

## Characterization of Odorants Causing an Atypical Aroma in White Pepper Powder (*Piper nigrum* L.) Based on Quantitative Measurements and Orthonasal Breakthrough Thresholds

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Application of the aroma extract dilution analysis on an extract of white pepper powder showing an intense fecal, cowshed-like off-flavor revealed 3-methylindole (fecal, swine-manure) and 4-methylphenol (fecal, horse-like) with the highest flavor dilution (FD) factors among the 22 odor-active compounds detected. In addition, high FD factors and/or undesirable odor qualities suggested 3-methylphenol (phenolic), butanoic acid (cheese-like), and 2- and 3-methylbutanoic acid (cheese-like) as well as pentanoic acid and hexanoic acid (cheese-like odors) as contributors to the malodor. Although the intensities of the off-note were clearly different in 50 commercial samples of white pepper, quantitation of 3-methylindole and 3- and 4-methylphenol as well as of the five short-chain acids by means of stable isotope dilution assays showed similar concentrations in most of the samples. Storage of a freshly ground white pepper powder for up to 7 months revealed a significant decrease in the typical odor qualities of white pepper and an increase in the fecal odor note with storage time. Because the concentrations of the odorants mentioned above were not much changed during storage, possibly very volatile odorants, such as  $\alpha$ -pinene, which are able to mask the malodor, are lost during storage of, in particular, pepper powders. On the basis of odor activity values, which were calculated using breakthrough thresholds, in particular, 3-methylindole, 4-methylphenol, 3-methylphenol, and butanoic acid could be suggested as the main sources of the fecal off-flavor.

**KEYWORDS:** Pepper; aroma; off-flavor; aroma extract dilution analysis; skatole; 3-methylindole; 4-methylphenol; 3-methylphenol; stable isotope dilution analysis

### INTRODUCTION

Different types of pepper are used all over the world to evoke a hot taste in various foods. Besides its hot taste, the characteristic aroma is another important quality attribute of, in particular, white and black peppers.

Black and white peppers are produced from the fruits of the same plant (*Piper nigrum* L.). For black pepper manufacturing, the unripe fruits are harvested when fully grown, but still of green color. Sun-drying leads to a brownish-black color and the typical shriveled surface. White pepper is mainly produced from orange to red ripe fruits. By soaking the fruits in water, these undergo a retting process during which the skin and the outer parts of the mesocarp are softened and are then mechanically removed. The remaining pale-fawn or grayish core is sun-dried and assigned as white pepper.

The aroma of white pepper clearly differs from that of black pepper. However, sometimes a more or less intense, unpleasant aroma note is detectable, particularly in white pepper. This malodor is often described as cowshed- or fecal-like. Occasionally, also phenolic and cheesy odor notes are attributed to white

pepper off-flavor. Besides its effect on the quality of the white pepper itself, the fecal off-odor sometimes turned out to be a severe problem for food-producing companies, because it may be transferred into the final product.

Many investigations on the volatile constituents of pepper have been performed in the past, leading to the identification of 273 compounds so far (1). However, only a few authors have attempted to evaluate the sensory impact of individual white pepper volatiles (2–5). Nitz (2) applied an aroma extract dilution analysis (AEDA) on a distillate obtained from Indonesian white pepper by extraction with liquid carbon dioxide. Among the compounds showing high flavor dilution (FD) factors,  $\alpha$ -pinene (2,6,6-trimethylbicyclo[3.1.1]hept-2-ene), linalool (3,7-dimethyl-1,6-octadien-3-ol),  $\beta$ -damascenone [(2E)-1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one], eugenol (4-allyl-2-methoxyphenol), skatole (3-methylindole), *m*-cresol (3-methylphenol), guaiacol (2-methoxyphenol), and piperonal (1,3-benzodioxole-5-carbaldehyde) were identified, but the authors neither evaluated the contribution of the single compounds identified to the overall aroma of white pepper nor reported any contribution to an off-odor.

A quantitative study on aroma-active pepper constituents was recently performed on a sample of white pepper from Brazil

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eliciting an intense fecal-like off-note (3–5). On the basis of odor activity values (OAV; ratio of concentration to odor threshold) in starch, 3-methylindole was suggested as the major contributor to this off-flavor. Another odorant exhibiting a fecal odor quality was identified as 4-methylphenol (*p*-cresol).

However, although 3-methylindole and 4-methylphenol were also identified in a few further white pepper samples (3), it is still open (i) whether 3-methylindole and 4-methylphenol occur only occasionally in white peppers, (ii) whether their occurrence is related to a specific geographical origin, and (iii) whether 3-methylindole and 3- and 4-methylphenol are formed during storage of white pepper or are generated during the retting process.

The aim of the present study was, therefore, (i) to re-evaluate the key odorants in white pepper samples eliciting a pronounced off-odor, (ii) to quantify all odorants exhibiting an untypical odor quality in a representative number of commercial white pepper samples, and (iii) to monitor changes in these odorants during storage of powdered white pepper as well as during storage of entire peppercorns.

## MATERIALS AND METHODS

**Pepper Samples.** Samples of white pepper powders or whole corns (WP-01–25 and WP-34–49) were provided by German spice trading companies, members of the Fachverband der Gewürzindustrie e.V. (German Spice Association). Samples WP-26–33 were purchased at a local supermarket. Black pepper samples BP-1–8 were either obtained from German spice traders or purchased at a supermarket.

For the storage trial, freshly ground white pepper was stored in polyethylene bags in portions of 50 g at room temperature (20–22 °C) in the dark. After 0.5, 1, 2, 3, 5, and 7 months, the sample was frozen at –60 °C prior to the analysis. Fresh, undried pepper fruits were collected on a farm in Thailand.

**Chemicals.** The following chemicals were obtained from commercial sources (IUPAC names following): butanoic acid, 3-methyl-3-buten-1-ol, 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane), *p*-cymene (1-isopropyl-4-methylbenzene), 2-isobutyl-3-methoxypyrazine, (*R*)-limonene [(*R*)-4-isopropenyl-1-methylcyclohexene], (*S*)-limonene [(*S*)-4-isopropenyl-1-methylcyclohexene], (±)-linalool (3,7-dimethyl-1,6-octadien-3-ol), 2-methoxyphenol, (±)-2-methylbutanal, 3-methylbutanal, 3-methylphenol, 4-methylphenol, [<sup>2</sup>H<sub>8</sub>]-4-methylphenol, methylpropanal, 2-methylbutanoic acid, 3-methylbutanoic acid, 3-methylindole, myrcene (7-methyl-3-methylenoocta-1,6-diene), (–)- $\alpha$ -pinene [(1*S*,5*S*)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene], (–)- $\beta$ -pinene [(1*S*,5*S*)-6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane], piperonal (1,3-benzodioxole-5-carbaldehyde), and tris(triphenylphosphine)rhodium(I) chloride were from Aldrich, Sigma-Aldrich Chemie, Taufkirchen, Germany. Hexanoic acid, (*R*)-linalool [(*R*)-3,7-dimethyl-1,6-octadien-3-ol], and pentanoic acid were from Fluka, Sigma-Aldrich Chemie, Taufkirchen, Germany. Dichloromethane, diethyl ether, ethanol (Lichrosolv), eugenol (4-allyl-2-methoxyphenol), methanol (Lichrosolv), pentane, potassium permanganate, sodium sulfate, silica gel 60 (0.040–0.63 mm; mesh 230–400), and toluene (Lichrosolv) were from Merck, Darmstadt, Germany. Deuterium gas (99.7% purity) was from Messer-Griesheim, Krefeld, Germany. Dichloromethane, diethyl ether, and *n*-pentane were freshly distilled before use. Silica gel was purified by overnight stirring with hydrochloric acid (32%) according to a procedure described by Esterbauer (6).

**Syntheses.** [<sup>3,4-<sup>2</sup>H<sub>2</sub>]-3-Methylbutanoic Acid. The synthesis was performed by deuteration of 3-methyl-3-buten-1-ol followed by an oxidation of the alcohol into the corresponding acid.</sup>

[<sup>3,4-<sup>2</sup>H<sub>2</sub>]-3-Methylbutan-1-ol. In a deuterium atmosphere maintained by using a deuterium-filled balloon, tris(triphenylphosphine)rhodium(I) chloride [Wilkinson-catalyst (12); 500 mg; 0.54 mmol] was suspended in toluene (15 mL). With continuous stirring, the red suspension turned orange. Then, 3-methyl-3-buten-1-ol (2.15 g; 25 mmol) in toluene (15 mL) was added, and stirring was continued for 2 h. The solution was diluted with *n*-pentane (50 mL) and applied onto</sup>

a water-cooled (12 °C) glass column (20 cm × 1.5 mm i.d.) filled with silica gel (20 g). After elution with *n*-pentane (200 mL), the [<sup>3,4-<sup>2</sup>H<sub>2</sub>]-3-methylbutan-1-ol formed was eluted with diethyl ether (200 mL), and the residual catalyst was removed by solvent-assisted flavor evaporation [SAFE; (7)] at 10 mPa.</sup>

MS-EI (*m/z*: intensity in %) 44 (100), 51 (91), 43 (73), 45 (69), 71 (66), 42 (58), 57 (51), 72 (41), 59 (33), 55 (22), 46 (20), 41 (18), 40 (16), 58 (12), 39 (11), 47 (9), 70 (6), 54 (3), 73 (3), 60 (2); MS-CI (*m/z*: intensity in %) 73 (100; M + H<sup>+</sup> – H<sub>2</sub>O).

[<sup>3,4-<sup>2</sup>H<sub>2</sub>]-3-Methylbutanoic Acid. After careful removal of the solvent, [<sup>3,4-<sup>2</sup>H<sub>2</sub>]-3-methylbutan-1-ol was oxidized into [<sup>3,4-<sup>2</sup>H<sub>2</sub>]-3-methylbutanoic acid in an alkaline aqueous solution using potassium permanganate (8). Compared to a heterogeneously catalyzed deuteration, usually resulting in nonuniformly labeled products (examples in refs 8–11), uniformly dideuterated 3-methylbutanoic acid was obtained. Thus, earlier findings about precise deuterations using Wilkinson catalysts were confirmed (12–15).</sup></sup></sup>

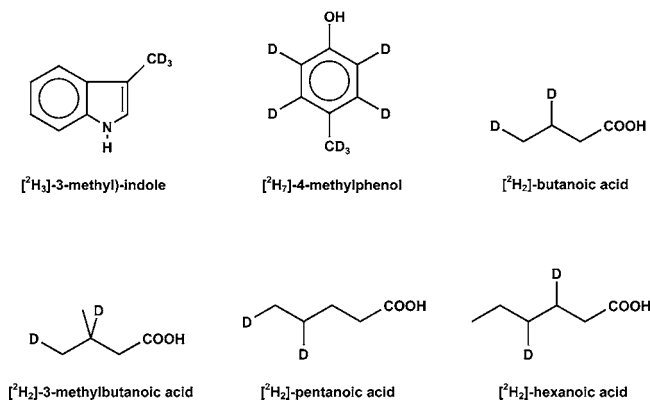
Yield: 1.76 g (67%); MS-EI (*m/z*: intensity in %) 60 (100), 45 (33), 43 (27), 61 (25), 42 (23), 89 (9), 88 (9), 47 (7), 46 (7), 44 (7), 40 (7), 41 (5), 39 (5), 70 (2), 62 (2), 59 (2), 57 (2), 87 (1), 71 (1), 69 (1), 58 (1), 5 (1); MS-CI (*m/z*: intensity in %) 105 (100; M + H<sup>+</sup>).

The following deuterium-labeled compounds were synthesized according to published procedures: [<sup>3,4-<sup>2</sup>H<sub>2</sub>]-butanoic acid (16), [<sup>3,4-<sup>2</sup>H<sub>2</sub>]-hexanoic acid (3, 4), 3-([<sup>2</sup>H<sub>3</sub>]-methyl)indole (17), and [4,5-<sup>2</sup>H<sub>2</sub>]-pentanoic acid (3, 4).</sup></sup>

**Isolation and Fractionation of Pepper Volatiles.** Whole peppercorns were powdered in an ice-cold mill (–10 °C; IKA, Staufen, Germany) prior to the extraction. The powder (5 g) was extracted by stirring in dichloromethane (100 mL) at ambient temperature for 1 h. After filtration, the residue was extracted again (50 mL). The combined extracts were dried over anhydrous sodium sulfate. After filtration, the volatiles were isolated by SAFE distillation (7) at 40 °C. Acidic compounds were removed by treatment with aqueous sodium hydrogen carbonate (0.5 mol/L; three portions of 200 mL total volume) to obtain the neutral and basic volatiles (NBV). The alkaline aqueous phase was washed twice with dichloromethane (50 mL), acidified (pH 2.0) with hydrochloric acid (6 M), and finally extracted with dichloromethane (3 × 50 mL) to yield the acidic volatiles (AV). NBV and AV were dried over anhydrous sodium sulfate and each concentrated to 1 mL using a Vigreux column (50 cm × 1 cm) and a microdistillation.

**High-Resolution Gas Chromatography–Olfactometry (HRGC-O).** For HRGC-O, a gas chromatograph type 8160 (Fisons Instruments, Mainz, Germany) was used. Helium at a pressure of 70 kPa served as the carrier gas. Samples were applied by cold-on-column injection onto capillaries DB-5 or DB-FFAP (both 30 m, 0.32 mm i.d., 0.25  $\mu$ m film thickness; J&W Scientific, Agilent Technologies, Waldbronn, Germany). The end of the capillary was connected to a deactivated Y-shaped glass splitter (Chromatographie Handel Mueller, Fridolfing, Germany) dividing the effluent of the column into two equal parts, which were then transferred via two deactivated fused silica capillaries (50 cm × 0.25 mm) to a sniffing port and a flame ionization detector (FID), respectively. The sniffing port consisted of a cylindrically shaped aluminum device (80 mm length, 25 mm diameter) with a beveled top and a central drill hole (2 mm) housing the capillary. It was mounted on a detector base of the GC and heated to 220 °C. The FID was operated at 250 °C with hydrogen (20 mL/min) and air (200 mL/min). Nitrogen (30 mL/min) was used as the makeup gas. Injection of the samples (0.5  $\mu$ L) was performed at an oven temperature of 40 °C. After 2 min, the temperature was raised by 6 °C/min to 190 °C and then by 12 °C/min to 240 °C. The final temperature was held for 10 min. During a GC run, the panelist placed his nose closely above the top of the sniffing port and evaluated the odor of the chromatographic effluent. When an odor was recognized, the retention time was marked in the chromatogram and the odor quality was assigned on the basis of sensory training with pure reference odorants (18).

**Aroma Extract Dilution Analysis.** For AEDA, fractions NBV and AV were stepwise diluted 1+1 using dichloromethane as the solvent to obtain dilutions of 1:1, 1:2, 1:4, 1:8, 1:16, ... of the original extracts (18). Dilution was continued until no odorant could be detected by GC-O. Each odorant was thus assigned a FD factor representing the last dilution in which the odorant was detectable.



**Figure 1.** Structures of the isotopically labeled internal standards used in the stable isotope dilution assays.

**Structural Assignments.** For identification, mass spectra were recorded using a gas chromatograph 5890 series II (Hewlett-Packard, Waldbronn, Germany) connected to a sector field mass spectrometer type MAT 95 S (Finnigan, Bremen, Germany). Mass spectra in the electron ionization mode (MS/EI) were recorded at 70 eV ionization energy and mass spectra in the chemical ionization mode (MS/CI) at 115 eV using isobutane as the reactant gas. Identification of all odorants was performed by comparing their retention indices on the two stationary phases detailed above and their mass spectra as well as their sensory properties (odor quality and odor threshold determined by GC-O) with those of reference compounds.

**Quantitation by Stable Isotope Dilution Assays. General Procedure.** Fresh, undried pepper fruits were powdered in frozen state as described above and intensely mixed with the same amount of anhydrous sodium sulfate prior to the extraction. This mixture or the pepper powder (1–5 g) was suspended in dichloromethane (100 mL) and the labeled internal standards (for structures and position of labeling see **Figure 1**), dissolved in either dichloromethane or diethyl ether (0.5 mL), were added. Extraction and SAFE distillation were performed as described above. The distillate was fractionated into the NBV and the AV as detailed above, but using aqueous sodium hydroxide (2 mol/L) instead of sodium hydrogen carbonate for separation.

**Phenols and Acids.** For the determination of the carboxylic acids and the phenols, the AV (1  $\mu\text{L}$ ) was analyzed on a capillary column (DB-FFAP) installed in a GC (CP 3800, Varian, Darmstadt, Germany) and coupled to a mass spectrometer (Saturn 2000, Varian) running in the CI mode with methanol as reactant gas. The oven temperature was held at 40  $^\circ\text{C}$  for 2 min, then raised by 10  $^\circ\text{C}/\text{min}$  to 100  $^\circ\text{C}$ , by 4  $^\circ\text{C}/\text{min}$  to 180  $^\circ\text{C}$ , and, finally, by 20  $^\circ\text{C}/\text{min}$  to 240  $^\circ\text{C}$ . In the mass chromatogram, areas under the peaks at the respective mass traces were recorded, and concentrations were calculated as described recently (21). The following ions were selected: butanoic acid,  $m/z$  89;  $[3,4\text{-}^2\text{H}_2]$ -butanoic acid,  $m/z$  91; 3-methylphenol,  $m/z$  109; 4-methylphenol,  $m/z$  109;  $[^2\text{H}_7]$ -4-methylphenol,  $m/z$  115–116; hexanoic acid,  $m/z$  117;  $[3,4\text{-}^2\text{H}_2]$ hexanoic acid,  $m/z$  119; 3-methylbutanoic acid,  $m/z$  103;  $[3,4\text{-}^2\text{H}_2]$ -3-methylbutanoic acid,  $m/z$  105; pentanoic acid,  $m/z$  103;  $[4,5\text{-}^2\text{H}_2]$ pentanoic acid,  $m/z$  104–106.

**3-Methylindole.** For the determination of 3-methylindole, the volatiles present in the NBV were separated on the FFAP column installed in the first GC (Trace GC, 2000 series, Thermo Finnigan, Egelsbach, Germany) of a two-dimensional GC-GC-MS system using a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland). The oven temperature was held at 40  $^\circ\text{C}$  for 2 min, then raised by 20  $^\circ\text{C}/\text{min}$  to 160  $^\circ\text{C}$  and finally by 4  $^\circ\text{C}/\text{min}$  to 240  $^\circ\text{C}$ . During the elution of 3-methylindole and  $[^2\text{H}_3]$ -3-methylindole, the effluent of the first column was quantitatively transferred into a cold trap (SGE, Darmstadt, Germany) using a moving column stream switching system (Thermo Finnigan) (19). After discontinuation of the cooling, the trapped material was transferred onto the second column (DB-1701) installed in a GC (CP 3800, Varian). Starting at 40  $^\circ\text{C}$ , the oven temperature was quickly raised by 20  $^\circ\text{C}/\text{min}$  to 120  $^\circ\text{C}$  and then by 6  $^\circ\text{C}/\text{min}$  to 240  $^\circ\text{C}$ . The effluent was transferred into an ion trap mass spectrometer (Saturn 2000, Varian) running in the CI mode with methanol as reactant gas.

**Table 1.** Concentrations of 12 White Pepper Odorants Used in the Pepper Aroma Recombinate (3)

odorant	$\mu\text{g}/\text{kg}$ of starch	odorant	$\mu\text{g}/\text{kg}$ of starch
(-)-limonene	2230	1,8-cineole	48.4
(-)- $\beta$ -pinene	2130	(+)-linalool	28.6
(+)-limonene	1490	methylpropanal	6.50
(-)- $\alpha$ -pinene	1176	3-methylbutanal	3.14
piperonal	104	2-methylbutanal	1.10
(-)-linalool	81.4	eugenol	0.96

Concentrations of 3-methylindole were determined using the intensities of the ions  $m/z$  132 for the unlabeled and  $m/z$  135 for the labeled 3-methylindole.

**Dry Matter.** For better comparison, values are expressed as amount per dry matter, which was determined by drying pepper samples (5–15 g) in nickel dishes (height = 2.7 cm, diameter = 7 cm) at 105  $^\circ\text{C}$  until constant.

**Determination of Breakthrough Thresholds.** In a recent publication, an aroma recombine had been developed by our group based on the concentrations of key odorants as these occurred in a white pepper sample, which completely matched the aroma of the spice (3). On the basis of these quantitative data, an ethanolic solution (0.5 mL) of 12 selected key pepper aroma compounds in the amounts given in **Table 1** was added to a commercial starch (500 g; Mondamin, Bestfoods, Heilbronn) and homogenized by intense shaking for 1 h (pepper aroma recombine). 3-Methylindole, the cresols, and the short-chain fatty acids were omitted from the recombine due to their undesirable aroma attributes described in ref 3. The overall aroma of this “reduced” aroma recombine (RAR) was described as typically pepper-like in a sensory panel test.

For the determination of breakthrough thresholds, the RAR was singly administered with increasing concentrations of either 3-methylindole, 3- or 4-methylphenol, butanoic acid, or a mixture of 2- and 3-methylbutanoic acid dissolved in ethanol (0.2 mL). As reference, the RAR was only administered with ethanol (0.2 mL). Therefore, at the end, the test samples contained 13 odorants, with 1 odorant varying in concentration, whereas the reference sample contained 12 odorants.

Each test sample was then orthonasally evaluated in a series of triangular tests against two reference samples as previously described (22). For this purpose, the samples (1.5 g each) were placed into cylindrical ground neck glasses (height = 7 cm; i.d. = 3.5 cm). Evaluations were performed in a specially equipped sensory room at  $21 \pm 1$   $^\circ\text{C}$  in daylight. For determination of the breakthrough threshold, trained panelists indicated in which sample the aroma quality of the respective odorant could be perceived without being masked by the others. For each panelist an individual threshold was determined from the sample with the lowest concentration detected. The breakthrough threshold was then obtained as the geometrical mean of the individual thresholds of 10 panelists.

**Aroma Profiles of Pepper Samples.** Ten trained panelists were asked to orthonasally evaluate the intensity of seven major odor qualities in the overall aroma of ground pepper, as determined in preliminary tests, on a scale from 0 to 3 with 0 = not perceivable, 1 = weak, 2 = medium, and 3 = intense. Odor qualities were specified by using the following reference compounds: 3-methylindole (fecal), butanoic acid (cheese-like), 3-methylphenol (phenolic),  $\alpha$ -pinene (pine needle), myrcene (geranium-like), linalool (flowery), and (*R*)-limonene (citrus-like).

## RESULTS

**Aroma Extract Dilution Analysis.** To locate odor-active compounds in a sample of white pepper (WP-01) exhibiting a characteristic intense fecal off-odor, a distillate obtained by extraction/SAFE distillation of the powder (5 g) was analyzed by AEDA. A small portion of the distillate, when sniffed on a strip of filter paper, clearly represented the typical aroma of the pepper powder as well as the fecal, cowshed-like odor note.

In the FD factor range of 32–1024, 20 odor-active areas were detected. The results of the identification experiments in



**Table 2.** Most Aroma-Active (FD  $\geq$  16) Compounds or Areas, Respectively, Detected in a Distillate from a White Pepper Powder Eliciting a Strong Fecal Off-odor (WP-01)

odorant <sup>b</sup>	odor quality <sup>c</sup>	RI <sup>a</sup> on		FD factor
		DB-5	FFAP	
$\alpha$ -pinene	resinous, pine needle	932	1008	1024
linalool	flowery, citrus-like	1100	1537	1024
3-methylindole	fecal, swine manure	1387	2495	512
4-methylphenol <sup>d</sup>	fecal, horse-like	1077	2074	256
unknown <sup>e</sup>	fruity	1135	1425	256
piperonal	sweet, aniseed-like	1334	2225	256
unknown <sup>e</sup>	spicy	1687	2235	256
myrcene	geranium-like	991	1152	128
unknown <sup>e</sup>	terpene-like	1028	1182	128
3-methylphenol <sup>d</sup>	phenolic	1079	2083	128
unknown <sup>e</sup>	woody	1247	nd	128
eugenol	clove-like	1356	2163	128
unknown <sup>e</sup>	spicy	1726	2250	128
unknown <sup>e</sup>	spicy	1381	nd	64
unknown <sup>e</sup>	smoky	1530	nd	64
butanoic acid <sup>d</sup>	cheese-like	nd	1621	64
<i>p</i> -cymene	terpene-like	1022	1254	32
2-methoxyphenol <sup>d</sup>	smoky	1088	1852	32
2-isobutyl-3-methoxy-pyrazine	bell-pepper-like	1181	1517	32
2/3-methylbutanoic acid <sup>d</sup>	cheese-like	nd	1661	32
pentanoic acid <sup>d</sup>	cheese-like	nd	1731	16
hexanoic acid <sup>d</sup>	cheese-like	nd	1840	16

<sup>a</sup> Retention index; nd = not determined. <sup>b</sup> The odorant was identified by comparing it with the reference compound on the basis of the following criteria: (i) retention indices on the capillaries detailed in the table, (ii) mass spectra obtained by MS/EI and MS/CI, and (iii) odor quality and odor threshold determined by GC-O. <sup>c</sup> Odor quality perceived at the sniffing port. <sup>d</sup> Compound was identified in fraction AV. <sup>e</sup> The mass spectra obtained were too weak for an unequivocal identification.

combination with the FD factors (**Table 2**) revealed  $\alpha$ -pinene and linalool followed by 3-methylindole to have the highest odor activities among the compounds sensorially detected. These results confirmed previous data published on key odorants of white pepper (3).

Besides 3-methylindole, 4-methylphenol and 3-methylphenol were identified with high FD factors. Five compounds, namely, butanoic, pentanoic, and hexanoic acid as well as 2- and 3-methylbutanoic acid, might also act as the cause of the malodor, because of their cheese-like, rancid odor qualities (**Table 2**).

Because the study was aimed at identifying "off-odorants", the further experiments were focused on compounds eliciting odors close to the overall off-note.

**Quantitative Measurements.** The application of the AEDA on a few samples of white pepper powders showing either no or only a slight malodor resulted in the same aroma compounds, but somewhat differing in the FD factors (data not shown). However, it was not possible to find a clear correlation between the overall aroma of the respective pepper sample and the FD factors of the eight odorants exhibiting unpleasant odor qualities. Therefore, in a first series of quantitative experiments, eight odorants proposed as contributors to the off-odor on the basis of their odor quality were quantified in 19 pepper samples, including WP-01 and WP-02, which both elicited the most intense off-aroma. The data summarized in **Table 3** showed clear differences in the concentrations of 3-methylindole (3-MI) ranging from 0.7 to 2.7 mg/kg pepper. Although WP-01 was among the samples highest in 3-MI, no clear correlation of its concentration with the overall aroma impression of the 19 pepper samples was found, because WP-13 and WP-14, which were even higher in 3-MI, did not show a pronounced off-aroma.

**Table 3.** Concentrations of 3-Methylindole (3-MI), 4-Methylphenol (4-MP), 3-Methylphenol (3-MP), Butanoic Acid (BA), 2- and 3-Methylbutanoic Acid (MBA; Sum of Isomers), Pentanoic Acid (PA), and Hexanoic Acid (HA) in 19 Commercial White Pepper Samples<sup>a</sup>

sample	concn <sup>b</sup> (mg/kg)						
	3-MI	4-MP	3-MP	BA	MBA	PA	HA
WP-01	2.4	<b>19</b>	<b>26</b>	<b>180</b>	<b>9.5</b>	<b>60</b>	<b>79</b>
WP-02	1.3	3.9	6.1	45	4.0	33	47
WP-03	<b>0.7</b>	<b>1.9</b>	3.0	<b>19</b>	2.6	25	28
WP-04	1.9	3.7	11	41	4.8	42	45
WP-05	1.3	3.5	6.6	46	4.9	38	46
WP-06	1.6	3.4	5.4	27	2.6	26	30
WP-07	1.8	2.7	5.2	60	8.0	42	47
WP-08	0.9	4.0	19	33	3.5	39	48
WP-09	2.3	2.2	<b>0.8</b>	57	5.2	34	40
WP-10	2.1	3.3	2.2	33	3.5	30	39
WP-11	2.1	7.9	19	37	4.0	31	32
WP-12	2.0	5.0	13	38	4.4	28	30
WP-13	<b>2.7</b>	5.5	4.9	81	4.5	38	55
WP-14	2.6	7.4	4.6	73	7.5	36	38
WP-15	1.2	2.6	9.9	61	2.5	32	32
WP-16	2.0	5.7	13	23	6.5	<b>22</b>	<b>23</b>
WP-17	1.6	3.3	4.8	23	5.7	24	23
WP-18	1.6	4.8	13	43	3.0	27	32
WP-19	1.0	2.9	3.8	45	<b>2.5</b>	29	34
range	0.7–2.7	1.9–19	0.8–26	19–180	2.5–9.5	22–60	23–79

<sup>a</sup> Lowest and highest values in bold. <sup>b</sup> Values determined in triplicates differed by not more than  $\pm 5\%$ .

4-Methylphenol (4-MP) varied between 1.9 and 19 mg/kg (**Table 3**). Also for this odorant, WP-01 was highest among all samples analyzed. But, again, the intensity of the overall off-aroma quality in the samples was not in agreement with high concentrations of 4-MP.

The concentrations of 3-methylphenol determined in the 19 samples lay between 0.8 and 26 mg/kg, and again sample WP-01 was highest.

Among the short-chain fatty acids, butanoic acid showed the largest variation, between 19 and 180 mg/kg. The highest concentrations were also determined in WP-01. The same was true for hexanoic and pentanoic acid, which both were highest in WP-01 as compared to all other samples analyzed (**Table 3**).

These results proposed the eight odorants as indicators for an off-odor, but in summary, the quantitative results did not give a clear idea of which concentration of a single odorant should not be exceeded to avoid off-odor notes in white pepper.

It might be speculated that after the grinding of peppercorns, the off-odorants might be newly formed or others, eliciting positive aroma attributes, might be lost. To follow this idea, several pepper samples available as intact peppercorns were cooled in liquid nitrogen and then ground, and the concentrations of the eight odorants were immediately analyzed by stable isotope dilution assays.

None of the 14 samples investigated exhibited a pronounced off-aroma after grinding, thus suggesting that possibly lower amounts of the off-odorants under consideration might be present. However, the results obtained showed that 3-methylindole was present in concentrations of 1.1–3.6 mg/kg (**Table 4**). Compared to the amounts in the pepper powders, the concentrations were in the same concentration range or were even higher (cf. **Tables 3** and **4**; WP-24). Also, the two phenols as well as the two cheese-like smelling acids did not differ from the concentration ranges quantified in the pepper powders (cf. **Tables 3** and **4**). Therefore, these data suggested that a formation of the eight pepper odorants during storage of pepper powder is unlikely. This point will be further addressed below.

**Table 4.** Concentrations of 3-Methylindole (3-MI), 4-Methylphenol (4-MP), 3-Methylphenol (3-MP), Butanoic Acid (BA), and 2- and 3-Methylbutanoic Acids (MBA; Sum of Isomers) in 14 Samples of Whole Corns of White Pepper of Different Brands<sup>a</sup>

sample	concn <sup>b</sup> (mg/kg)				
	3-MI	4-MP	3-MP	BA	MBA
WP-20	1.3	9.0	37	63	3.6
WP-21	1.8	9.5	32	42	4.0
WP-22	2.9	7.9	<b>2.1</b>	<b>92</b>	9.0
WP-23	2.9	6.0	13	47	5.6
WP-24	<b>3.6</b>	<b>15</b>	<b>47</b>	52	5.0
WP-25	2.7	8.3	16	63	<b>10</b>
WP-26	2.0	7.0	7.9	56	5.3
WP-27	1.7	7.8	9.5	50	5.0
WP-28	<b>1.1</b>	5.4	11	51	5.1
WP-29	1.2	<b>3.7</b>	4.1	<b>26</b>	<b>3.2</b>
WP-30	1.3	4.3	2.7	73	6.3
WP-31	2.4	6.6	14	67	7.3
WP-32	1.3	3.9	4.0	63	5.9
WP-33	1.3	4.7	8.2	86	4.9
range	1.1–3.6	3.7–15	2.1–47	26–92	3.2–10

<sup>a</sup> Lowest and highest values in bold. <sup>b</sup> Values determined in triplicates differed by not more than  $\pm 5\%$ .

**Peppers from Defined Geographical Origin.** In a last series of experiments, 16 pepper samples consisting of 4 samples of pepper powder from Muntok and 3 powders from Sarawak, 3 samples of peppercorns from Muntok, and 2 peppercorns from Sarawak as well as 1 sample of peppercorns from Brazil, Pontianak, Samarinda, and Sulawesi were analyzed for the seven odorants. The concentrations determined differed among all samples analyzed (Table 5), but the amounts lay in the same concentration ranges as determined in the commercial samples analyzed before (Tables 3 and 4). No clear correlation was found between the amounts of the odorants and a given provenance.

Furthermore, although the concentration of, for example, 3-MI, in powdered WP-44, was the highest of all 50 samples analyzed, no pronounced off-odor was detected in this sample. The same was true for WP-46 showing the highest concentrations of butanoic acid or WP-49 showing the highest concentration of hexanoic acid.

In summary, the data suggested that all odorants under investigation have to be regarded as characteristic aroma constituents of white pepper. However, the results do not provide any explanation for the sporadic appearance of a fecal-like off-odor in white pepper samples.

**Sensory Experiments.** According to the OAV concept, odorants do contribute to the overall aroma if they exceed their odor threshold in a given matrix (18). Because starch is the major nonvolatile constituent of pepper (23), first, the odor threshold of seven aroma compounds in starch were determined and their OAVs were calculated on the basis of the concentration ranges determined (Table 6).

The data revealed that all eight odorants should contribute to the pepper aroma, because their concentrations clearly exceeded their odor thresholds in starch. In particular, 3-methylindole was present with very high OAVs between 3000 and 16000.

The calculation of OAVs is a useful tool to predict a contribution of single odorants to an overall aroma. However, any masking or additive effect caused by other key aroma compounds cannot be evaluated. Another approach to determine the potential of a single odorant as a cause of off-flavors is the determination of its "breakthrough" odor threshold. This can

be done in sensory experiments by adding increasing amounts of the odorant to the food itself. Using the food as the reference, panelists approximate the concentration at which they can detect the odor quality of the compound under investigation. This concept has been successfully applied previously, for example, in the characterization of a metallic off-flavor in buttermilk (24).

A new concept to evaluate the contribution of a single odorant suspected to contribute to an off-odor is to use a food aroma recombine, which matches the aroma of the food, based on a mixture of reference odorants in the "natural" concentrations. By varying the amounts of single, possibly off-odor-causing substances in the overall aroma recombine, their breakthrough thresholds can be determined in triangular tests using the recombine lacking in the respective odorant as the blank.

Using an aroma recombine made by mixing 12 key pepper odorants matching the typical aroma of white pepper powder (Table 1), the breakthrough thresholds of five aroma compounds were determined (Table 7). On the basis of these thresholds, the OAVs were then recalculated. The results showed that 3-methylphenol, 3-methylindole, and 4-methylphenol clearly exceeded their breakthrough thresholds in samples showing the highest concentrations of the respective odorants by factors of nearly 100 and above (Table 7). By contrast, the acids, in particular 2- and 3-methylbutanoic acid, showed much lower odor activities.

It is, however, interesting that even the lower concentrations in some samples were sufficient to reach an OAV > 1. Because such samples were not ascribed as having an off-odor, the release from the pepper matrix is another important, yet unconsidered, influence on the OAV.

**Aroma Changes during Storage of White Pepper.** Spice traders occasionally observed that the characteristic off-flavor is not detected in white pepper immediately after grinding of whole peppercorns, but is elicited when the powder is stored for a longer period of time. On the basis of the fact that the commercial samples of pepper powders analyzed so far had been stored under different conditions, a model study was conducted starting from freshly ground white pepper. After storage for 14, 30, 60, 90, 150, and 210 days in sealed plastic bags, the seven major odor qualities were evaluated in their intensities by a trained sensory panel.

The data clearly showed that after 90 days, four positive aroma qualities such as pine needle, geranium-leaf-like, flowery, and citrus-like were significantly decreased, whereas the fecal, cheesy, and phenolic aromas became more pronounced (Figure 2). These results suggest that, for example, 3-methylindole, 3-methylphenol, and 4-methylphenol might have been increased during storage. However, the quantitative data revealed (Table 8) that 3-methylindole, 3-methylphenol, 4-methylphenol, and butanoic acid were even decreased to a certain extent during storage.

These data suggested that the stronger off-flavor observed after storage for 210 days was not due to the formation of off-odorants, but most probably resulted from the loss of white pepper odorants, which are possibly able to mask the off-odor in the freshly ground samples.

In summary, the data point to 3-methylindole, 3-methylphenol, and 4-methylphenol, although natural constituents of white pepper, as off-odorants, particularly in stored pepper powder. It can be assumed that certain odorants, such as  $\alpha$ -pinene, are lost because of their higher volatility as compared to the odorants under investigation and, thus, the aroma qualities of the above-mentioned compounds become more prominent in the overall aroma.

**Table 5.** Concentrations of 3-Methylindole (3-MI), 4-Methylphenol (4-MP), 3-Methylphenol (3-MP), Butanoic Acid (BA), 2- and 3-Methylbutanoic Acid (MBA; Sum of Isomers), Pentanoic Acid (PA), and Hexanoic Acid (HA) in White Pepper Powders and Whole Peppercorns from Distinct Geographical Origins<sup>a</sup>

sample	type	origin	concn <sup>b</sup> (mg/kg)						
			3-MI	4-MP	3-MP	BA	MBA	PA	HA
WP-34	powder	Muntok	1.2	3.2	8.1	45	2.6	30	33
WP-35	powder	Muntok	<b>1.0</b>	3.4	13	42	2.6	28	33
WP-36	powder	Muntok	2.2	4.3	4.1	<b>34</b>	3.4	34	45
WP-37	powder	Muntok	1.3	<b>2.6</b>	4.8	44	<b>2.4</b>	<b>26</b>	<b>29</b>
WP-38	powder	Sarawak	2.6	5.1	3.0	55	5.6	29	35
WP-39	powder	Sarawak	3.3	7.2	7.1	53	6.5	36	46
WP-40	powder	Sarawak	1.9	3.9	<b>2.5</b>	42	4.4	31	30
WP-41	whole corn	Muntok	1.7	6.0	16	84	4.3	37	44
WP-42	whole corn	Muntok	2.3	8.3	14	73	5.5	43	60
WP-43	whole corn	Muntok	1.9	8.6	22	73	4.2	35	55
WP-44	whole corn	Pontianak	<b>3.8</b>	<b>32</b>	<b>105</b>	119	<b>8.1</b>	<b>62</b>	74
WP-45	whole corn	Samarinda	1.1	4.1	3.0	40	3.2	31	38
WP-46	whole corn	Sulawesi	1.6	5.2	4.2	<b>155</b>	5.6	61	73
WP-47	whole corn	Sarawak	2.1	4.3	12	77	3.4	36	47
WP-48	whole corn	Sarawak	1.7	5.4	2.5	47	4.8	34	36
WP-49	whole corn	Brazil	2.8	14	41	108	6.6	57	<b>117</b>
range			1.0–3.8	2.6–32	2.5–105	34–155	2.4–8.1	26–62	29–117

<sup>a</sup> Lowest and highest values in bold. <sup>b</sup> Values determined in triplicates differed by not more than  $\pm 5\%$ .

**Table 6.** Concentration Ranges, Orthonasal Odor Thresholds, and Odor Activity Values (OAV) of Eight White Pepper Odorants

compound	concn <sup>a</sup> (mg/kg of pepper)	odor threshold <sup>b</sup> (mg/kg of starch)	OAV <sup>c</sup>
3-methylindole	0.7–3.6	0.00023	3000–16000
4-methylphenol	1.9–32	0.13	15–250
3-methylphenol	0.8–105	nd <sup>d</sup>	nd
butanoic acid	19–180	0.10	190–1800
2/3-methylbutanoic acid <sup>e</sup>	2.4–10 <sup>f</sup>	0.024	100–420
pentanoic acid	22–62	4.9	4–13
hexanoic acid	23–117	11	2–11

<sup>a</sup> Data from **Tables 3–5**. <sup>b</sup> Data taken from ref 3. <sup>c</sup> Calculated by dividing the concentrations by the odor thresholds in starch. <sup>d</sup> nd = not determined. <sup>e</sup> The odor threshold of 3-methylbutanoic acid is 0.006 mg/kg in starch. <sup>f</sup> Sum of isomers.

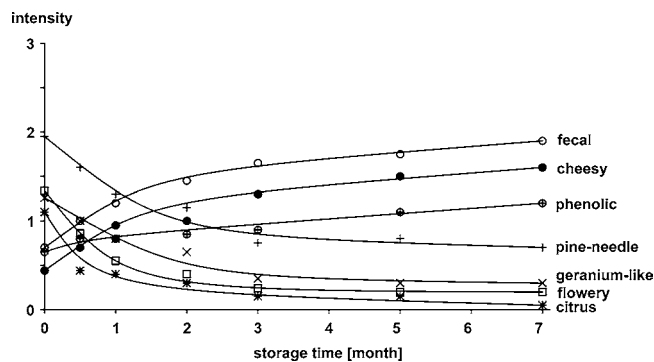
**Table 7.** Concentration Ranges and Odor Activity Values (OAV) Calculated on the Basis of Orthonasal Breakthrough Values

compound	concn (mg/kg of pepper)	breakthrough threshold (mg/kg)	OAV <sup>a</sup>
3-methylindole	0.7–3.6	0.035	20–100
4-methylphenol	2–32	0.33	6–100
3-methylphenol	1–105	0.38	3–280
butanoic acid	19–180	7.5	3–24
2/3-methylbutanoic acid	3–10 <sup>b</sup>	3.0	<1–3

<sup>a</sup> Calculated by dividing the concentration by the odor threshold in a recombine. <sup>b</sup> Sum of isomers.

### Concentrations in Fresh Pepper and in Black Pepper.

Because of their potential to spoil the aroma of, in particular, ground white pepper during storage, it was of interest to gain first insights into the source and formation of 3-methylindole as well as 3- and 4-methylphenol and butanoic acid. For this purpose, fresh pepper fruits, collected in Thailand, were analyzed. Because for black pepper production, green, unripe fruits and for white pepper manufacturing mainly fully ripe orange to red pepper fruits are used, also the influence of the ripening state on the concentrations in fresh pepper fruits was elucidated. Pepper fruits were picked from the spikes and sorted according to their state of ripeness into three categories: (1) green, unripe fruits; (2) fully ripe, red fruits; and (3) overripe, already brownish discolored fruits. Quantitation of 3-methylindole,

**Figure 2.** Changes in the intensities of seven aroma attributes in the overall aroma of a white pepper powder during storage.**Table 8.** Changes in the Concentrations of Four Odorants in White Pepper during Storage

odorant	concn (mg/kg) after						
	0 days	14 days	30 days	60 days	90 days	150 days	210 days
3-methylindole	1.38	1.34	1.15	0.98	0.90	0.72	0.65
3-methylphenol	7.5	6.9	8.3	5.6	4.7	4.0	3.3
4-methylphenol	3.6	3.5	3.5	2.7	1.9	1.7	1.6
butanoic acid	69	66	72	50	56	42	35

**Table 9.** Concentrations of 3-Methylindole (3-MI), 4-Methylphenol (4-MP), 3-Methylphenol (3-MP), and Butanoic Acid (BA) in Fresh, Undried Pepper Fruits of Different Ripeness States (Thailand)

ripeness state	color	concn (mg/kg)			
		3-MI	4-MP	3-MP	BA
unripe	green	<0.01	0.06	0.05	0.8
ripe	orange to red	<0.01	0.04	0.03	2.1
overripe	brown	<0.01	0.03	0.04	1.7

4-methylphenol, 3-methylphenol, and butanoic acid in these samples indicated very low concentrations independently from the grade of maturation (**Table 9**). Therefore, these data clearly proved that the off-odorants are not formed during fruit ripening.

To gain first insights into the influence of the fermentation step used in white pepper production, several samples of black



**Table 10.** Concentrations of 3-Methylindole (3-MI), 4-Methylphenol (4-MP), 3-Methylphenol (3-MP), and Butanoic Acid (BA) in Eight Samples of Black Pepper (Whole Corns)

sample	concn (mg/kg)			
	3-MI	4-MP	3-MP	BA
BP-1	≤0.01	0.15	0.13	7.5
BP-2	≤0.01	0.20	0.17	11
BP-3	≤0.01	0.10	0.16	5.2
BP-4	≤0.01	0.07	0.13	3.7
BP-5	≤0.01	0.04	0.09	4.2
BP-6	≤0.01	0.13	0.24	6.9
BP-7	0.01	0.13	0.33	7.5
BP-8	≤0.01	0.22	0.17	2.7
range	≤0.01	0.04–0.22	0.09–0.33	2.7–11

pepper were analyzed because this type of pepper does not undergo any fermentation. The concentrations found in black pepper (Table 10) were much lower than those in white pepper. 3-Methylindole was hardly detectable (≤0.01 mg/kg), and the concentrations of 4-methylphenol (≤0.2 mg/kg) and 3-methylphenol (≤0.3 mg/kg) were negligible as compared to those found in white pepper. Also, butanoic acid showed clearly lower concentrations (3–10 mg/kg).

In summary, the data suggest that 3-methylindole, 4-methylphenol, 3-methylphenol, and butanoic acid are crucial odorants for the development of the characteristic fecal off-odor in white pepper samples, although these aroma compounds are ubiquitously present in white pepper. Lewis et al. (25) reported on some experimental trials done in Indonesia and Malaysia, the major white pepper producing countries today. They found that a “bad smell” was developed when keeping green pepper fruits in jute bags in running water for 14 days, also causing a “somewhat unpleasant smell” in the final pepper.

It can, thus, be assumed that the fermentation of ripe pepper fruits used to remove the fruit flesh is the main reason for the formation of the odorants described above. Further studies will be performed to elucidate the formation of these compounds during the fermentation step.

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