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Decoding the Key Aroma Compounds of a Hungarian-Type Salami by Molecular Sensory Science Approaches

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Application of aroma extract dilution analysis on an extract/distillate prepared from a Hungarian-type salami and subsequent identification experiments led to the identification of 51 of 55 odor-active compounds detected in the flavor dilution (FD) factor range of 16−4096. Nineteen of these compounds are reported for the first time as aroma components of dry-fermented sausages, among them 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon), *trans*-4,5-epoxy-(*E*)-2-decenal, and bis(2-methyl-3-furyl) disulfide. The highest FD factors were found for 2-methoxyphenol (smoky, sweet), 2-methoxy-4-(2-propenyl)phenol (clove-like), 2-methoxy-4-(*E*)-(1-propenyl)phenol (clove-like), and acetic acid (pungent, sour). Forty-five aroma compounds were subsequently quantified using stable isotope dilution assays, and their odor activity values (OAVs; ratio of concentration to odor threshold) were calculated on the basis of odor thresholds in oil. The highest OAVs were calculated for acetic acid, acetaldehyde, 3-(methylthio)propanal, phenylacetaldehyde, 2-methoxyphenol, and 2-acetyl-1-pyrroline. A model mixture containing 33 odorants in the same concentrations as they occurred in the sausage was prepared in a matrix consisting of 44% sunflower oil and 56% aqueous phosphate buffer. A comparison of the overall aroma of this model mixture with the original showed a very high similarity, suggesting that the key aroma compounds were successfully identified.

KEYWORDS: Dry-fermented sausage; odor thresholds; aroma extract dilution analysis; odor activity value; aroma reconstitution

INTRODUCTION

Salami is a raw, fermented sausage consisting of raw, comminuted pork and/or beef meat, fat, and additives, such as curing salt, spices, sugar, and a starter culture. To obtain the typical texture and aroma, this mixture is stuffed into casings, then fermented, and finally dried. Salami was first manufactured in Italy about 270 years ago (*1*), and today a great variety of salami types exist as a result of regional manufacturing differences and aroma preferences. In northern and central Europe, smoked salamis are preferred, and ripening is controlled by the addition of lactic acid-producing starter cultures, whereas in southern Europe, salami is slowly air-dried and mostly mold-ripened. Hungarian-type salami is a specialty in salami production, because it is first slightly smoked and mold-ripened afterward.

First investigations on salami volatiles succeeded in the identification of several short-chain volatile acids, such as butanoic acid and 3-methylbutanoic acid (*2*) and aldehydes, such as hexanal, nonanal, and 2- and 3-methylbutanal (*2*−*5*). Later, terpenes, such as limonene, linalool, and α-pinene, aromatic compounds, such as 2-methoxyphenol, phenylacetaldehyde, and esters, as well as sulfur- and nitrogen-containing compounds, such as dimethyl disulfide or 2,6-dimethylpyrazine, were reported as salami constituents (*6*−*9*). As a result, to date, more than 400 volatile compounds have been identified in different types of dry-fermented sausages (*10*).

Volatility, however, is only a limited criterion to predict the contribution of a single volatile to food aromas (*11*). To become an odorant, a compound must exceed a certain concentration in the air above a given food, thus enabling reaction with human odorant receptors in the nasal cavity. However, because it is not yet possible to directly measure the "active" concentration of a volatile at a certain odorant receptor, screening methods, such as gas chromatography−olfactometry (GC-O), and dilution to odor threshold techniques, such as the aroma extract dilution (AEDA), have been developed to separate the bulk of odorless volatiles from the key aroma compounds in a given food extract (*11*). However, so far only a very few studies on salami aroma using such techniques have been performed.

Schmidt and Berger (*12*) were among the first to study the odor-active volatiles in self-prepared dry-fermented sausages by application of GC-O. They determined the highest odor activities for diallyl disulfide, diallyl sulfide, 3-(methylthio)-1-propene (allylmethyl sulfide), acetic acid, 3-methylbutanoic acid, ethyl butanoate, propyl 3-methylbutanoate, linalool, and 2-methoxy-4-(2-propenyl)phenol (eugenol) in extracts isolated by a molecular distillation technique. Further investigations on the key aroma compounds of dry-fermented sausages were

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performed by Blank et al. (*13*), who investigated the aroma of an Italian-type salami. These authors reported acetic acid, 3-methylbutanoic acid, and butanoic acid as well as hexanal, 3-(methylthio)propanal, 2-acetyl-1-pyrroline, and two unidentified compounds with a sulfury and a roasty/nutty flavor, respectively, as the most odor-active constituents. Their results suggested, however, that further, yet unknown, compounds may be important for the characteristic flavor of dry-fermented sausages. Very recently, Marco et al. (*14*) reported on the influence of different curing salts on aroma volatiles in dry-fermented sausages. They found higher odor activities for, for example, phenylacetaldehyde and 3-methylbutanal in sausages prepared with added nitrate.

Another, but less directed, approach to reveal important odorants is the quantitative analysis of the entire set of volatiles followed by a statistical correlation of certain odor notes determined by a sensory panel. Following such protocols, the mature flavor of salami was found to correlate with the presence of ethyl esters (*15*) or with high concentrations of branched-chain aldehydes, such as 2- and 3-methylbutanal and 2-methylpropanal (*16*).

However, results of systematic approaches combining analytical methods with human odor perception in each analytical step including the calculation of odor activity values (OAVs) and re-engineering of the overall aroma by aroma reconstitution experiments have not yet been applied to unravel the aroma of dry-fermented sausages. Such approaches, recently assigned as "molecular sensory science" (*17*), have been successfully applied to mimick the aroma and, also, the taste of several foods, for example, black tea (*18*, *19*) or cocoa (*20*, *21*).

One of the major demands in the food industry is the production of traditional foods with their superior aroma, but using shorter, cost-effective processes. Thus, there is a need to know the blueprint of the natural aroma, that is, the natural composition of key food aroma compounds. On the basis of such knowledge, food processing steps can systematically be optimized by assessing the changes in key aroma compounds occurring in each manufacturing step. However, it is a prerequisite for this approach that the key odorants of a product have been characterized. The purpose of this investigation was, therefore, to characterize the key aroma compounds in the typical flavor of a high-quality, dry-fermented sausage by means of methods of "molecular sensory science" (*17*). By this approach, in the first step the key aroma compounds are decoded on the basis of their molecular structures and their concentrations, followed by re-engineering of the aroma in a model matrix using reference compounds in the natural concentrations occurring in the food itself.

MATERIALS AND METHODS

Salami Sample. Several batches of a high-quality salami of Hungarian origin, the so-called "Hungarian Winter salami" (Pick), were purchased from a local supermarket. It consisted of raw pork meat, bacon, salt, spices, sugars, and sodium nitrite. Prior to the 3 month ripening process, the salami was exposed to beech tree smoke for 2 weeks. During the ripening period, the salami surface was covered with mold. Aroma profile analyses and extract preparations were performed immediately after purchase. The remaining material was cut into pieces of about 100 g and stored under vacuum at −60 °C for further analyses.

Chemicals. The following reference aroma compounds were obtained from the commercial sources given in parentheses: acetaldehyde, acetic acid, 2-acetyl-2-thiazoline, 1-allyl-3,4-(methylendioxy) benzole (safrole), 2,3-butandione, butanoic acid, (*E*,*E*)-2,4-decadienal, 2,3-diethyl-5-methylpyrazine, 2,6-dimethoxyphenol, 6,6-dimethyl-2-methylbicyclo-[3,1,1]-2-heptene (α-pinene), (*R*)-3,7-dimethyl-1,6-octadien-3-ol ((*R*)-linalool), 2,6-dimethylphenol, 1,8-epoxy-*p*-menthene (1,8-cineole), ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl butanoate, 2-ethyl-3,5-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 3-ethylphenol, 4-ethylphenol, hexanal, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon), 2-furylmethanthiol, 3-isopropyl-2-methoxypyrazine, 3-isobutyl-2-methoxypyrazine, 2-methyl-3-furanthiol, 7-methyl-3-methylen-1,6-octadiene (myrcene), 2-methyl propanal, 3-methylbutanal, 2-methylbutanal, 2-methylbutanoic acid, 3-methylbutanoic acid, 3-methylindole (skatole), 4-methyl-2-methoxyphenol, 3-methylphenol, 4-methylphenol, 3-(methylthio)propanal (methional), 3-(methylthio)-1-propene (allylmethyl sulfide), propanoic acid, 2-propen-1-thiole (allylmercaptan), 4-propyl-2-methoxyphenol, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4-nonadienal, nonanal, (*E*)-2-nonenal, phenylacetaldehyde, and 2-phenylacetic acid (Aldrich, Sigma-Aldrich Chemie, Taufkirchen, Germany); (*R*,*S*)-3,7-dimethyl-1,6-octadien-3-ol ((*R*,*S*)-linalool), methanthiol, and 2-phenylethanol (Fluka, Neu-Ulm, Germany); 5,6-benzo-2-pyrone (cumarin), 4-hydroxy-3-methoxybenzaldehyde (vanillin), 2-methoxy-4-(2-propenyl)phenol (eugenol), and 2-methoxyphenol (guaiacol) (Merck, Darmstadt, Germany); 4-ethyl-2-methoxyphenol, 2-methoxy-4-(*E*)-(1-propenyl)-phenol (*trans*-isoeugenol), and 1-octen-3-one, (Lancaster, Mühlheim, Germany); bis(2-methyl-3-furyl) disulfide and 2-hydroxy-3,5-dimethyl-2-cyclopenten-1-one (Acros, Geel, Belgium).

Deuterium oxide (^2H_2O > 99.8%), lithium aluminum deuteride ($LiAl^2H_4$ > 99% ^2H), and tetrahydrofuran (THF < 0.005% H_2O) were obtained from Aldrich, Sigma-Aldrich Chemie. 2-Nonyn-1-ol, methyl octanoate, and (+)-*p*-menth-1-ene were obtained from Fluka. Dess−Martin periodane was from Lancaster.

Ethanol, diethyl ether, dichloromethane, potassium hydroxide, calcium chloride, silica gel 60, sodium carbonate, sodium chloride, sodium citrate, sodium sulfate anhydrous, perchloric acid (10%), hydrochloric acid (32%), and sulfuric acid (97%) were obtained from Merck.

Diethyl ether, pentane, and dichloromethane were distilled prior to use. The sunflower oil (Vita d'Or) used in the sensory experiments was purchased from a local supermarket and underwent a SAFE distillation prior to use to remove volatile constituents.

Syntheses. *Reference Aroma Compounds*. The following reference compounds were synthesized according to the literature cited: 2-acetyl-1-pyrroline (*22*), *trans*-4,5-epoxy-(*E*)-2-decenal (*23*), 2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one (*24*), 5-methyl-2-methoxyphenol (*25*), (*Z*)-2-nonenal, and (*Z*)-1,5-octadien-3-one (*26*).

Isotopically Labeled Compounds. [2,3-$^2H_2$]-(*E*)-2-Nonenal was prepared by deuteration of 2-nonyn-1-ol followed by oxidation of the [2,3-$^2H_2$]-(*E*)-2-nonenol obtained according to ref*27*.

The target compound was characterized by the following mass spectrometric data: MS-EI, *m*/*z* (%) 41 (100), 43 (88), 55 (40), 40 (39), 57 (34), 85 (30), 56 (30), 42 (30), 72 (29), 71 (29), 83 (28), 82 (27), 59 (23), 69 (18), 81 (14), 70 (14), 44 (12), 58 (11), 95 (11), 84 (10), 68 (10), 142 (9); MS-CI, isobutane, *m*/*z* (%) 143 (100), 125 (31), 83 (15), 82 (11), 126 (9), 144 (7), 85 (7), 84 (6), 142 (5), 141 (4), 99 (4), 127 (3), 124 (3), 114 (3).

The following isotopically labeled standards were synthesized according to the literature cited: 2-acetyl-[$^2H_{2-5}$]-1-pyrroline (*22*); bis(2-[$^2H_3$]-methyl-3-furyl) disulfide (*28*); [$^2H_2$]-butanoic acid (*29*); [$^2H_4$]-(*E*,*E*)-2,4-decadienal (*30*); 2,3-diethyl-[$^2H_3$]-5-methylpyrazine (*31*); [$^2H_2$]-linalool (*32*); [$^2H_3$]-ethyl butanoate (*33*); [$^2H_3$]-2-ethyl-3,5-dimethylpyrazine and [$^2H_3$]-2-ethyl-3,6-dimethylpyrazine (*31*); [$^2H_{2-4}$]-4-ethyl-2-methoxyphenol (*34*); [$^2H_3$]-ethyl 2-methylbutanoate, [$^2H_3$]-ethyl 3-methylbu tanoate, and [$^2H_3$]-ethyl 2-methylpropanoate (*35*); [$^2H_2$]-3-ethylphenol (*36*); [$^2H_2$]-2-furylmethanthiol (*28*); [$^2H_4$]-hexanal (*37*); [$^{13}C_2$]-3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (*38*); 3-isopropyl-2-methoxypyrazine (*39*); 3-isobutyl-2-methoxypyrazine (*40*); [$^2H_3$]-2-methoxy-4-methylphenol and [$^2H_3$]-2-methoxy-5-methylphenol (*25*); [$^2H_3$]-2-methoxyphenol (*31*); [$^2H_4$]-2-methoxy-4-(*E*)-(1-propenyl)phenol and [$^2H_{2-4}$]-4-propyl-2-methoxyphenol (*34*); [$^2H_2$]-3-methylbutanal (*41*); [$^2H_2$]-3-methylbutanoic acid (*42*); [$^2H_3$]-3-(methylthio)propanal (*28*); [$^2H_2$]-(*E*,*E*)-2,4-nonadienal and [$^2H_2$]-1-octen-3-one (*30*); and [$^{13}C_2$]-phenylacetaldehyde (*43*).

[1,2-$^{13}C_2$]-Acetaldehyde, [$^2H_3$]-acetic acid, [$^2H_7$]-4-methylphenol, and [$^{13}C_2$]-2-phenylacetic acid were supplied by Sigma-Aldrich.

Determination of the Concentrations of the Isotopically Labeled Compounds. The concentrations of the labeled compounds were determined by GC-FID using methyl octanoate as internal standard. To take into account the different detector responses, first, an FID response factor was determined by GC analysis of a solution containing defined amounts of the respective unlabeled compound and methyl octanoate. In a second step, a defined amount of methyl octanoate was added to a defined volume of the solution containing the labeled compound. After analysis by GC-FID, the concentration of the labeled compound was calculated from the peak areas in the GC chromatogram using the FID response factor determined for the unlabeled analyte.

Isolation of Salami Volatiles. Salami (30 g, without skin) was cut into pieces, frozen with liquid nitrogen, mixed with 30 g of anhydrous sodium sulfate and powdered in a blender. The powder was extracted three times with diethyl ether (total volume = 500 mL) after soaking in the solvent for 2 h, 16 h (overnight), and finally 1 h, respectively. After drying over anhydrous sodium sulfate, the volatiles were isolated using the solvent-assisted flavor evaporation (SAFE) technique (*44*). To separate the acidic volatile fraction (AF) from the neutral/basic volatile fraction (NBF), the distillate was extracted with aqueous sodium carbonate solution (3 × 50 mL; pH 10.0). Then, the organic layer was washed with an aqueous saturated sodium chloride solution (2 × 30 mL). The combined aqueous solutions were adjusted to pH 2 by the addition of hydrochloric acid (32%), and the acidic volatiles were extracted with diethyl ether (3 × 50 mL). Both solutions containing either the NBF or the AF, respectively, were dried over anhydrous sodium sulfate and concentrated at 42 °C to a final volume of 400 μL by distilling off the solvent by means of a Vigreux column followed by microdistillation (*23*).

Separation of Volatiles by Column Chromatography. For the identification experiments, the volatiles were isolated from about 500 g of salami as described above. The concentrated distillate (4 mL) was fractionated by column chromatography using five water-cooled glass columns (29 cm × 1 cm i.d.) filled with a slurry of purified silica gel 60 (7% water) in pentane (*23*). Fractionation into five fractions (fractions A−E) was performed using pentane/diethyl ether mixtures of increasing polarity (100:0, 90:10, 70:30, 50:50, 0:100, v/v, 100 mL each). Each fraction was concentrated to a final volume of 200 μL at 42 °C.

High-Resolution Gas Chromatography−Olfactometry (HRGC-O). HRGC was performed by means of a gas chromatograph type 8000 Top series (Fisons Instruments, Mainz, Germany) using the following capillary columns and temperature programs: DB-FFAP (30 m × 0.32 mm i.d.; 0.25 μm film thickness) (J&W Scientific, Folsom, CA), 40 °C, 2 min, (6 °C/min) 180 °C, 0 min, (10 °C/min) 230 °C, 10 min; DB-5 (30 m × 0.25 mm i.d.; 0.25 μm film thickness) (J&W Scientific), 40 °C, 2 min, (6 °C/min) 70 °C, 0 min, (4 °C/min) 140 °C, 0 min, (15 °C/min), 250 °C, 10 min; DB-1701 (30 m × 0.25 mm i.d.; 0.25 μm film thickness) (J&W Scientific), 40 °C, 2 min, (8 °C/min) 240 °C, 10 min; and BGB-176 (30 m × 0.32 mm i.d.; 0.25 μm film thickness) (BGB Analytik AG, Anwil, Switzerland), 40 °C, 2 min, (4 °C/min) 170 °C, 0 min, (40 °C/min) 230 °C, 5 min. The samples were injected at 40 °C by means of the cold on-column technique.

For HRGC-O, the effluent was evenly split at the end of the column between a flame ionization detector (FID) and a heated sniffing port (180 °C) using a Y-shaped glass splitter and two deactivated fused silica capillaries (30 cm × 0.1 mm i.d.). Helium adjusted to a flow rate of 1.5 mL/min served as the carrier gas. Retention indices (RI) of the odorants were calculated from the retention times of *n*-alkanes by linear interpolation (*18*).

Aroma Extract Dilution Analysis (AEDA). For AEDA, the concentrated extracts of NBF and AF were stepwise diluted 1:1 using diethyl ether as the solvent to obtain dilutions of 1:1, 1:2, 1:4, 1:8, 1:16, and up to 1:4096 of the original extracts (*11*). Sniffing of dilutions was continued until no odorant could be detected by GC-O. Each odorant was thus assigned a flavor dilution factor (FD factor) representing the last dilution in which the odorant was still detectable. The evaluation of the original extracts was performed by four panelists, in particular, to agree upon the odor-active areas detectable and also on their odor qualities. AEDA was finally performed by three panelists in separate runs, and the FD factors determined were averaged.

Static Headspace−Olfactometry (SHO). SHO was performed using the equipment described by Guth and Grosch (*45*). Salami (15 g) was ground, and the powder obtained was equilibrated in a 300 mL Teflon-sealed flask at 35 °C for 30 min. To determine the relative intensities of the odorants, decreasing volumes (20−0.25 mL) were withdrawn from individual samples by means of a gastight syringe and were injected into the purge and trap system operating in the desorption mode. The headspace volatiles were collected at −100 °C in a precooled trap. After the inlet had been heated to 200 °C, the volatiles were flushed onto an DB-1701 column (30 m × 0.32 mm i.d.; 0.32 μm film thickness). The oven was held at 0 °C for 3 min, and then the temperature was raised at 6 °C/min to 230 °C. The volatiles were detected by FID and GC-O as described above.

HRGC−Mass Spectrometry (HRGC-MS). For the identification experiments, 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) at 70 eV in the electron impact mode (MS-EI) and at 115 eV in the chemical ionization mode (MS-CI; reagent gas, isobutane) using the capillaries and the oven programs described above.

Quantitation of Odorants by Stable Isotope Dilution Assays (SIDA). Volatile Isolation. Depending on the concentrations of the aroma compounds determined in preliminary experiments, different amounts of salami (from 2 to 100 g) were used to obtain concentrations between 1 and 5 μg/mL of the respective compound in the extract used for quantitation. Prior to the extraction, the salami powder was spiked with defined amounts of the 36 internal standards listed in **Table 1**. Solvent extraction and SAFE distillation were performed as described above. However, diethyl ether was substituted by dichloromethane in the second and third extraction steps, except for the quantitation of 2- and 3-methylbutanal and allylmethyl sulfide.

For the quantitation of acetaldehyde, salami (15 g) was spiked with the labeled standard in a 300 mL septum-sealed flask and equilibrated at 35 °C for 30 min prior to the headspace analysis.

For the quantitation of 2-methyl-3-furanthiol and 2-furanmethanethiol, the salami powder (100 g) was spiked with both internal standards and extracted twice with dichloromethane (150 mL). The combined extracts were concentrated, and the target compounds were enriched by reaction with *p*-hydroxymercuribenzoic acid and subsequent quantitation of the liberated thiols by dynamic headspace HRGC-MS (*46*).

In a few cases, compounds were quantified using a structurally related labeled internal standard, for example, [$^2H_2$]-3-methylbutanal for 2-methylbutanal or [$^2H_{2-4}$]-2-methoxy-4-propylphenol for 2-methoxy-4-(2-propenyl)phenol. For the quantitation of α-pinene, (+)-*p*-menth-1-ene was used as the internal standard.

Quantitation by Mass Spectrometry. Quantitation was carried out by means of three different HRGC-MS systems using the capillaries mentioned above. Acids and phenols (except for 2-methoxy-5-methylphenol) were quantified using a Varian gas chromatograph 3800 (Varian, Darmstadt, Germany) coupled to an ion trap mass spectrometer Saturn 2000 (Varian). The quantitation of the majority of the aroma compounds was performed by means of a two-dimensional gas chromatography system consisting of a Trace 2000 gas chromatograph (Thermoquest, Egelsbach, Germany) coupled to a Varian GC 3800 using the MSCC system to transfer to volatiles to the second column. Acetaldehyde, 2-methyl-3-furanthiol, and 2-furanmethanethiol were quantified using a gas chromatograph CP 9001 (Chrompack, Frankfurt, Germany) with a purge-and-trap system TCT/PTI 4001 (Chrompack) coupled to a quadrupole mass spectrometer INCOS XL (Finnigan MAT, Bremen, Germany). Mass spectra were generated in the chemical ionization mode using methanol as reagent gas.

A response factor was determined for each compound by analyzing mixtures consisting of defined amounts of the unlabeled odorants and the respective labeled standards in three different mass ratios (3:1, 1:1, and 1:3). The response factors calculated from the relative intensities of the peak areas of the selected ions and their concentrations are summarized in **Table 1**.

Determination of Isomeric Distributions. The enantiomeric ratio of linalool was determined by two-dimensional (TD) HRGC-MS using a chiral BGB-176 capillary as second column (*32*). For the differentiation

Table 1. Isotopically Labeled Standards, Selected Ions, and Response Factors (RF) Used in the Stable Isotope Dilution Assays

labeled standard	ion (*m*/*z*)[a]	ion (*m*/*z*)[b]	RF[c]
[$^{13}C_2$]-acetaldehyde	47	45	0.99
[$^{2}H_3$]-acetic acid	78	75	0.93
[$^{2}H_{2-5}$]-2-acetyl-1-pyrroline	114−117	112	0.98
[$^{2}H_6$]-bis(2-methyl-3-furyl) disulfide	233	227	0.98
[$^{2}H_2$]-butanoic acid	105	103	0.92
[$^{2}H_4$]-(*E*,*E*)-2,4-decadienal	156−158	153	0.98
[$^{2}H_3$]-2,3-diethyl-5-methylpyrazine	154	151	1.01
[$^{2}H_3$]-ethyl butanoate	120	117	1.00
[$^{2}H_3$]-2-ethyl-3,5-dimethylpyrazine	140	137	0.80
[$^{2}H_3$]-2-ethyl-3,6-dimethylpyrazine	140	137	0.92
[$^{2}H_{2-4}$]-4-ethyl-2-methoxyphenol	155−157	153	1.00
[$^{2}H_3$]-ethyl 2-methylbutanoate	134	131	0.98
[$^{2}H_3$]-ethyl 3-methylbutanoate	134	131	0.95
[$^{2}H_3$]-ethyl 2-methylpropanoate	120	117	0.99
[$^{2}H_2$]-3-ethylphenol	125	123	0.85 0.95[d]
[$^{2}H_2$]-2-furanmethanethiol	83	81	0.80 1.38[e]
[$^{2}H_4$]-hexanal	105	101	0.87
[$^{13}C_2$]-3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	131	129	1.00
[$^{2}H_3$]-2-isobutyl-3-methoxypyrazine	170	167	0.90
[$^{2}H_3$]-2-isopropyl-3-methoxypyrazine	154	151	0.98
[$^{2}H_2$]-linalool	139	137	0.87
[$^{2}H_3$]-2-methoxy-4-methylphenol	142	139	1.00
[$^{2}H_3$]-2-methoxy-5-methylphenol	142	139	1.02
[$^{2}H_3$]-2-methoxyphenol	128	125	0.96
[$^{2}H_4$]-2-methoxy-4-(*E*)-(1-propenyl)phenol	169	165	0.95
[$^{2}H_{2-4}$]-2-methoxy-4-propylphenol	169−171	165	1.02 0.94[f]
[$^{2}H_2$]-3-methylbutanal	89	87	0.99 0.80[g]
[$^{2}H_2$]-3-methylbutanoic acid	119	117	0.93
[$^{2}H_3$]-3-(methylthio)propanal	108	105	1.01
[$^{2}H_7$]-4-methylphenol	115−116	109	0.88 0.94[h]
[$^{2}H_2$]-(*E*,*E*)-2,4-nonadienal	141	139	0.91
[$^{2}H_2$]-(*E*)-2-nonenal	143	141	0.90 0.86[j]
[$^{2}H_2$]-1-octen-3-one	129	127	0.80
[$^{13}C_2$]-phenylacetaldehyde	123	121	1.00
[$^{13}C_2$]-2-phenylacetic acid	139	137	1.00

[a] Ion used to monitor the internal standard. [b] Ion used to monitor the analyte. [c] MS response factor. [d] Response factor for 4-ethylphenol. [e] Response factor for 2-methyl-3-furanthiol. [f] Response factor for 2-methoxy-4-(2-propenyl)phenol. [g] Response factor for 2-methylbutanal. [h] Response factor for 3-methylphenol. [j] Response factor for (*Z*)-2-nonenal.

of 2- and 3-methylbutanoic acid, the acidic fraction was analyzed by means of HRGC-MS in the EI mode using the FFAP capillary column. To evaluate the distribution of the characteristic mass fragments *m*/*z* 74 for 2-methylbutanoic acid and *m*/*z* 60 for 3-methylbutanoic acid, a calibration curve was prepared by analyzing defined mixtures of both isomers.

Determination of Odor Thresholds. To mimic the matrix of the salami (44% fat), odor thresholds were determined in odorless sunflower oil. To guarantee the absence of contaminating odorants, all reference aroma components were first analyzed by HRGC-O and purified by distillation, if necessary (*47*). A defined amount of the odorant in ethanol (10 μL) was pipetted into a Teflon vessel with 25 mL of sunflower oil and stirred for 2 min. The sample was judged by a panel of 10 trained assessors as described below by means of the triangular test. Twenty-five milliliters of sunflower oil was used as control, and the samples were presented in increasing concentrations (*47*). Odor thresholds were calculated according to the method in section 35 LMBG, methods 00.90-7 and 00.90-9.

Aroma Profile Analyses (APA). For the APA, 13 assessors were recruited from the German Research Center for Food Chemistry and were trained to describe and recognize the odor qualities of about 80 odorants (*47*). The following 12 aroma descriptors, represented by the aroma compounds given in parentheses, were evaluated: garlic-like (allylmethyl sulfide); pepper-like (freshly ground, black pepper); fruity (ethyl 2-methylbutanoate); roasty, popcorn-like (2-acetyl-1-pyrroline); cooked potato-like (methional); sour (acetic acid); sweaty (3-methylbutanoic acid); fatty ((*E*,*E*)-2,4-nonadienal); honey-like, sweet (phenylacetaldehyde); meat-like (bis(2-methyl-3-furyl) disulfide); smoky (2-methoxyphenol and 3-methylphenol); and seasoning-like (sotolon).

The evaluation of the orthonasal aroma (smell) of the sausage was performed in the following way: Thin slices of salami were presented to the panelists in glass vessels, and they were asked to rate the intensities of the 12 odor qualities using a 7-point linear scale of 0 (not perceivable), 0.5, 1.0, 1.5, ..., 3.0 (strong). The results obtained at three different sessions were averaged for each odor note and plotted in a spider web diagram. The values judged by the single assessors differed by not more than 20%. Sensory analyses were performed in a sensory panel room in 21 ± 1 °C at three different sessions.

Aroma Recombination. To re-engineer the natural salami aroma, a stock solution using deodorized sunflower oil was prepared containing all 29 compounds showing OAVs ≥1, and in addition bis(2-methyl-3-furyl) disulfide, (*E*,*E*)-2,4-decadienal, (*E*,*E*)-2,4-nonadienal, and (*E*)-2-nonenal in 10-fold concentration levels as compared to those in salami. To achieve concentration levels of the odorants equal to those in the salami, this stock solution was diluted 1:10 using the following two matrices: The matrix for model 1 was pure sunflower oil; for model 2, a matrix consisting of 44% sunflower oil and 56% phosphate buffer (pH 6.3) was used to simulate the natural content of fat and the actual pH value of the salami. The solutions (25 mL) were presented in glass vessels; two evaluations were done per session. The aroma profiles of the two models were described in the same way as described above for the salami. In a separate session, the overall similarity of the aroma of the freshly sliced salami and models 1 and 2, respectively, was estimated using a 7-point scale from 0 to 3.

Omission Experiments. Three model mixtures omitting the following odorants were prepared: OM 1 without (*E*)-2-nonenal, (*E*,*E*)-2,4-nonadienal, and (*E*,*E*)-2,4-decadienal; OM 2 without 2-methyl-3-furanthiol and bis(2-methyl-3-furyl) disulfide; and OM 3 without 3-isopropyl-2-methoxypyrazine. Each model mixture was presented to the sensory panel (13 panelists) in comparison to the complete model in a triangle test, and the significance α of the detected difference was calculated (*18*).

RESULTS AND DISCUSSION

Identification of Odor-Active Compounds. In a preliminary session, the sensory panel hedonically evaluated the aroma of various commercial, high-priced dry-fermented sausages of the northern (smoked) and southern European styles (air-dried). As a result, the Hungarian winter salami, which is slightly smoked and mold-ripened afterward, was favored by a group of 20 trained panelists on the basis of this hedonic evaluation. This sausage was, thus, chosen for this investigation.

To evaluate whether the overall aroma can be isolated by solvent extraction and SAFE distillation, one small drop of the distillate obtained was evaluated on a strip of filter paper. The headspace immediately evoked the characteristic aroma of the salami, thereby confirming that the key odorants were exhaustively extracted/distilled.

For the identification experiments, the distillate obtained from 30 g of salami was separated into the AF and the NBF to avoid interference of coeluting substances. By application of HRGC-O on both fractions, a great variety of odor qualities were perceived, such as fatty, honey-like, sweaty, meat-like, popcorn-like, and phenolic, but no characteristic salami-like smelling area was detectable. Sniffing of serial dilutions of the NBF revealed 47 odorants, and 8 additional odor-active regions were detected in the AF in the FD factor range of 16−4096, respectively (**Table 2**).

Table 2. Most Odor-Active Compounds (FD ≥ 16) Identified in the Hungarian Salami

no.	aroma compound [a]	aroma quality [b]	fraction [c]	RI[d] on FFAP	RI[d] on DB-5	RI[d] on OV-1701	FD factor[e]	earlier reported[f] as volatile constituent of dry sausages
1	2-and 3-methylbutanal[g]	malty	NBF	916	651	728	64	*5*
2	3-(methylthio)-1-propene (allylmethyl sulfide)	garlic-like	NBF	948	695	740	32	*9*
3	ethyl 2-methylpropanoate	fruity	NBF	964	753	816	32	*8*
4	α-pinene	pine-like	NBF	1009	930	946	32	*6*
5	ethyl butanoate	fruity	NBF	1028	802	861	32	*9*
6	ethy 2-methylbutanoate	fruity	NBF	1044	849	909	64	*8*
7	ethyl 3-methylbutanoate	fruity	NBF	1063	853	913	32	*8*
8	hexanal	green, grassy	NBF	1079	801	885	16	*3*
9	1-octen-3-one	mushroom-like	NBF	1293	974	1068	256	*49*
10	2-acetyl-1-pyrroline	roasty, popcorn-like	NBF	1325	924	1012	256	*48*
11	(*Z*)-1,5-octadien-3-one[h]	geranium-like	NBF	1366	979	1081	16	
12	nonanal	citrus-like, soapy	NBF	1383	1106	1195	16	*4*
13	3-isopropyl-2-methoxypyrazine	earthy, pea-like	NBF	1423	1049	1141	64	
14	2-furylmethanthiol	roasty, sulfurous	NBF	1427	912	995	128	*48*
15	acetic acid	pungent, sour	AF	1436	nd	733	2048	*2*
16	3-(methylthio)propanal (methional)	cooked potato-like	NBF	1447	907	1043	512	*50*
17	2-ethyl-3,5(6)-dimethylpyrazine	earthy	NBF	1447	1084	1154	16	
18	2,3-diethyl-5-methylpyrazine	earthy	NBF	1480	1151	1218	32	
19	unknown	roasty, sulfurous	NBF	1495	nd	nd	32	
20	(*Z*)-2-nonenal	fatty, tallowy	NBF	1499	1147	1261	32	*51*
21	3-isobutyl-2-methoxypyrazine	earthy, pea-like	NBF	1513	1184	1235	16	
22	(*E*)-2-nonenal	fatty, tallowy	NBF	1522	1160	1274	32	*8*
23	propanoic acid	sweaty	AF	1524	nd	nd	16	*2*
24	(*R*/*S*)-linalool	flowery	NBF	1542	1100	1199	64	*6*
25	unknown	fatty	NBF	1614	nd	nd	16	
26	butanoic acid	sweaty, cheese-like	AF	1619	836	979	256	*2*
27	phenylacetaldehyde	honey-like	NBF	1632	1041	1183	512	*7*
28	unknown	flowery, honey-like	NBF	1651	nd	nd	16	
29	2- and 3-methylbutanoic acid[g]	sweaty	AF	1661	867	1030	512	*3*
30	(*E*,*E*)-2,4-nonadienal	fatty	NBF	1693	1217	1358	16	*51*
31	2-acetyl-2-thiazoline	roasty, popcorn-like	NBF	1747	1103	1244	32	
32	2-hydroxy-3,5-dimethyl-2-cyclopenten-1-one	caramel-like	AF	1790	1204	1056	16	
33	2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one	seasoning-like	AF	1832	1226	1073	32	
34	2-methoxyphenol (guaiacol)	smoky, sweet	NBF	1853	1083	1230	4096	
35	2-phenylethanol	honey-like	NBF	1903	1111	1277	32	*13*
36	2,6-dimethylphenol	phenolic	NBF	1907	nd	1284	32	
37	5-methyl-2-methoxyphenol	smoky, sweet	NBF	1935	1183	1330	1024	
38	4-methyl-2-methoxyphenol	sweet	NBF	1952	1189	1342	32	
39	*trans*-4,5-epoxy-(*E*)-2-decenal	metallic	NBF	1997	1384	1550	32	
40	4-ethyl-2-methoxyphenol	clove-like	NBF	2023	1271	1430	512	
41	4-methylphenol	horse-like, phenolic	NBF	2077	1072	1307	128	
42	3-methylphenol	phenolic	NBF	2087	1074	1311	64	*6*
43	4-propyl-2-methoxyphenol	clove-like	NBF	2100	nd	1522	512	
44	bis(2-methyl-3-furyl) disulfide	cooked meat-like	NBF	2138	1534	1648	128	
45	2-methoxy-4-(2-propenyl)phenol (eugenol)	clove-like	NBF	2164	1350	1517	2048	
46	4-ethylphenol	phenolic	NBF	2171	1165	1396	256	
47	3-ethylphenol	phenolic	NBF	2178	1168	1402	512	
48	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon)	seasoning-like	AF	2195	1105	1359	256	
49	unknown	rubber-like, phenolic	NBF	2252	n.d.	n.d.	32	
50	2,6-dimethoxyphenol	smoky, sweet	NBF	2271	1346	1548	256	
51	2-methoxy-4-(*E*)-(1-propenyl)phenol (*trans*-isoeugenol)	clove-like	NBF	2350	1455	1632	2048	
52	coumarin	sweet	NBF	2458	1442	1693	16	
53	3-methylindole (skatole)	fecal-like	NBF	2486	1391	1637	32	*13*
54	2-phenylacetic acid	honey-like	AF	2559	1262	1513	64	*13*
55	4-hydroxy-3-methoxybenzaldehyde (vanillin)	vanilla-like, sweet	NBF	2585	1410	1637	32	

[a] Identification was performed by comparing the following criteria: retention index on three capillary columns detailed in the table; odor quality and odor threshold perceived at the sniffing port; mass spectra obtained by MS-CI and MS-EI with reference compounds. [b] Odor quality perceived at the sniffing port. [c] Acidic (AF) and neutral/basic (NBF) fraction. [d] Linear retention index. nd, not determined (the compound was not detected during HRGC-O on this capillary column). [e] Flavor dilution factor. [f] Reported earlier as volatile compound of salami in the literature cited. [g] The stereochemistry was determined during quantitation. [h] The MS signal was too weak for an unequivocal interpretation. Tentative identification is based on the remaining criteria given in footnote [a].

To identify the compounds responsible for these odors, their odor qualities and intensities as well as their retention indices on three capillary columns were compared to data available in an in-house database containing more than 500 previously characterized reference aroma compounds. To obtain enough material for mass spectrometry, the volatiles were isolated from 500 g of salami and the NBF was further fractionated by column chromatography on silica gel (*23*). The single fractions were

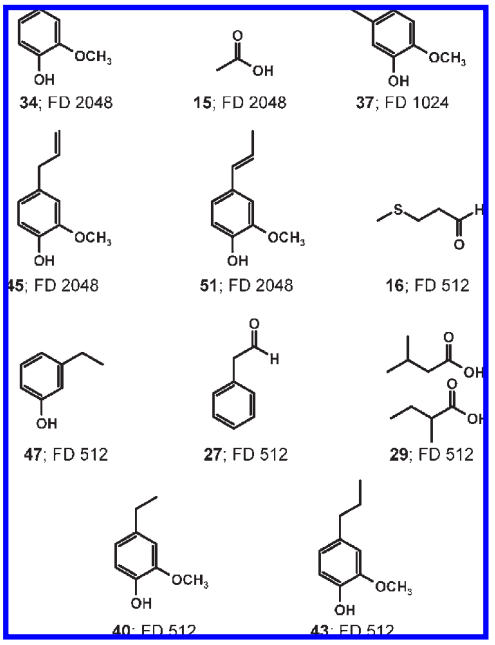

Figure 1. Structures of key odorants identified in the Hungarian-type salami.

concentrated, and the respective odorants were located in the fractions by GC-O. By application of GC-MS, in most cases by this procedure mass spectra were obtained, which could be compared to the spectra of reference odorants.

In the NBF, the compound with the highest FD factor of 4096 was identified as 2-methoxyphenol (**34**) (**Figure 1**) followed by the two structurally related phenolic compounds eugenol (2-methoxy-4-(2-propenyl)phenol; **45**) and *trans*-isoeugenol (2-methoxy-4-(*E*)-(1-propenyl)phenol; **51**). The last mentioned two methoxyphenols showed almost similar MS spectra but were clearly differentiated by their retention indices (**Table 2**).

Further phenolic compounds with high FD factors were 5-methyl-2-methoxyphenol (**37**), 4-propyl-2-methoxyphenol (**43**), 4-ethyl-2-methoxyphenol (**40**), and 3-ethylphenol (**47**). In addition, phenylacetaldehyde (**27**) and methional (**16**) showed high FD factors (**Figure 1**). In the acidic fraction, the highest FD factor of 2048 was found for acetic acid (**15**), followed by 2- and 3-methylbutanoic acid (**29**).

In agreement with data (*12*) reported for a self-prepared, unsmoked salami, acetic acid, 3-methylbutanoic acid, and eugenol were among the compounds with the highest FD factors. By contrast, diallyl disulfide, which had shown the highest FD factor in the previous study (*12*), was not even detected as an odorant in the Hungarian-type salami. However, this could be due to different ingredients used in the manufacturing process.

Altogether 51 of the 55 aroma-active compounds detected in the FD factor range of 16−4096 were identified (**Table 2**), among which 19 compounds are reported here for the first time as salami constituents. Among them, *trans*-isoeugenol, 5-methyl-2-methoxyphenol, 3-ethylphenol, 4-ethylphenol, 4-propyl-2-methoxyphenol, and sotolone showed very high FD factors, although most of them gave no FID signal. Thus, enrichment by column chromatography prior to GC-O and MS analysis was necessary for their unequivocal identification. These results suggested that, besides the different types of salami previously analyzed, the lacking FID signal might be another reason these odorants have so far not been reported in salami.

During distillation and concentration steps prior to the AEDA, highly volatile odorants may get lost and, consequently, their aroma contribution might be underestimated. Thus, SHO was performed to bridge this gap. Compared to the results of the AEDA, additionally acetaldehyde, methanthiol, 2-methylpropanal, 2-propene-1-thiol (allyl-1-thiol), and 2,3-butandione were detected by GC-O. However, with the exception of acetaldehyde reaching an FD factor of 16 (data not shown), these compounds showed comparatively low FD factors (< 16).

Quantitation of Important Odorants and Calculation of Odor Activity Values (OAVs). To perform aroma recombination experiments, exact quantitative data of the key aroma compounds in the salami are needed. Therefore, using a stock of isotopically labeled compounds available in our group, a total of 45 compounds were quantified in the Hungarian salami by means of stable isotope dilution assays. The data showed (**Table 3**) that by far the highest concentration was present for acetic acid (1.2 g/kg), followed by phenylacetaldehyde, butanoic acid, 2-methoxyphenol, eugenol, 4-methyl-2-methoxyphenol, 3-methylphenol, 4-ethyl-2-methoxyphenol, 2-phenylacetic acid, 2,6-dimethoxyphenol, and 4-methylphenol, which all occurred in amounts of > 1 mg/kg.

On the other hand, very low concentrations of < 1 μg/kg were determined for, for example, 2-methyl-3-furanthiol and five pyrazines. Quantitative analyses of 2−10 different extracts, prepared in separate experiments from the same batch of the salami, revealed some differences in concentrations up to a factor of 2, for example, for ethyl 3-methylbutanoate (**Table 3**). However, these differences may be due to the natural variations of aroma compounds in dry-fermented sausages, because a rather large variation was reported to be common for meat products (*16*).

To get an idea of how the concentrations of the key odorants are related to their aroma contribution, OAVs were calculated for all compounds quantified. The odor thresholds, in particular of allylmethyl sulfide, 5-methyl-2-methoxyphenol, 3-methylphenol, 3-ethylphenol, eugenol, 2,6-dimethoxyphenol, α-pinene, 4-methyl-2-methoxyphenol, and 4-ethylphenol in oil, were not available in the literature and were, thus, determined in this study (**Table 4**).

Calculation of OAVs (**Table 4**) revealed by far the highest OAV for acetic acid (8830), followed by acetaldehyde (1610), methional (740), phenylacetaldehyde (330), 2-methoxyphenol (213), and 2-acetyl-1-pyrroline (140). The latter result confirmed previous data suggesting 2-acetyl-1-pyrroline to be an important flavor compound of mold-ripened salami (*48*). Eight additional odorants exceeded their odor thresholds by 10 times or more, namely, butanoic acid, 3-methylbutanoic acid, 3-methylbutanal, 3- and 4-methylphenol, sotolon, 3-ethylphenol, 5-methyl-2-methoxyphenol, and allylmethyl sulfide. With comparatively low OAVs, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl 2-methylpropanoate, and (*R*)-linalool are suggested to contribute less to the salami aroma. These compounds had earlier been proposed as aroma compounds in an Italian salami (*12*).

Although high FD factors of 2048 were found for eugenol and *trans*-isoeugenol, both compounds exhibiting a clove-like smell, they were not likely to influence the overall aroma of the salami under investigation. For eugenol, a low OAV of 7 was calculated, and the concentration of *trans*-isoeugenol did even not reach its odor threshold in oil. This discrepancy can be explained by the fact that during AEDA the entire amount of a given odorant is vaporized and, thus, easily reaches the human olfactory receptors, whereas the concentrations in the air above a matrix depend on the volatility of an odorant and are, without

Table 3. Concentrations of 45 Important Aroma Compounds in the Hungarian Salami

aroma compound	concn (μg/kg)	range (μg/kg)	n^a
acetic acid	1190000	1060000−1420000	6
phenylacetaldehyde	7910	6410−9490	4
butanoic acid	6560	6060−7030	4
2-methoxyphenol	3620	3290−3910	4
eugenol	3500	3300−3850	6
4-methyl-2-methoxyphenol	2520	2510−2540	2
3-methylphenol	2150	2110−2190	2
4-ethyl-2-methoxyphenol	1900	1700−2050	4
2-phenylacetic acid	1880	1800−1960	2
2,6-dimethoxyphenol	1610	1600−1620	2
4-methylphenol	1040	1040	2
4-ethylphenol	846	845−847	2
3-methylbutanoic acid	725	553−980	8
4-propyl-2-methoxyphenol	454	453−455	2
α-pinene	413	386−440	2
3-ethylphenol	399	395−403	2
acetaldehyde	387	357−418	2
2-methylbutanoic acid	346	264−468	8
trans-isoeugenol	325	240−444	4
allylmethyl sulfide	212	204−220	4
3-methylbutanal	156	138−174	4
methional	148	121−187	10
(*R*)-linalool	147	144−149	2
5-methyl-2-methoxyphenol	129	129−129	2
hexanal	88	86−90	2
2-methylbutanal	85	84−86	2
(*E*)-2-nonenal	61	57−63	4
ethyl butanoate	23	20−26	4
2-acetyl-1-pyrroline	14	12−16	6
1-octen-3-one	7.3	5.7−9.3	4
(*E*,*E*)-2,4-decadienal	6.3	5.6−7.2	4
(*E*,*E*)-2,4-nonadienal	7.1	5.8−8.7	4
(*Z*)-2-nonenal	6.6	6.2−7.5	4
ethyl 2-methylpropanoate	2.8	2.5−3.0	4
bis(2-methyl-3-furyl) disulfide	2.3	1.6−3.2	6
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	2.0	1.9−2.1	4
ethyl 3-methylbutanoate	2.0	1.4−2.9	6
2-furanmethanethiol	1.9	1.7−2.2	3
ehyl 2-methylbutanoate	1.5	1.2−1.7	6
2-methyl-3-furanthiol	0.72	0.63−0.82	2
2-ethyl-3,6-dimethylpyrazine	0.66	0.65−0.67	2
2-ethyl-3,5-dimethylpyrazine	0.58	0.57−0.58	2
3-isobutyl-2-methoxypyrazine	0.38	0.36−0.39	2
2,3-diethyl-5-methylpyrazine	0.24	0.22−0.23	2
3-isopropyl-2-methoxypyrazine	<0.10	<0.10	2

[a] Number of replicates.

Table 4. Orthonasal Odor Thresholds and Odor Activity Values of 45 Important Aroma Compounds in the Hungarian Salami

aroma compound	odor threshold (μg/kg of oil)	ref	OAV[a]
acetic acid	135	*52*	8830
acetaldehyde	0.24	*52*	1610
methional	0.20	*53*	740
phenylacetaldehyde	24	*54*	330
2-methoxyphenol	17	*56*	213
2-acetyl-1-pyrroline	0.10	*55*	140
butanoic acid	147	*29*	45
3-methylbutanoic acid	24	*52*	30
3-methylbutanal	5.9	*53*	26
4-methylphenol	68	*57*	15
allylmethyl sulfide	22	*b*	10
5-methyl-2-methoxyphenol	13	*b*	10
3-methylphenol	190	*b*	10
sotolon	0.20	*58*	10
3-ethylphenol	44	*b*	9
eugenol	512	*b*	7
2,6-dimethoxyphenol	263	*b*	6
ethyl 2-methylbutanoate	0.28	*52*	5
2-furanmethanethiol	0.40	*56*	4
(*R*)-linalool[c]	37	*22*	4
2-phenylacetic acid	360	*36*	4
ethyl 3-methylbutanoate	0.68	*52*	3
4-propyl-2-methoxyphenol	157	*34*	3
α-pinene	274	*b*	2
ethyl 2-methylpropanoate	1.35	*52*	2
4-methyl-2-methoxyphenol	1485	*b*	2
4-ethyl-2-methoxyphenol	792	*34*	2
2-methyl-3-furanthiol	0.56	*46*	1
4-ethylphenol	627	*b*	1
2-methylbutanal	152	*57*	<1
ethyl butanoate	31	*53*	<1
hexanal	326	*30*	<1
1-octen-3-one	11	*30*	<1
2-ethyl-3,6-dimethylpyrazine	62	*58*	<1
2,3-diethyl-5-methylpyrazine	0.60	*58*	<1
2-ethyl-3,5-dimethylpyrazine	2.4	*58*	<1
(*Z*)-2-nonenal	4.9	*32*	<1
3-isobutyl-2-methoxypyrazine	0.90	*58*	<1
(*E*)-2-nonenal	978	*30*	<1
2-methylbutanoic acid	203	*34*	<1
(*E*,*E*)-2,4-nonadienal	2720	*30*	<1
(*E*,*E*)-2,4-decadienal	196	*30*	<1
bis(2-methyl-3-furyl) disulfide	204	*59*	<1
trans-isoeugenol	978	*34*	<1
3-isopropyl-2-methoxypyrazine	0.075	*60*	<4

[a] Odor activity values were calculated by dividing the concentration of the aroma compound by the odor threshold. [b] Odor thresholds newly determined in this study. [c] The odor acitivity value of (*R*)-linalool was calculated using the amount of (*R*)-linalool (61.1%) and the odor threshold of (*R*)-linalool (37 μg/kg). The amount of (*S*)-linalool, the odor threshold of which is about 80 times higher compared to that of the (*R*)-isomer (*61*), was neglected.

doubt, substantially lower. Thus, the significance of eugenol for the salami aroma reported in ref *12* could not be confirmed for the product analyzed in our study. A further 15 odorants showed OAVs of <1 and, thus, should not contribute to the aroma of the Hungarian-type salami. Among these compounds were several fatty acid degradation products such as hexanal, (*E*)-2-nonenal, and (*E*,*E*)-2,4-nonadienal as well as all pyrazines quantified.

Flavor Reconstitution and Omission Experiments. Because no single aroma compound was found to possess a typical salami-like odor, aroma reconstitution experiments were performed to reveal if the composition of the identified odorants in their "natural" concentrations were able to simulate the typical aroma of the Hungarian-type salami.

The first experiment was carried out using pure sunflower oil as the matrix (model 1), which was spiked with all aroma compounds showing OAVs of ≥ 1 (**Table 4**). Additionally, (*E*)-2-nonenal (fatty), (*E*,*E*)-2,4-decadienal (fatty), (*E*,*E*)-2,4-nonadienal (fatty), and bis(2-methyl-3-furyl) disulfide (meat-like) were included in the reconstitution experiments to judge whether these compounds might be responsible for the "fatty" and "meat-like" odor impressions detected by the sensory panel in the aroma profile analysis. A trained sensory panel evaluated the aroma of the model mixture containing these 33 compounds as well as the aroma of the salami using 12 aroma descriptors (**Figure 2**). Although most odor qualities of model 1 were judged to have similar intensities as for the original salami aroma, the sour odor note was rated much higher as compared to the original salami aroma. Therefore, the overall similarity of

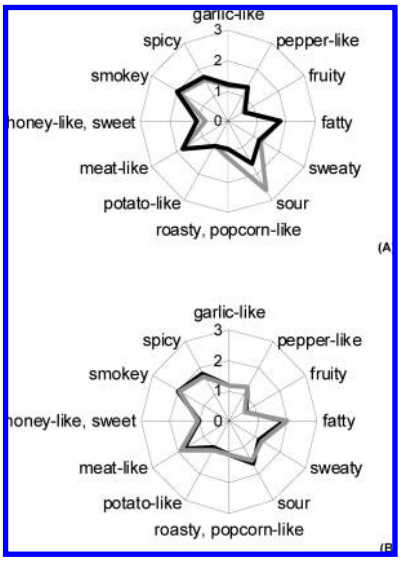

Figure 2. Comparative aroma profile analyses of (**A**) the Hungarian-type salami (black) and model mixture 1 (gray) consisting of 33 odorants dissolved in pure sunflower oil and (**B**) the Hungarian-type salami (black) and the model mixture 2 (gray) consisting of 33 odorants dissolved in phosphate buffer (pH 6.3) and sunflower oil (56:44; by vol).

the aroma of the model with the original salami aroma was judged to be only 2.1 of 3.0.

For a better simulation of the salami matrix, a second model was prepared using 44% of sunflower oil and 56% phosphate buffer to achieve the original pH of 6.3 (model 2). Evaluation of the aroma in comparison to the original salami flavor revealed that now all odor notes were estimated with nearly identical intensities for model mixture 2 and the original salami. Even the sour odor note was rated with a high similarity of 1.5 points in comparison with 1.6 points for the original salami. Moreover, the degree of overall similarity was judged by the sensory panel with a value of 2.8 of 3.0.

These data confirm that the key aroma compounds of the Hungarian-type salami were successfully identified and quantified in this investigation. Furthermore, the reconstitution experiments clearly demonstrated the influence of the respective matrix on the release of flavor compounds. In particular, the low odor threshold of acetic acid in oil (135 μg/kg; (*52*)) compared to that in water (180000 μg/kg; (*47*)) resulted in an overestimation of the sour odor quality in model 1, which was prepared in pure sunflower oil.

Omission experiments were finally performed to determine whether the set of compounds with OAVs of <1 actually contributed to the "fatty" and "meat-like" flavor notes described in the APA. Two model mixtures were prepared in a matrix of 44% of sunflower oil and 56% of phosphate: model OM 1, in which (*E*)-2-nonenal, (*E*,*E*)-2,4-decadienal, and (*E*,*E*)-2,4-nonadienal were omitted, and model OM 2, omitting bis(2-methyl-3-furyl) disulfide and 2-methyl-3-furanthiol. Both were presented to the sensory panel in triangle tests in comparison to the entire model mixture.

The data showed that neither the omission of the three fatty-smelling compounds in model OM 1 nor the omission of the two cooked meat-like smelling compounds in model OM 2 were judged to be significant, because only 9 or 6 panelists, respectively, recognized the deviating mixture in 24 tests. Furthermore, also no significant difference was found between the entire model 2 and model OM 3 omitting 3-isopropyl-2-methoxypyrazine.

The results suggest short-chain fatty acids and fermentation products, such as acetic acid, acetaldehyde, butanoic acid, and 3-methylbutanoic acid as well as amino acid degradation products such as methional, phenylacetadehyde, and 3-methylbutanal, as important aroma compounds of the Hungarian-type salami under investigation. Moreover, numerous phenols contributed to the overall smoky, phenolic note. Although it might be speculated how these odorants were formed or simply transferred from the raw materials during salami manufacturing, systematic studies comparing the amounts of odorants already present in the raw materials are needed to draw the right conclusions. These studies are currently underway.

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