
52	unknown	coconut-like	2566	nd	8	
53	phenylacetic acid	honey-like	2573	1270	128	
54	4-hydroxy-3-methoxybenzaldehyde	vanilla-like	2586	1404	8	

[a]The compounds were identified by comparison of its odor quality and intensity, the retention index on capillaries FFAP and DB-5, and mass spectra (MS-EI, MS-CI) with data of reference compounds. [b]Odor quality perceived at the GC sniffing port. [c]Flavor dilution factor determined by AEDA on capillary FFAP. [d]Previously identified as volatile in processed (heated and/or dried) white mushrooms. [e]No unequivocal mass spectra could be obtained. Identification is based on the remaining criteria given in footnote *a*. [f]Identification was based on data given in ref 33. [g]nd, not determined.

indicated that, as found for various other foods, no single aroma compound is likely to represent the typical aroma of pan-fried mushrooms. In the absence of such a "character impact odorant", usually a set of several key odorants is responsible for the overall aroma impression and, thus, aroma reconstitution experiments are a useful tool to evaluate the odor contribution of single compounds to the overall aroma of a given food.[9,30] A model mixture containing the 13 odorants with OAVs ≥1 in their actual concentrations (Table 4) was, thus, prepared in aqueous solution, and the odor attributes of the model mixture and freshly prepared pan-fried mushrooms were then judged in a comparative descriptive aroma profile test using 10 aroma descriptors: caramel, earthy, malty, seasoning, mushroom, coconut-like, cooked potato, flowery/honey-like, popcorn-like, and sweaty. The results of the aroma profile analyses are presented in Figure 3. Overall, the sensory panel agreed that the recombinate elicited the typical aroma of pan-fried mushrooms, whereas the odor qualities earthy and popcorn were rated slightly lower than in the mushrooms. The opposite was true for the odor qualities caramel, seasoning, coconut, flowery/honey, and sweaty, whereas the odor descriptors malty, mushroom, and cooked potato were rated similarly in both the recombinate and the fried mushrooms. Furthermore, the overall similarity of the model mixture and the original mushrooms was judged to be 2.4 of 3.0, thus corroborating the data obtained and showing that the overall aroma of pan-

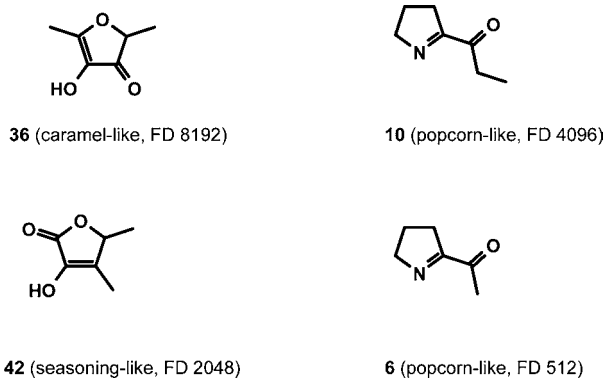

Figure 2. Structures of odorants in pan-fried mushrooms showing the highest FD factors (numbering refers to Table 2).

Table 3. Concentrations of 25 Key Aroma Compounds in Pan-Fried Mushrooms (*Agaricus bisporus*)

odorant	concn (μg/kg fresh wt)	range (μg/kg)	n^a
acetic acid	8800	8400−9200	2
4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	1200	1200−1200	2
3-methylbutanal	440	410−470	2
phenylacetic acid	260	240−270	2
3-methylbutanoic acid	200	180−220	4
2-methylpropanoic acid	180	180−180	2
3-(methylthio)propanal	170	170−170	2
phenylacetaldehyde	140	140−140	2
2-methylbutanoic acid	65	59−72	4
butanoic acid	47	39−55	4
(*Z*)-6-dodeceno-γ-lactone	6.5	6.3−6.8	2
2,5-dimethyl-3-ethylpyrazine	6.4	6.0−7.3	4
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	5.7	5.5−5.8	2
2-acetyl-1-pyrroline	5.3	4.2−7.0	4
1-octen-3-ol	5.0	3.1−6.0	3
1-octen-3-one	1.1	1.0−1.3	4
2,6-dimethyl-3-ethylpyrazine	0.6	0.5−0.6	2
γ-nonalactone	0.6	0.5−0.6	2
γ-dodecalactone	0.5	0.4−0.6	3
2,3-diethyl-5-methylpyrazine	0.4	0.4−0.4	2
γ-undecalactone	0.3	0.2−0.4	2
3-methylnonane-2,4-dione	0.3	0.3−0.3	2
2-propionyl-1-pyrroline	0.2	0.2−0.4	4
δ-decalactone	0.2	0.1−0.2	4
δ-nonalactone	0.1	0.1−0.1	2

[a]Number of replicates.

Table 4. Orthonasal Odor Thresholds and Odor Activity Values (OAVs) of Key Odorants in Pan-Fried Mushrooms

odorant	odor threshold in water (μg/L)	OAV[a]
3-methylbutanal	0.50[b]	885
3-(methylthio)propanal	0.43[b]	399
2-acetyl-1-pyrroline	0.053[b]	100
1-octen-3-one	0.016[b]	68
2-propionyl-1-pyrroline	0.0067[c]	36
4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	40[b]	31
phenylacetaldehyde	5.2[c]	27
2,3-diethyl-5-methylpyrazine	0.031[b]	14
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	0.49[b]	12
3-methylnonan-2,4-dione	0.046[c]	6
2,5-dimethyl-3-ethylpyrazine	0.28[c]	2
γ-dodecalactone	0.43[b]	1
(*Z*)-6-dodeceno-γ-lactone	5.4[b]	1
3-methylbutanoic acid	490[b]	<1
2,6-dimethyl-3-ethylpyrazine	25[c]	<1
γ-undecalactone	2.1[b]	<1
acetic acid	99000[b]	<1
γ-nonalactone	9.7[b]	<1
phenylacetic acid	6100[b]	<1
2-methylbutanoic acid	2200[b]	<1
butanoic acid	2400[b]	<1
2-methylpropanoic acid	16000[b]	<1
δ-nonalactone	11[c]	<1
δ-decalactone	31[b]	<1
1-octen-3-ol	45[c]	<1

[a]Odor activity value (ratio of concentration to odor threshold). [b]Detection threshold in water.[3] [c]Detection threshold in water newly determined in this study.

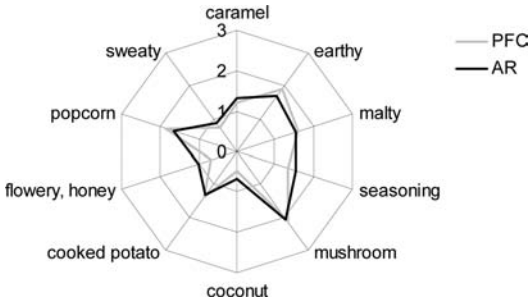

Figure 3. Comparative aroma profiles of pan-fried mushrooms (PFC; gray) and the aroma recombinate (AR; black).

fried mushrooms can closely be mimicked by mixing the following 13 odorants: 3-methylbutanal, 3-(methylthio)-propanal, 2-acetyl-1-pyrroline, 1-octen-3-one, 2-propionyl-1-pyrroline, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, phenylacetaldehyde, 2,3-diethyl-5-methylpyrazine, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, 3-methylnonane-2,4-dione, 2-ethyl-3,5-dimethylpyrazine, γ-dodecalactone and (*Z*)-6-dodeceno-γ-lactone, in their actual concentrations present in the pan-fried mushrooms.

The malty smelling 3-methylbutanal has already been described as a constituent of cooked dried white mushrooms by MacLeod and Panchasara,[7] and Misharina et al.[8] have reported 3-(methylthio)propanal as a volatile component of dried mushrooms. Also, 1-octen-3-one has been detected in various heat-treated mushrooms before.[6−8] An investigation of the impact of these three compounds on the aroma of heat-treated mushrooms, however, has not yet been performed.

The popcorn-like smelling aroma compounds 2-acetyl-1-pyrroline and 2-propionyl-1-pyrroline are reported as aroma compounds of heat-treated mushrooms for the first time in this study. The same is true for 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one as well as for 2,3-diethyl-5-methylpyrazine and 3,5-dimethyl-2-ethylpyrazine. All six compounds are known to be formed in carbohydrate/amino acid degradation reactions and, thus, were characterized often as key odorants in other processed foods. However, the ratio of concentrations is different in foods such

as popcorn,[15] roasted peanuts, or roasted hazelnuts and, thus, a different overall aroma is generated from the respective mixtures.

Identification of Odor-Active Compounds in Raw Mushrooms. To investigate the influence of the heat treatment on the formation of odor-active compounds, the volatiles of raw white mushrooms from the batch were characterized for comparison.

Application of the AEDA revealed 35 odorants in the FD factor range of 4−4096 (Figure 4 and Table 5). The highest FD

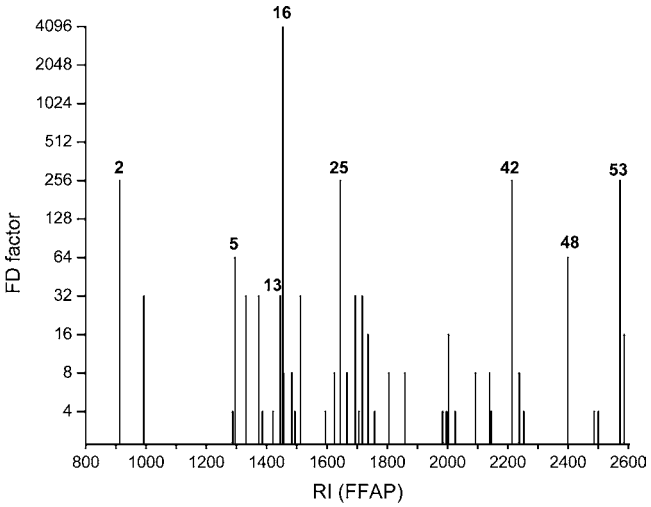

Figure 4. Flavor dilution chromatogram (FD) of the volatile fraction isolated from raw mushrooms.

factor of 4096 was found for **16** (cooked potato-like), followed by **2** (malty), **25** (flowery, honey-like), **42** (seasoning-like), and **53** (honey-like). The identification experiments revealed methional (**16**), 3-methylbutanal (**2**), and phenylacetaldehyde (**25**) followed by 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**42**), phenylacetic acid (**53**), 1-octen-3-one (**5**), and (*Z*)-6-γ-dodecenolactone (**48**) as key odorants in the raw mushrooms (Figure 5). Besides 1-octen-3-one, the other typical mushroom-like smelling compound 1-octen-3-ol (**13**) reached an FD factor of only 32, along with 2,3-butanedione (**3**, buttery), 2-acetyl-1-pyrroline (**6**, popcorn-like), (*Z*)-1,5-octadien-3-one (**7**, geranium-like, metallic), 2-isobutyl-3-methoxypyrazine (**18**, earthy, green bell pepper), (*E*,*E*)-2,4-nonadienal (**27**, fatty, green), and 3-methylnonane-2,4-dione (**28**, hay-like, fishy) (Table 5).

Quantitation of Selected Odorants in Raw Mushrooms. Following the procedure already applied in the quantitation of odor-active compounds in the pan-fried mushrooms, 23 odorants were selected for quantitation using SIDAs. The highest concentrations were found for acetic acid (14 mg/kg), 1-octen-3-ol (6.2 mg/kg), phenylacetaldehyde (5.4 mg/kg), and 3-methylbutanal (2.5 mg/kg) (Table 6). Further compounds with concentrations >50 μg/kg were 2-methylpropanoic acid, 3-(methylthio)propanal, phenylacetic aicd, and 1-octen-3-one. On the other hand, low concentrations were found for 3,5-dimethyl-2-ethylpyrazine, 2,5-dimethyl-3-ethylpyrazine, and 2,3-diethyl-5-methylpyrazine. Calculation of OAVs revealed the highest OAV of 5000 for the malty smelling 3-methylbutanal, followed by the typical mushroom-like smelling 1-octen-3-one and phenylacetaldehyde exhibiting a flowery, honey-like odor note (Table 6). With OAVs > 100, 3-(methylthio)propanal, 1-octen-3-ol, and 3-methylnonane-2,4-dione are also likely to contribute to the overall aroma of raw mushrooms. Interestingly, only 10 of the 23 odorants investigated were found with concentrations exceeding their odor thresholds.

The high OAV found for the typical mushroom-like smelling 1-octen-3-one (**5**; Table 5) in the raw mushrooms is well in line with data reported by Fischer and Grosch,[5] who had determined the highest FD factor for the ketone and had suggested a main role of 1-octen-3-one in the aroma of raw mushrooms. Also, the lower aroma impact of the corresponding 1-octen-3-ol[5] was corroborated by our data. Despite the comparably high concentration of the alcohol in raw mushrooms, because of its high odor threshold in water, only a lower OAV was calculated (Table 6). In this study, an odor threshold of 45 μg/L of water was newly determined, which seems rather high as compared to threshold values reported in former studies, for example, 0.1 μg/L[2] or even 0.01 μg/L.[36] However, commercially available solutions of 1-octen-3-ol are likely to contain small amounts of 1-octen-3-one, thus simulating a lower odor threshold for the alcohol because of the clearly lower odor threshold of the ketone (0.016 μg/L).[32] In our study, the purity of 1-octen-3-ol was controlled by GC-O analysis.

A comparison of the quantitative results obtained for the raw mushrooms with those obtained for the pan-fried mushrooms showed that 1-octen-3-ol and 1-octen-3-one were found in clearly higher concentrations in the raw mushrooms (by factors of 1000 and 75, respectively). Because both odorants are known to be formed after disruption of the mushroom tissue by an enzymatic degradation of linoleic acid,[37,38] the results obtained for the raw mushrooms are well in-line with the literature. However, the much lower amounts in the pan-fried material suggested either a loss by distillation, a degradation of both compounds during pan-frying, or an inhibition of a possible enzymatic formation by the heat treatment (Table 7).

On the other hand, several odorants were present in clearly higher amounts in the pan-fried mushrooms than in the raw mushrooms. This was the case for the roasty, popcorn-like smelling compound 2-acetyl-1-pyrroline, which has previously been identified as a degradation product of proline in the presence of reducing carbohydrates.[39] This odorant was found in a 10-fold higher concentration in the pan-fried mushrooms. The also roasty, popcorn-like smelling homologue 2-propionyl-1-pyrroline (**10**), known to be formed via similar pathways,[39] was detectable only in the pan-fried mushrooms, but not in the raw mushrooms. Thus, an increase in its amount due to heat treatment is likely, because a high FD factor of 4096 was found for **10** in the pan-fried mushrooms.

Furthermore, all pyrazines were present in higher concentrations in the pan-fried mushrooms. However, because only a short heat treatment was applied, the increase in pyrazine concentrations was rather low.

By contrast, phenylacetaldehyde, 3-methylbutanal, and 3-(methylthio)propanal were substantially higher in the raw mushrooms compared to the pan-fried mushrooms. Because the formation of these aldehydes by a thermally induced Strecker degradation of their parent amino acids is well-known,[40] the data are surprising because the contrary would have been expected. Further studies on the time course of the formation of several odorants from raw mushroom tissue showed an increase of, in particular, the Strecker aldehydes (data not shown). Thus, because the raw mushrooms were minced before extraction, and an extraction time of ∼3 h at

Table 5. Key Aroma Compounds (FD ≥ 4) Identified in Raw Mushrooms (*Agaricus bisporus*)

no.	odorant[a]	odor quality[b]	retention index on FFAP	retention index on DB-5	FD[c]	previously identified[d]
1	2-methylpropanal[e]	malty	800	603	16	
2	3-methylbutanal	malty	913	661	256	2, 34
3	2,3-butanedione	buttery	993	605	32	
4	octanal	citrus-like, green	1287	1003	4	5
5	1-octen-3-one	mushroom-like	1295	977	64	2, 35
6	2-acetyl-1-pyrroline	popcorn-like	1331	916	32	
7	(*Z*)-1,5-octadien-3-one[e]	geranium-like, metallic	1373	984	32	
8	nonanal	citrus-like, soapy	1386	1106	4	
11	(*E*)-2-octenal	fatty	1421	1062	4	5, 34, 35
13	1-octen-3-ol	mushroom-like	1445	991	32	2, 5, 6, 34
15	acetic acid	vinegar-like	1454	619	8	34
16	3-(methylthio)propanal	cooked potato-like	1456	912	4096	
17	2,3-diethyl-5-methylpyrazine	earthy	1484	1156	8	
18	2-isobutyl-3-methoxypyrazine[e]	earthy, green bell pepper	1512	1178	32	
23	4-isopropyl-1-methyl-1-cyclohexen-4-ol	earthy, seasoning-like	1595	1179	4	
24	butanoic acid	sweaty	1624	806	8	34, 35
25	phenylacetaldehyde	flowery, honey	1644	1041	256	2, 6
26	2- and 3-methylbutanoic acid	sweaty	1667	nd[g]	8	34, 35
27	(*E*,*E*)-2,4-nonadienal	fatty, green	1695	1251	32	
28	3-methylnonane-2,4-dione	hay-like, fishy	1717	1244	32	
30	pentanoic acid	sweaty, fruity	1736	888	16	34, 35
31	2-acetyl-2-thiazoline[e]	roasty, popcorn-like	1758	1102	4	
32	(*E*,*E*)-2,4-decadienal[e]	fatty	1805	1317	8	
33	2,3-dihydro-5-hydroxy-6-methyl-4*H*-pyran-4-one[f]	caramel-like	1859	nd	8	
34	*trans*-4,5-epoxy-(*E*)-2-decenal	metallic	2003	1379	16	
35	γ-nonalactone	coconut-like	2026	1369	4	
38	4-methylquinazoline[e]	minty, rubber-like	2093	1340	8	
39	unknown	seasoning-like	2139	nd	8	
40	γ-decalactone	peach-like	2146	1470	4	
42	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	seasoning-like	2214	1113	256	
43	(*R*)-δ-decenolactone[e]	peach-like	2238	1483	8	
44	unknown	green, seasoning-like	2253	nd	4	
48	(*Z*)-6-dodeceno-γ-lactone	peach-like	2399	1664	64	
50	3-methylindole[e]	fecal	2500	1388	4	
53	phenylacetic acid	honey-like	2573	1270	256	
54	4-hydroxy-3-methoxybenzaldehyde	vanilla-like	2586	1404	16	

[a]The compound was identified by comparison of its odor quality and intensity, the retention indices on capillaries FFAP and DB-5, and mass spectra (MS-EI, MS-CI) with data of reference compounds. [b]Odor quality perceived at the GC sniffing port. [c]Flavor dilution factor determined by AEDA on capillary FFAP. [d]Previously identified as volatile in raw white mushrooms. [e]No unequivocal mass spectra was obtained. Identification is based on the remaining criteria given in footnote *a*. [f]As no reference compound was available, identification is based on the data given in ref 33. [g]nd, not determined.

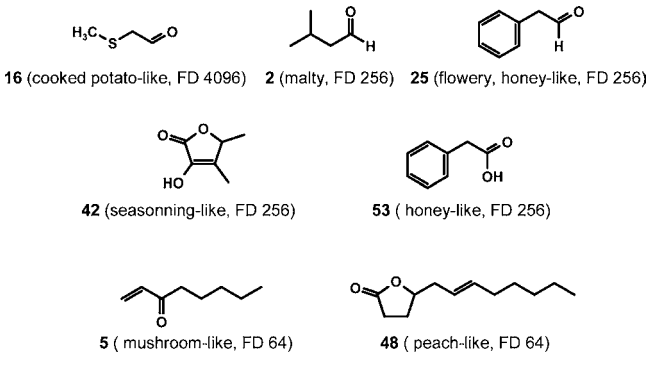

Figure 5. Structures of odorants in raw mushrooms showing the highest FD factors (numbering refers to Table 5).

room temperature was applied, it is possible that the aldehydes are formed during extraction by an enzymatic degradation of amino acids. However, although it is known that microorganisms, such as baker's yeast, are able to enzymatically degrade amino acids into the corresponding aldehydes by the so-called "Ehrlich" mechanism,[41] this reaction has not yet been described for mushroom tissue before. Furthermore, baker's yeast usually reduces the aldehydes into the corresponding alcohols, a reaction that was not observed here. In summary, the results clearly show that the character impact odorants of raw mushrooms, 1-octen-3-one and 1-octen-3-ol, do not much contribute to the aroma of mushrooms after a thermal processing. Because obviously enzymes present in the raw cut mushroom tissue are able to generate the malty, honey-like smelling odorants 3-methylbutanal and phenylacetaldehyde, the overall aroma present after mushroom processing can clearly be influenced by the time the raw, ground tissue is allowed to react.

dx.doi.org/10.1021/jf4006752 | J. Agric. Food Chem. 2013, 61, 3804−3813

Table 6. Concentrations and Odor Activity Values (OAVs) of 23 Aroma Compounds in Raw Mushrooms (*Agaricus bisporus*)

odorant	concn (μg/kg)	range (μg/kg)	n^a	OAV
acetic acid	14000	11000−17000	4	<1
1-octen-3-ol	6200	3500−8500	2	138
phenylacetaldehyde	5400	5200−5500	2	1038[b]
3-methylbutanal	2500	2300−2700	2	5000
2-methylpropanoic acid	440	430−450	2	<1
3-(methylthio)propanal	310	310−310	2	721
phenylacetic acid	140	110−170	2	<1
1-octen-3-one	74	57−91	2	4625
3-methylbutanoic acid	16	14−19	2	<1
butanoic acid	14	13−15	2	<1
(*Z*)-6-dodeceno-γ-lactone	7.8	2.5−18	5	1
2-methylbutanoic acid	7.0	6.0−8.1	2	<1
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	2.5	2.5−2.6	2	5
3-methylnonane-2,4-dione	1.0	0.82−1.1	2	21[b]
γ-nonalactone	0.9	0.72−1.1	4	<1
γ-dodecalactone	0.9	0.85−0.95	2	2
γ-undecalactone	0.4	0.29−0.46	2	<1
2-acetyl-1-pyrroline	0.4	0.11−0.64	2	7
δ-decalactone	0.1	0.11−0.13	2	<1
δ-nonalactone	0.09	0.088−0.095	2	<1[b]
2,6-dimethyl-3-ethylpyrazine	0.08	0.071−0.086	2	<1[b]
2,5-dimethyl-3-ethylpyrazine	0.05	0.050−0.059	2	<1[b]
2,3-diethyl-5-methylpyrazine	0.008	0.0073−0.0094	2	<1

[a]Number of batches analyzed. [b]The following odor thresholds (μg/kg water) were newly determined: phenylacetaldehyde (5.2), 1-octen-3-ol (45), 3-methyl-2,4-nonandione (0.046), 3,5-dimethyl-2-ethylpyrazine (0.28), 2,5-dimethyl-3-ethylpyrazine (25) and γ-nonalactone (11). Data were taken from ref 33.

Table 7. Comparison of Selected Aroma Compounds Known To Be Generated from Carbohydrate/Amino Acid Degradation Showing Differences in Their Concentrations between Raw and Pan-Fried White Mushrooms

	concn (μg/kg)	
odorant	raw	pan-fried
2-acetyl-1-pyrroline	0.4	5.3
2,5-dimethyl-3-ethylpyrazine	0.08	0.6
2,6-dimethyl-3-ethylpyrazine	0.05	6.4
2,3-diethyl-5-methylpyrazine	0.008	0.4
2- and 3-methylbutanal	2500	440
phenylacetaldehyde	5400	140
3-(methylthio)propanal	310	170

Comprehensive measurements to clarify the time course of the formation of odorants from raw mushroom tissue as well as labeling experiments are underway to prove the enzymatic formation of Strecker aldehydes.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Petra Steinhaus for careful preparation of the manuscript.

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