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Identification and Synthesis of New Volatile Molecules Found in Extracts Obtained from Distinct Parts of Cooked Chicken

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Supporting Information

ABSTRACT: Several chicken parts (skin, fat, juice) were cooked in different ways (roasting, simmering) and investigated separately for their volatile composition. In-depth GC/MS analysis of the separate fractions revealed several unknown molecules. Mass spectra interpretation allowed us to identify nine molecules for the first time in chicken, including cyclic aldehydes, cyclic ketones, and new δ-lactones containing an unsaturated linear chain. Identification was confirmed by chemical synthesis followed by comparison of the mass spectra and linear retention indices. The natural occurrence of five of these molecules is reported here for the first time in a natural product.

KEYWORDS: chicken, Bresse chicken, poultry, roasted fat, cooked fat, roasted skin, roasted juice, aroma compounds, GC/MS

INTRODUCTION

The complexity of the flavor of cooked meat results from the large number of thermally induced reactions which can occur during heating. In particular, the Maillard reaction and the degradation of lipids account for the wide range of volatiles found in cooked meat. The volatile composition of cooked and roasted chicken meat has been extensively analyzed and reviewed in the literature.[1,2] The compounds identified in all types of meat include the entire range of aroma chemicals, but those that contribute highly to overall aroma range from aldehydes to sulfur-containing components to heterocycles (furans, pyrroles, pyrazines, thiazoles, thiophenes, oxazoles, pyridines).

Saturated and unsaturated aldehydes bearing 6−10 carbons constitute the major part of the volatile compounds found in cooked meat. Compared with beef meat, chicken meat contains a higher proportion of unsaturated fatty acids in the triglycerides, which produce more unsaturated volatile aldehydes upon degradation. As these aldehydes generally have a strong green, fatty, tallow aroma, they are thought to have a major role in the meat aroma. In particular, 2,4-decadienal is well-known as a major volatile component of cooked and roasted chicken, contributing to its fatty aroma.[1] An obvious oxidation product of 2,4-decadienal, *trans*-4,5-epoxy-trans-2-decenal, was reported by Shi and Ho as having "probably an important flavor contribution of fried chicken".[2] Several dienals and trienals (C_{10}−C_{14}) were also isolated by Harker and Begemann in cooked chicken and compared with model substances.[3] Werkhoff et al. reported many aroma-active compounds identified for the first time in chicken, including 5-alkylcyclopentene-1-carbaldehydes and methyl-branched long-chain saturated aliphatic aldehydes.[4]

Sulfur-containing components are generally found in smaller amounts in chicken meat than in beef meat, but some of them may still contribute to the overall meaty aroma because of their very low odor threshold. Gasser and Grosch identified 2-methyl-3-furanthiol and its disulfide, bis(2-methyl-3-furyl) disulfide, as well as 2-furfurylthiol and methional, as primary odorants of chicken broth.[5] Thialdine (2,4,6-trimethyl-5,6-dihydro-1,3,5-dithiazine) and 2,4,6-trimethyl-1,3,5-trithiane were reported by Tang et al. in fried chicken [6] and described as being useful for the creation of chicken flavors.[7] More recently, Werkhoff et al. identified new alkyl-substituted 1,2,4-trithiolanes, as well as new aliphatic sulfur-containing components.[4]

Heterocyclic compounds found in chicken, including furans, thiophenes, pyrazines, thiazoles, pyridines, and trithiolanes, have been reviewed by Shibamoto.[8] Furans, especially 2-alkylfurans, are known to be present in chicken meat and fat.[6] Many alkylpyrazines have been identified in chicken meat and recognized as important trace flavor compounds for their roasted, nut-like, or toasted character.[2] Numerous thiazoles and some thiophenes have been reported in fried chicken by Tang et al.[6] Several pyridines have also been identified in chicken meat or fat. 2-Methylpyridine was identified by Horvat in 1976,[9] and Tang et al. later identified pyridine, 2-methylpyridine, 2-butylpyridine, 2-pentylpyridine, 3-ethylpyridine, and 4-ethylpyridine in fried chicken.[6]

Compared with chicken meat, the skin and dripping fat of roasted chicken have been investigated less extensively. In 1986, Noleau and Toulemonde quantified the volatile compounds found in a roasted skin extract obtained by Likens−Nickerson

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extraction.[10] More than 200 compounds were identified and quantified. Later, they isolated the volatiles of roasted chicken fat by Leybold distillation.[11] The analysis revealed a large amount of aldehydes (59%) and acids (20%), whereas many alcohols, ketones, and lactones were found in lower amounts. Esters, furans, pyrazines, pyrroles, and thiazole were found in trace amounts, and some compounds remained unidentified.

In the present study, aroma extracts of different tonalities were obtained from distinct parts of cooked chicken (skin, fat, juice) prepared with different cooking procedures. The volatile composition of each extract was analyzed by GC/MS with the aim of better understanding the complexity of chicken flavor and identifying new aroma molecules.

MATERIALS AND METHODS

Freshly distilled pentane (analytical grade, SDS, France) and diethyl ether (analytical grade, SDS, France), dichloromethane (atrasol, SDS, France) were used for sample preparation and fractionation. The Leybold (or short-path, or thin-film) distillation unit was purchased from Leybold-Heraeus (Oetikon, Switzerland) more than 30 years ago. A similar apparatus is currently commercialized by ChemTech (Lockforf, IL), model KDL-4.

Sample Preparation. A special variety of chicken, poulet de Bresse AOC (Miéral, France), was used for this study (AOC, "Appelation d'Origine Contrôlée," is an official certification of origin and high quality from the French Department of Agriculture). This chicken variety is well-known for its gustative quality because of a high fat content. The abdominal fat of 20 chickens was removed before they were roasted for 1 h in an oven at 180 °C. After 30 min, the juice was regularly used to baste the chicken, thereby increasing the grilling of the skin. After 1 h, the dripping fat and the juice were collected and separated in a funnel to provide both *roasted fat* (fatty fraction) and *roasted juice* (aqueous phase). *Cooked fat* was obtained by gently simmering the abdominal fat (2 kg) in a pan with 8% added water for 2 h. After filtration of the brown residues, a yellow shiny oil was obtained. It was stored at −18 °C before Leybold distillation.

Leybold Distillation of Cooked Fat: Extracts A, B, and C. The cooked fat was gently melted in a water bath (50 °C). Molecular distillation of cooked fat (2 kg) was performed on Leybold laminar distillation equipment at 60 °C (0.06−0.03 mbar, 400 rpm) in 10 batches of 200 g. The oil feeding rate was about 100 mL/h. For each batch, the coldfinger (−12 °C) of the Leybold distillation unit was rinsed with pentane (15 mL), and the combined batches were concentrated to 5 mL by using a Vigreux column to give the "cooked fat Leybold distillate," extract A. Similarly, the trap (−195 °C) of the Leybold unit was rinsed three times by using 15 mL of pentane, and the combined rinses were dried over $MgSO_4$ and concentrated to a volume of 2 mL by using a Vigreux column to give the "cooked fat Leybold trap," extract B.

For one batch, the volatiles were captured by solid-phase microextraction (SPME) for 15 min directly from the trap with a gray notched fiber (DVB/CAR/PDMS, 1 cm, Supelco 57328-U) while the trap was defrosting. The fiber was then desorbed in a gas chromatograph/mass spectrometer (GCMS 6890/5972, Agilent, equipped with a polar Supelcowax10 capillary column) for 5 min, in splitless mode for 30 s. The temperature program was as described in the following sections. The extract obtained is called "cooked fat Leybold SPME," extract C hereafter.

Fractionation of the Cooked Fat Leybold Distillate Extract A. Cooked fat Leybold distillate extract A (1 g) was fractionated by flash chromatography over silica gel (32−63, 60 Å, Brunschwig) with a gradient of pentane/diethyl ether, from 100:0 to 0:100 in 111 tubes of 25−30 mL, which were grouped according to their qualitative odor evaluation on a paper strip. The resulting 10 fractions, called A-1 to A-10, were concentrated over a Vigreux column and then under a gentle argon flux to volumes of about 30−200 μL. Fraction A-7 (40 mg) (eluted with pentane:diethyl ether 50:50) had to be treated with deactivated alumina to remove fatty acids before GC/MS analysis. Deactivated alumina was obtained by thoroughly mixing with 4% of water and stirring for 2 h using a rotavapor. Fraction A-7 was percolated through 650 mg of deactivated alumina in a Pasteur pipet, resulting in A-7Al.

Preparation of Roasted Fat (Likens−Nickerson): Extract D. Roasted fat (56 g) in deionized water (560 mL) was codistilled with pentane (150 mL) for 1 h 30 min at atmospheric pressure in Likens−Nickerson equipment. The organic phase was dried over $MgSO_4$ and concentrated over a Vigreux column, yielding the "roasted fat (Likens−Nickerson)," extract D (20 mg).

Preparation of Roasted Juice Hydrodistillate: Extract E. Roasted juice (1400 g) in deionized water (600 mL) was hydrodistilled under reduced pressure by using the following program at 200 rpm: 25 mbar at 45 °C for 1 h 15 min, 20−10 mbar at 55 °C for 15 min. The aqueous distillate was saturated with sodium chloride and extracted with pentane:dichloromethane 2:1 (3 × 90 mL). The combined organic phase was dried over $MgSO_4$. The solvent was removed by using a Vigreux column. The residual solvent was further removed under a gentle argon flux, yielding the "roasted juice hydrodistillate," extract E (ca. 2 g).

Preparation of Roasted Skin Hydrodistillate: Extract F. The skin of four roasted chickens (220 g) was cut into small pieces, put into deionized water (850 mL), and hydrodistilled under reduced pressure with the following program at 200 rpm: 25 mbar at 43 °C for 1 h, and then 20−15 mbar at 50 °C for 30 min. The aqueous distillate was saturated with sodium chloride and extracted with pentane:dichloromethane 2:1 (3 × 90 mL). The combined organic phases were dried over $MgSO_4$. The solvent was removed by using a Vigreux column. The residual solvent was further removed under a gentle argon flux, yielding the "roasted skin hydrodistillate," extract F (ca. 300 mg).

Fractionation of the Roasted Skin Hydrodistillate Extract F. Roasted skin hydrodistillate (∼200 mg) was fractionated by flash chromatography over silica gel (32−63, 60 Å, Brunschwig) with a gradient pentane:diethyl ether, from 100:0 to 0:100, resulting in 97 tubes of 5−10 mL, which were grouped according to qualitative odor evaluation on a paper strip. The resulting 10 fractions, called F-1 to F-10, were concentrated over a Vigreux column and a gentle argon flux.

Odor Evaluations of the Extracts A, B, D−F and Fractions A1−10 and F1−10. The extracts and fractions were evaluated on a paper strip by expert flavorists who used in-house descriptors.

Cooked fat Leybold distillate extract A: "heavy, sweet, fatty, creamy"; cooked fat Leybold trap extract B: "animal, honey, heavy, malty, lactonic, cocoa"; roasted fat extract D: "roasted, fatty, pungent, malty, sweaty"; roasted juice extract E: "grilled, fatty, cardboard, woody"; roasted skin extract F: "typical, grilled, roasted skin".

Flash chromatography fractions: A-1: "fresh, sulfury, grilled"; A-2: "grilled, nutty "; A-3: "fatty, green, aldehydic"; A-4: "sweet, nutty, pyrazinic, paint, fatty, caramel"; A-5: "phenolic, animalic, dusty"; A-6: "peanut, fatty, green, roasted"; A-7: "lactonic, peachy, creamy"; A-8: "sweet, creamy, lactonic, caramel, creamy, burnt"; A-9: "cooked, creamy, coffee"; A-10: "burnt, acidic"; F-1: "coal, hydrocarbon, petrol"; F-2: "weak"; F-3: "sweet, fatty, cardboard"; F-4: "fishy, aldehydic, decanal-like, citrus"; F-5: "coconut, creamy, lactonic"; F-6: "fatty, chicken skin, burnt, pyrazinic"; F-7: "acetyl pyrazine-like, cereal"; F-8: "fruity, baked, coconut, lactonic, burnt"; F-9: "acidic, sweaty, animalic, cooked"; F-10: "sweaty, vegetable".

GC/MS: General Conditions. GC/MS analyses were performed on a GCMS 6890/5972 (Agilent) equipped with a polar column Supelcowax10 capillary column (30 m × 0.25 mm, film thickness 0.25 μm); oven temperature program: 50 °C, 5 min isotherm and then 5 °C/min to 240 °C; injector temperature 250 °C; transfer line temperature 250 °C; carrier gas: helium, constant flow rate 1 mL/min; split ratio 1:50. Analyses were repeated on a GCMS 6890/5973 (Agilent)

equipped with a nonpolar column SPB-1 capillary column (30 m × 0.25 mm, film thickness 1 μm); oven temperature program: 60 °C, 5 min isotherm and then 5 °C/min to 250 °C; injector temperature 250 °C; transfer line temperature 250 °C; carrier gas: helium, constant flow rate 1 mL/min; split ratio 1:50.

Mass spectra were generated at 70 eV at a scan range from *m/z*: 27−350. Linear retention indices (LRIs) were determined after injection of a series of *n*-alkanes (C_5−C_{28}) under identical conditions.

Alternatively, some analyses were performed on a Varian 300-MS triple quadruple GC/MS system equipped with a CombiPal autosampler, two split/splitless injectors, a VF-WAXms column (30 m × 0.25 mm, film thickness 0.25 μm) and a VF-1 ms column (30 m × 0.25 mm, film thickness 0.25 μm) installed on each of the injectors connected to the transfer line (methyl deactivated fused silica tubing, 0.25 mm × 30 cm) via a fused silica Y connector (Supelco, PA, PN 23631). VF-1 and VF-WAX columns are equivalent to the widely used DB-1 and DB-Wax columns. The transfer line temperature was set at 250 °C, and the ion source temperature was set at 175 °C. The GC conditions were as follows: injector temperature 250 °C; both columns were operated at a constant flow of 0.7 mL/min; oven temperature program: 40 °C held for 5 min, 5 °C/min to 240 °C and held for 10 min, total run time 55 min; split ratio 1:5; injection volume: 1 μL. MS parameters at electron ionization (EI) mode: electron energy 70 eV, mass range *m/z* 29 to *m/z* 450, scan time 0.25 s. MS parameters at chemical ionization (CI) mode: CI gas pressure 3.00 Torr, mass range *m/z* 60 to *m/z* 350, and scan time 0.1 s for single ion monitoring (SIM).

Identification of Components. The volatile compounds were identified by GC/MS (see Supporting Information) and confirmed by comparison of their mass spectra and LRIs with those of reference samples. If only one method was applied (MS data alone or LRI alone), the identification was "tentative". These reference samples were purchased from Fluka-Sigma-Aldrich (Buchs, Switzerland), Acros Organics (Geel, Belgium), or SDS Carlo-Erba (Val de Reuil, France), or synthesized in-house using standard procedures. The structures of all reference samples were unequivocally confirmed in-house by NMR spectroscopy. The synthetic procedures and characterizations of all newly identified molecules in chicken are given in detail hereafter.

High-Resolution Gas Chromatography Time-of-Flight Mass-Spectrometry (HR GC-TOF-MS). Roasted juice hydrodistillate, extract E, was injected on a GCT Premier (Waters, Milford, MA) with a SPB-1 column (30 m × 0.25 mm, film thickness 1.0 μm film, Supelco). Oven: 60 °C for 5 min, 5 °C/min to 250 °C; injector temperature 250 °C; transfer line temperature 250 °C; carrier gas: helium; constant flow rate 1.0 mL/min; split ratio 1:10; injection volume: 1.0 μL. The acquisition time was set to 0.5 s with an interscan delay of 0.01 s over a mass range of 1−350 Da. Spectra were recorded with an electron energy of 70 eV, emission current of 612 μA, trap current of 200 μA, and source temperature of 200 °C. Calibration was performed by using heptacosa (perfluorotributylamine, MS grade, Apollo Scientific Ltd., Bradbury, UK). Calibration data were collected for 1 min in centroid mode. A total of 60 spectra were summed to generate a 16-point calibration curve from *m/z* 69 to 614 Da. The curve was fitted to a second-order polynomial such that the standard deviation of the residuals was 0.001 amu or lower. Heptacosa was continuously introduced into the ion source, and the ion *m/z* 218.9856 was used as a lock mass. Mass spectra and molecular formula were obtained by using MassLynx software (Waters). The difference δ between the exact mass calculated from the molecular formula and that measured is calculated by the software and expressed in ppm: $\delta = (M_{measured} - M_{calculated})/M_{calculated} \times 10^6$.

Semipreparative Normal Phase High-Performance Liquid Chromatography (NP-HPLC). NP-HPLC experiments were performed on an Agilent 1100 system equipped with a quaternary pump, a single wavelength UV detector, and an autosampler (loop of 100 μL). The column was a Luna Silica ^{2} (3 μm, 150 × 1.0 mm; Phenomenex). Solvent A: heptane; solvent B: heptane/diethyl ether (1/1). Gradient: 0% B, 1 min; 0−15% B in 12 min linear; 15% B isocratic for 2.9 min; 15−100% B in 3.5 min, rinse for 7 min and equilibration at 0% B for 15 min. Flow: 0.35 mL/min (initial pressure was 240 bar). Detection: UV at 250 nm. The peaks seen at this wavelength were collected and injected directly onto the GC/MS (Varian). The peak corresponding to the unknown eluted as a shoulder peak at 12.6 min, just before the higher peak of 2,4-decadienal. Collection of the peak was made manually from five injections of extract F.

Microchemical Reactions. Reduction of the carbonyl: 0.5 mL of the collected peak solution was evaporated under a stream of nitrogen to remove most of the diethyl ether. Ethanol (0.7 mL, to avoid phase separation) and a few milligrams of sodium borohydride were added. The vial was vortexed for 1 min and left standing for 1 h. The reaction mixture was filtered over a PFTE syringe filter (0.45 μm) and injected onto the GC/MS (Varian).

Reduction of the double bond(s): To the same reaction mixture was added a few milligrams of 5% Pd−C. Hydrogen was gently bubbled for 1 min and left standing for 15 min. The same operation was repeated twice more. The reaction mixture was filtered and injected onto the GC/MS (Varian).

Synthesis. *Experimental Equipment.* All moisture-sensitive reactions were carried out under an argon atmosphere in oven- or flame-dried glassware. Purification by flash chromatography was performed over (done with) silica gel 32−63, 60 Å (Brunschwig). The yields of the syntheses were not optimized. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker DPX 400 spectrometer at 25 °C, with tetramethylsilane as the internal standard. Standard gradient-selected COSY, HSQC, and HMBC experiments were carried out on a Bruker Avance 500 spectrometer. Signal assignment was achieved by using Bruker NMR software TopSpin 2.0 (s: singlet; d: doublet; t: triplet; m: multiplet; brs: broad singlet; mc: centered multiplet).

2-Propyl-1-cyclopentanone ***2***. A solution of ethyl cyclopentanone-2-carboxylate **1** (Aldrich, 24 g, 0.157 mol, 1 equiv) in THF (200 mL) was added dropwise during 20 min to a mechanically stirred mixture of NaH (60% oil dispersion: 6.8 g, 0.17 mol, 1.1 equiv) in THF (300 mL) maintained between 15 °C and 25 °C. After a further 15 min at room temperature, a solution of 1-iodopropane (53.4 g, 0.314 mol, 2 equiv) in THF (200 mL) was added dropwise during 10 min. The mixture was then heated at reflux during 3 days. The cooled mixture was poured into cold sat. aq NH_4Cl (200 mL) and extracted (diethyl ether). Workup and concentration in vacuo afforded a residual oil (24.6 g) to which was added concd aq HCl (140 mL). The mixture was then heated at reflux (ca. 95 °C) during 20 h. The reaction was monitored by GC. The cooled mixture was poured into ice−water (250 mL) and extracted (diethyl ether). Workup and bulb-to-bulb distillation in vacuo (110−120 °C/8 mbar) afforded **2** (17 g, 88% yield) as a colorless oil (bp: 67−70 °C/18 mbar).

^{1}H NMR: (400 MHz, $CDCl_3$): δ 0.91 (t, J = 7.2, 3 H); 1.18−1.45 (m, 3 H); 1.46−1.58 (m, 1 H); 1.68−1.84 (m, 2 H); 1.94−2.35 (m, 5 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0 (q); 20.8 (t); 20.8 (t); 29.6 (t); 31.9 (t); 38.2 (t); 49.0 (d); 221.5 (s). MS (EI, 70 eV), *m/z* (%): 126(11); 67(6); 85(6); 84(100); 83(34); 70(7); 69(9); 67(8); 56(11); 55(33); 53(6); 43(4); 42(20); 41(28); 39(19).

2-Propyl-1-cyclopentanone p-Toluenesulfonylhydrazone ***4***. A solution of **2** (15 g, 0.119 mol, 1 equiv) and *p*-tolylsulfonylhydrazide (24.4 g, 0.131 mol, 1.1 equiv) in EtOH (180 mL) containing concd aq HCl (0.2 mL) was heated at reflux during 8 h. The yellow reaction mixture was cooled to room temperature and concentrated in vacuo to afford an yellow oil (43.1 g). Crystallization (diethyl ether:pentane 60:40) afforded **4** (26.1 g, 75% yield) as white crystals.

^{1}H NMR: (400 MHz, $CDCl_3$): δ 0.84 (t, J = 7.2 Hz, 3 H); 1.10−1.34 (m, 4 H); 1.54−1.68 (m, 2 H); 1.78−1.98 (m, 2 H); 2.04−2.16 (m, 1 H); 2.21−2.48 (m, 2 H); 2.43 (s, 3 H); 7.30 (d, J = 8.5 Hz, 2H); 7.85

(d, J = 8.5 Hz, 2H). ^{13}C NMR: (100 MHz, $CDCl_3$): δ 14.1 (q); 20.4 (t); 21.6 (q); 22.6 (t); 28.1 (t); 31.2 (t); 34.1 (t); 44.5 (d); 128.1 (d); 129.4 (d); 135.6 (s); 143.8 (s); 169.8 (s).

2-Pentyl-1-cyclopentanone p-Toluenesulfonylhydrazone ***5***. The same procedure was used as for compound **4**, using 2-pentyl-1-cyclopentanone **3** as starting material (available in house, 14.3 g, 92.9 mmol, 1 equiv) and giving white crystals of 2-pentyl-1-cyclopentanone *p*-toluenesulfonylhydrazone **5** (18.8 g, 63% yield).

^{1}H NMR: (400 MHz, $CDCl_3$): δ 0.87 (t, J = 7.2 Hz, 3 H); 1.10−1.35 (m, 8 H); 1.54−1.68 (m, 2 H); 1.77−1.98 (m, 2 H); 2.04−2.16 (m, 1 H); 2.22−2.48 (m, 2 H); 2.43 (s, 3 H); 7.29 (d, J = 8.1 Hz, 2 H); 7.57 (brs, 1H); 7.86 (d, J = 8.1, 2 H). ^{13}C NMR: (100 MHz, $CDCl_3$): δ 14.1 (q); 21.6 (q); 22.6 (t); 22.6 (t); 26.9 (t); 28.1 (t); 31.2 (t); 31.9 (t); 32.0 (t); 44.7 (d); 128.1 (d); 129.4 (d); 135.6 (s); 143.8 (s); 169.8 (s).

5-Propyl-1-cyclopentene-1-carbaldehyde ***6***. A 1.6 M hexane solution of *n*-butyllithium (64 mL, 102 mmol, 3 equiv) was added dropwise during 30 min to a stirred solution of **4** (10 g, 34 mmol, 1 equiv) in *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA, 75 mL) and THF (15 mL) at −45 °C under nitrogen. The deep-red solution was allowed to reach 10 °C during 1 h and then maintained at 15−18 °C for a further 75 min. Recooling to −5 °C (brown-green mixture) was followed by the dropwise addition of DMF (puriss., 6 g, 82 mmol, 2.4 equiv). A temperature rise to 8 °C with the appearance of a brown precipitate was observed. The reaction mixture was then stirred at 10 °C during 20 min. Dilution with 10% aq NaCl (100 mL) was followed by extraction (diethyl ether). The combined organic phase was washed with cold 5% aq HCl and then sat. aq NaCl. Workup, concentration in vacuo, flash chromatography (cyclohexane:ethyl acetate 95:5), and bulb-to-bulb distillation in vacuo (bp: 60−70 °C/2.5 mbar) afforded **6** (0.25 g, 5% yield), a colorless oil.

LRI (SWax) 1518, LRI (SPB-1) 1106. In natural extract: LRI (SWax) 1516; LRI (SPB-1) 1105. ^{1}H NMR: (400 MHz, $CDCl_3$): δ 0.90 (t, J = 7.2 Hz, 3H); 1.14−1.42 (m, 3 H); 1.66−1.81 (m, 2H); 2.15 (mc, 1H); 2.42−2.66 (m, 2 H); 2.92−3.02 (m, 1 H); 6.82 (mc, 1 H); 9.76 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.2 (q); 20.6 (t); 29.7 (t); 32.2 (t); 35.5 (t); 42.0 (d); 150.9 (s); 153.7 (d); 190.0 (d). MS (EI, 70 eV), m/z (%): 138(30); 123(15); 110(13); 109(33); 96(27); 95(48); 81(25); 79(18); 67(100); 41(27). HR-GC-TOF-MS: 138.1046 ($C_9H_{14}O$, +0.7 ppm).

5-Pentyl-1-cyclopentene-1-carbaldehyde ***7***. The same procedure was used as for compound **6**, using 2-pentyl-1-cyclopentanone *p*-toluenesulfonylhydrazone **5** (11 g, 34.1 mmol, 1 equiv) as starting material and giving **7** (1.55 g, 27% yield) as a pale-yellow oil (bp: 100−110 °C/0.8 mbar).

LRI (SWax) 1732, LRI (SPB-1) 1308. In natural extract: LRI (SWax) 1725; LRI (SPB-1) 1309. ^{1}H NMR: (400 MHz, $CDCl_3$): δ 0.88 (mc, 3 H); 1.16−1.38 (m, 7 H); 1.67−1.83 (m, 2 H); 2.08−2.21 (m, 1 H); 2.42−2.66 (m, 2H); 2.91−3.02 (m, 1 H); 6.82 (mc, 1 H); 9.76 (s, 1 H). ^{13}C NMR: (100 MHz, $CDCl_3$): δ 14.1 (q); 22.7 (t); 27.1 (t); 29.7 (t); 32.0 (t); 32.2 (t); 33.2 (t); 42.2 (d); 151.0 (s); 153.5 (d); 190.0 (d). MS (EI, 70 eV), m/z (%): 166(21); 137(10); 124(33); 123(18); 110(22); 109(25); 96(37); 95(49); 81(32); 79(24); 67(100); 41(36). HR-GC-TOF-MS: 166.1359 ($C_{11}H_{18}O$, +0.6 ppm).

2-Ethyl Cyclopentanone ***8***. Ethyl 2-oxocyclopentanecarboxylate **1** (Aldrich, 25 g, 160 mmol, 1 equiv), potassium carbonate (66 g, 423 mmol, 2.4 equiv), and ethyl iodide (37.5 g, 240 mmol, 1.5 equiv) in acetone (250 mL) were refluxed for 5 h. The reaction medium was filtered and evaporated. Then concd HCl (100 mL) was poured onto it, and the reaction was refluxed for 20 h, cooled, and extracted with heptane. The usual workup and distillation in vacuo with a Vigreux column yielded **8** (6.65 g, 37% yield, bp 53 °C/10 Torr).

^{1}H NMR: (AMX 360 MHz, $CDCl_3$): δ 0.94 (t, J = 7.2, 3 H); 1.26−1.38 (m, 1 H); 1.48−1.59 (m, 1 H); 1.71−1.84 (m, 2 H); 1.94−2.35 (m, 5 H). ^{13}C NMR (90 MHz, $CDCl_3$): δ 11.9 (q); 20.7 (t); 22.7 (t); 29.0 (t); 38.3 (t); 50.6 (d); 221.4 (s). MS (EI, 70 eV), m/z (%): 112(30); 97(5); 85(5); 84(100); 83(39); 70(6); 69(20); 68(30); 67(8); 57(6); 56(76); 55(70); 53(11); 51(6); 43(12); 42(36); 41(78); 39(50).

(Z)-N′-(2-Ethylcyclopentylidene)-4-methylbenzenesulfonohydrazide ***9***. The same procedure was used as for compound **4**, using 2-ethyl cyclopentanone **8** as starting material (6.65 g, 59.4 mmol, 1 equiv) and giving (*Z*)-*N*′-(2-ethylcyclopentylidene)-4-methylbenzenesulfonohydrazide **9** as a yellow powder (12.3 g, 74% yield). ^{1}H NMR and ^{13}C NMR were identical to those described in ref 12.

5-Ethyl 1-cyclopentene-1-carbaldehyde ***10***. To a −60 °C solution of **9** (4.5 g, 16.1 mmol, 1 equiv) in *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (40.5 mL) and THF (13.5 mL) was added *n*-butyllithium (40.5 mL of 1.6 M in hexane, 64.8 mmol, 4 equiv) over 45 min. The reaction was allowed to reach 0 °C and was stirred for 1.5 h until it changed from deep red to brownish-green. At −15 °C, DMF (4.5 mL, 58.0 mmol, 3.6 equiv) was added and was reacted for another 40 min at 5−10 °C. The reaction was poured onto 3.2 N HCl (190 mL) at 0 °C and was extracted with diethyl ether. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated to 40 mL; a GC/MS with decane as the internal standard showed about 430 mg of **10** (GC purity 46%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, J = 7.4 Hz, 3H); 1.36−1.25 (m, 1H); 1.85−1.69 (m, 2H); 2.19−2.10 (m, 1H); 2.64−2.45 (m, 2H); 2.96−2.91 (m, 1H); 6.85−6.83 (m, 1H); 9.77 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.4 (q); 25.8 (t); 29.1 (t); 32.2 (t); 43.5 (d); 150.5 (s); 153.9 (d); 190.1 (d). MS (EI, 70 eV), m/z (%): 124(53); 109 (28); 95(72); 93(11); 91(9); 81(14); 79(9); 77(8); 67(100); 65(17); 55(8); 41(22); 39(18).

3-(5-Ethyl-1-cyclopentenyl)-N-methoxy-N-methylacrylamide ***11***. Diethyl (*N*-methoxy-*N*-methylcarbamoylmethyl) phosphate (2.51 g, 12 mmol, 4 equiv) was added to a suspension of sodium methoxide (972 mg, 18 mmol, 6 equiv) in THF (42 mL) at room temperature. A solution of 35 mL of **10** (about 375 mg, 3 mmol, 1 equiv) and THF (12 mL) was added. A water bath was used to keep the reaction below 30 °C (slightly exothermic) for 1 h. The usual workup with dichloromethane as extraction solvent gave 4.2 g of crude product. The Weinreb amide **11** (405 mg, 65%) was obtained with a purity of 93% by flash chromatography (30% AcOEt in cyclohexane).

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.90 (t, J = 7.5 Hz, 3H); 1.38−1.28 (m, 1H); 1.68−1.60 (m, 1H); 1.80−1.74 (m, 1H); 2.10−2.04 (m, 1H); 2.40−2.33 (m, 1H); 2.51−2.43 (m, 1H); 2.86−2.82 (m, 1H); 3.27 (s, 3H); 3.71 (s, 3H); 6.13 (t, J = 2.9 Hz, 1H); 6.39 (d, J = 15.7 Hz, 1H); 7.45 (d, J = 15.7 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.4 (q); 25.8 (t); 29.3 (t); 31.8 (t); 32.5 (q); 45.0 (d); 61.7 (q); 115.2 (d); 139.2 (d); 139.4 (d); 144.9 (s); 167.7 (s). MS (EI, 70 eV), m/z (%): 209(6); 150 (16); 149(100); 131(21); 121(10); 119(5); 107(10); 105(9); 93(17); 91(29); 79(18); 77(10); 65(8); 55(25).

(E)-3-(5-Ethylcyclopent-1-enyl)acrylaldehyde ***12***. To a solution of the Weinreb amide **11** (250 mg, 1.14 mmol, 1 equiv) in diethyl ether (2 mL) kept at −15 °C was added $LiAlH_4$ (43 mg in 2 mL of diethyl ether) portionwise. The reaction was stirred at −15 °C for 30 min. The amide had completely reacted, as observed by GC/MS. Then diethyl ether (5 mL) was added, and the reaction was poured into cold 1% $KHSO_4$ (4 mL). The usual workup gave a crude product (199 mg), which was purified by flash chromatography (3% diethyl ether in pentane) to give pure **12** (61 mg, 34% yield).

LRI (SWax) 1853; LRI (SPB-1) 1296. In natural extract: LRI (SWax) 1855; LRI (SPB-1) 1299. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (t, J = 7.5 Hz, 3H); 1.34−1.21 (m, 1H); 1.66−1.56 (m, 1H); 1.84−1.77 (m, 1H); 2.16−2.06 (m, 1H); 2.46−2.38 (m, 1H); 2.57−2.48 (m, 1H); 2.84−2.79 (m, 1H); 6.10 (dd, J = 15.8, 7.8 Hz, 1H); 6.31 (t, J = 2.8 Hz, 1H); 7.24 (d, J = 15.8 Hz, 1H); 9.56 (d, J = 7.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.5 (q); 25.7 (t); 29.2 (t); 32.1 (t); 44.9 (d); 128.5 (d); 143.2 (d); 145.1 (s); 148.4 (d); 194.5 (d). MS (EI, 70 eV), m/z (%): 150 (42); 135 (5); 122 (20); 121 (100); 107(18); 103 (19); 94 (62); 93 (54); 91 (92); 79 (27); 77 (88); 65 (23); 63 (8); 55

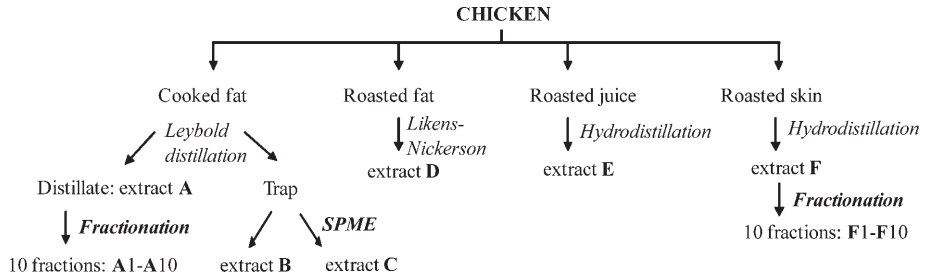


Figure 1. Extraction of the chicken parts (skin, fat, juice) investigated separately for their volatile composition. A = cooked fat Leybold distillate extract; B = cooked fat Leybold trap extract; C = cooked fat Leybold solid-phase microextraction (SPME) extract; D = roasted fat (Likens−Nickerson) extract; E = roasted juice hydrodistillate extract; F = roasted skin hydrodistillate extract.

(18); 53 (12); 51 (13); 41 (15); 39 (24). HR-GC-TOF-MS: 150.1044 ($C_{10}H_{14}O$, −0.7 ppm).

(E)-2-Butylidene-1-cyclopentanone ***14***. To a 500-mL flask under argon were added pyrrolidine (17.75 g, 0.25 mol), water (15.5 mL, 0.85 mol), and methyl *tert*-butyl ether (MTBE, 85 mL). The reaction mixture was cooled down with an ice bath, and acetic acid (15.00 g, 0.25 mol) was introduced dropwise within 30 min. Cyclopentanone **13** (84.00 g, 1.00 mol, 1 equiv) was added within 10 min and after an additional 10 min, butyraldehyde (89.28 g, 1.24 mol, 1.2 equiv) was added dropwise within 30 min. The reaction mixture was stirred for another 10 min and then refluxed for 4 h. MTBE was added, and the organic phase was separated and washed with HCl 20%, brine, H_2O/brine 1/1, and $NaHCO_3$ sat/brine 1/1, dried over $MgSO_4$, and concentrated. The residue was distilled over a Vigreux column under vacuum, giving (*E*)-2-butylidene-1-cyclopentanone **14** (43.96 g, 32% yield, purity GC/MS: 89%, bp: 86−90 °C/5.8 mbar).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.94 (t, J = 7.2 Hz, 3H); 1.40−1.58 (m, 2H); 1.89−1.98 (m, 2H); 2.10−2.16 (m, 2H); 2.33 (t, J = 7.7 Hz, 2H); 2.56−2.62 (m, 2H); 6.52−6.58 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.9 (q); 19.8 (t); 21.7 (t); 26.8 (t); 31.7 (t); 38.6 (t); 136.1 (d); 137.4 (s); 207.2 (s).

2-Butyl-2-cyclopenten-1-one ***15***. In a flask under argon, (*E*)-2-butylidene-1-cyclopentanone **14** (34.5 g, 0.25 mol) and iodine (35 mg, 0.14 mmol) were heated at 160 °C for 2 h. The mixture was distilled by using a Vigreux column under vacuum, giving 2-butyl-2-cyclopenten-1-one **15** (27.76 g, 80% yield, purity GC/MS: 92%, bp: 90−92 °C/12 mmHg).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.91 (t, J = 7.2 Hz, 3H); 1.28−1.39 (m, 2H); 1.41−1.51 (m, 2H); 2.13−2.20 (m, 2H); 2.37−2.41 (m, 2H); 2.54−2.59 (m, 2H); 7.29−7.32 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.9 (q); 22.5 (t); 24.5 (t); 26.5 (t); 29.9 (t); 34.6 (t); 146.5 (s); 157.3 (d); 210.0 (s).

2-Butyl-2,3-epoxy-1-cyclopentanone ***17***. A solution of 2-butyl-2-cyclopenten-1-one **15** (21.61 g, 157 mmol, 1 equiv) and aqueous hydrogen peroxide 30% (45 mL, 470 mmol, 3 equiv) in methanol (200 mL) was cooled to 15 °C. Then 6 N aqueous sodium hydroxide (13 mL, 79 mmol, 0.5 equiv) was added dropwise within 45 min under stirring. During the addition, the temperature was kept at 15−25 °C. The resulting mixture was stirred for 3 h at room temperature, poured into water (250 mL), extracted with diethyl ether, dried over $MgSO_4$, and concentrated. The residue was distilled by using a Vigreux column under vacuum, giving 2-butyl-2,3-epoxy-1-cyclopentanone **17** (7.9 g, 33% yield, purity GC/MS 98%, bp: 87−89 °C/6.8 mbar).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, J = 6.9 Hz, 3H); 1.29−1.44 (m, 4H); 1.72−2.15 (m, 4H); 2.24−2.41 (m, 2H); 3.78−3.80 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.9 (q); 22.3 (t); 22.8 (t); 23.8 (t); 26.6 (t); 31.7 (t); 63.0 (d); 63.7 (s); 210.9 (s).

2-Pentyl-2,3-epoxy-1-cyclopentanone ***18***. The same procedure was used as for compound **17**, using 2-pentyl-2-cyclopenten-1-one **16** (available in house, 30.40 g, 200 mmol) as starting material and giving 2-pentyl-2,3-epoxy-1-cyclopentanone **18** (11.85 g, 35% yield, purity GC/MS 95%, bp: 103.6 °C/6.4 mbar).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, J = 6.9 Hz, 3H); 1.24−1.44 (m, 6H); 1.72−2.14 (m, 4H); 2.24−2.41 (m, 2H); 3.78−3.80 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.9 (q); 22.3 (t); 22.4 (t); 24.1 (t); 31.7 (t); 31.9 (t); 63.0 (d); 63.7 (s); 210.8 (s).

3-Butyl-2-hydroxy-2-cyclopenten-1-one ***19***. A solution of 2-butyl-2,3-epoxy-1-cyclopentanone **17** (7.77 g, 50 mmol, 1 equiv) in acetic acid (35 mL) containing sulfuric acid (0.49 mL) was heated at 55 °C for 2 h. After removal of acetic acid in vacuo, the residue was diluted with diethyl ether and extracted with NaOH 5% (2×). The alkaline solution was acidified with HCl 10%, extracted with diethyl ether, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography by using pentane/diethyl ether 70/30. In order to further remove traces of acetic acid, the fractions were washed with aq $NaHCO_3$ sat., dried over $MgSO_4$, and concentrated, giving 3-butyl-2-hydroxy-2-cyclopenten-1-one **19** (1.00 g, 13% yield, purity GC/MS 99%).

LRI (SWax) 2097; LRI (SPB-1) 1288. In natural extract: LRI (SWax) 2095; LRI (SPB-1) 1287. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.94 (t, J = 7.3 Hz, 3H); 1.32−1.43 (m, 2H); 1.51−1.59 (m, 2H); 2.39−2.47 (m, 6H); 6.39 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.8 (q); 22.7 (t); 25.2 (t); 28.4 (t); 29.0 (t); 31.9 (t); 148.6 (s); 148.8 (s); 203.4 (s). MS (EI, 70 eV), m/z (%): 154 (16); 125(43); 112(100); 111(30); 99(27); 83(14); 70(8); 55(30); 41(12). HR-GC-TOF-MS: 154.0994 ($C_9H_{14}O_2$, −2.6 ppm).

2-Hydroxy-3-pentyl-2-cyclopenten-1-one ***20***. The same procedure was used as for compound **19**, using 2-pentyl-2,3-epoxy-1-cyclopentanone **18** (11.61 g, 69.1 mmol) as starting material and giving 2-hydroxy-3-pentyl-2-cyclopenten-1-one **20** (1.92 g, 17% yield, purity GC/MS 99%).

LRI (SWax) 2195; LRI (SPB-1) 1390. In natural extract: LRI (SWax) 2197; LRI (SPB-1) 1389. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, J = 7.1 Hz, 3H); 1.28−1.38 (m, 2H); 1.52−1.60 (m, 2H); 2.40−2.46 (m, 6H); 6.40 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0 (q); 22.4 (t); 25.2 (t); 26.5 (t); 28.6 (t); 31.8 (t); 31.9 (t); 148.8 (s); 148.9 (s); 203.5 (s). MS (EI, 70 eV), m/z (%): 168(18); 125(46); 112(100); 111(26); 99(34); 83(10); 55(25); 41(11). HR-GC-TOF-MS: 168.1146 ($C_{10}H_{16}O_2$, −2.4 ppm).

(8Z)-8-Tetradecen-5-olide ***28***. To a solution of trimethyl 4-bromoorthobutyrate **24** (Aldrich, 16.32 g, 72.0 mmol, 2.8 equiv) in diethyl ether (120 mL) at −78 °C was added *tert*-butyllithium (1.7 M in pentane, 76.32 mL, 130.0 mmol, 5 equiv) dropwise over 45 min. The reaction mixture was stirred at −78 °C for 1 h and then at 0 °C for 50 min. After recooling to −78 °C, (4*Z*)-decenal **21** (Acros, 3.96 g, 25.7 mmol, 1 equiv) was added dropwise over 5 min. The resulting light-yellow slurry was stirred at −78 °C for 30 min followed by 90 min at 0 °C. A 5% aq AcOH solution was added dropwise to the reaction mixture, followed by stirring at 0 °C for 40 min. The mixture was extracted with diethyl ether, dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography (pentane/diethyl ether: 7/3). The resulting crude

25 (2.85 g, 11.1 mmol, 1 equiv), KOH (1.85 g, 33.3 mmol, 3 equiv), H_2O (2.9 mL), and ethanol (16.5 mL) were refluxed for 2 h. After removal of ethanol, the residue was dissolved in water and concd H_2SO_4 until it reached pH 1. The mixture was extracted with diethyl ether, washed with brine, dried over $MgSO_4$, concentrated, and purified by flash chromatography by using pentane/diethyl ether 60/40, giving **28** (1.35 g, 54% yield, purity GC/MS 96%).

LRI (SWax) 2653; LRI (SPB-1) 1855. In natural extract: LRI (SWax) 2662; LRI (SPB-1) 1852. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, J = 6.8 Hz, 3H); 1.18−1.39 (m, 6H); 1.46−2.63 (m, 10H); 2.36−2.63 (m, 2H); 4.26−4.32 (m, 1H); 5.29−5.45 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (q); 18.5 (t); 22.6 (t); 22.7 (t); 27.2 (t); 27.8 (t); 29.4 (t); 29.5 (t); 31.5 (t); 35.8 (t); 79.9 (d); 128.0 (d); 131.4 (d); 1759); 55(49); 54(68); 41(40). MS (EI, 70 eV), m/z (%): 224 (2); 121(4); 110(100); 95(28); 82(31); 81(100); 68(43); 67(49); 55(39); 54(42); 41(36). HR-GC-TOF-MS: 224.1785 ($C_{14}H_{24}O_2$, +4.0 ppm).

(8E)-8-Tetradecen-5-olide ***29***. The same procedure was used as for compound **28**, using (4*E*)-decenal **22** (Acros, 3.96 g, 25.7 mmol, 1 equiv) as starting material and giving **29** (1.07 g, 37% yield, purity GC/MS 99%).

LRI (SWax) 2690; LRI (SPB-1) 1873. In natural extract: LRI (SWax) 2691; LRI (SPB-1) 1871. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, J = 6.8 Hz, 3H); 1.20−1.37 (m, 6H); 1.48−2.23 (m, 10H); 2.40−2.62 (m, 2H); 4.25−4.32 (m, 1H); 5.32−5.40 (m, 1H); 5.42−5.49 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (q); 18.5 (t); 22.5 (t); 27.8 (t); 27.9 (t); 29.2 (t); 29.5 (t); 31.4 (t); 32.5 (t); 35.7 (t); 79.8 (d); 128.5 (d); 131.8 (d); 171.9 (s). MS (EI, 70 eV), m/z (%): 224 (2); 121(4); 110(100); 95(28); 82(31); 81(71); 68(46); 67(47); 55(41); 54(46); 41(37). HR-GC-TOF-MS: 224.1782 ($C_{14}H_{24}O_2$, +2.7 ppm).

(6E)-6-Tetradecen-5-olide ***30***. The same procedure was used as for compound **28**, using (2*E*)-decenal **23** (Alfa Aesar, 5.00 g, 32.5 mmol, 1 equiv) as starting material and giving **30** (1.45 g, 20% yield, purity GC/MS 99%).

LRI (SWax) 2738; LRI (SPB-1) 1900. In natural extract: LRI (SWax) 2744; LRI (SPB-1) 1896. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86−0.91 (t, J = 6.9 Hz, 3H); 1.21−1.42 (m, 10H); 1.60−2.09 (m, 6H); 2.42−2.62 (m, 2H); 4.74−4.79 (m, 1H); 5.45−5.52 (m, 1H); 5.73−5.80 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (q); 18.2 (t); 22.7 (t); 28.4 (t); 28.9 (t); 29.1 (t); 29.1 (t); 29.5 (t); 31.8 (t); 32.2 (t); 80.8 (d); 127.9 (d); 134.6 (d); 171.5 (s). MS (EI, 70 eV), m/z (%): 224 (2); 164(11); 154(16); 136(20); 125(85); 112(41); 97(100); 84(46); 83(56); 70(52); 55(64), 42 (45); 41 (40). HR-GC-TOF-MS: 224.1786 ($C_{14}H_{24}O_2$, +4.5 ppm).

4-Methyl-2-pentylpyridine ***32***. A 50-mL reactor under argon was charged with 2-bromo-4-methylpyridine **31** (2.0 g, 11.6 mmol, 1 equiv) and THF (10 mL). The reaction was cooled to −78 °C, and Pd(dppf)$_2$Cl$_2$ (0.18 g, 0.232 mmol, 2 mol % cat., dppf: 1,1′-di(diphenyl-

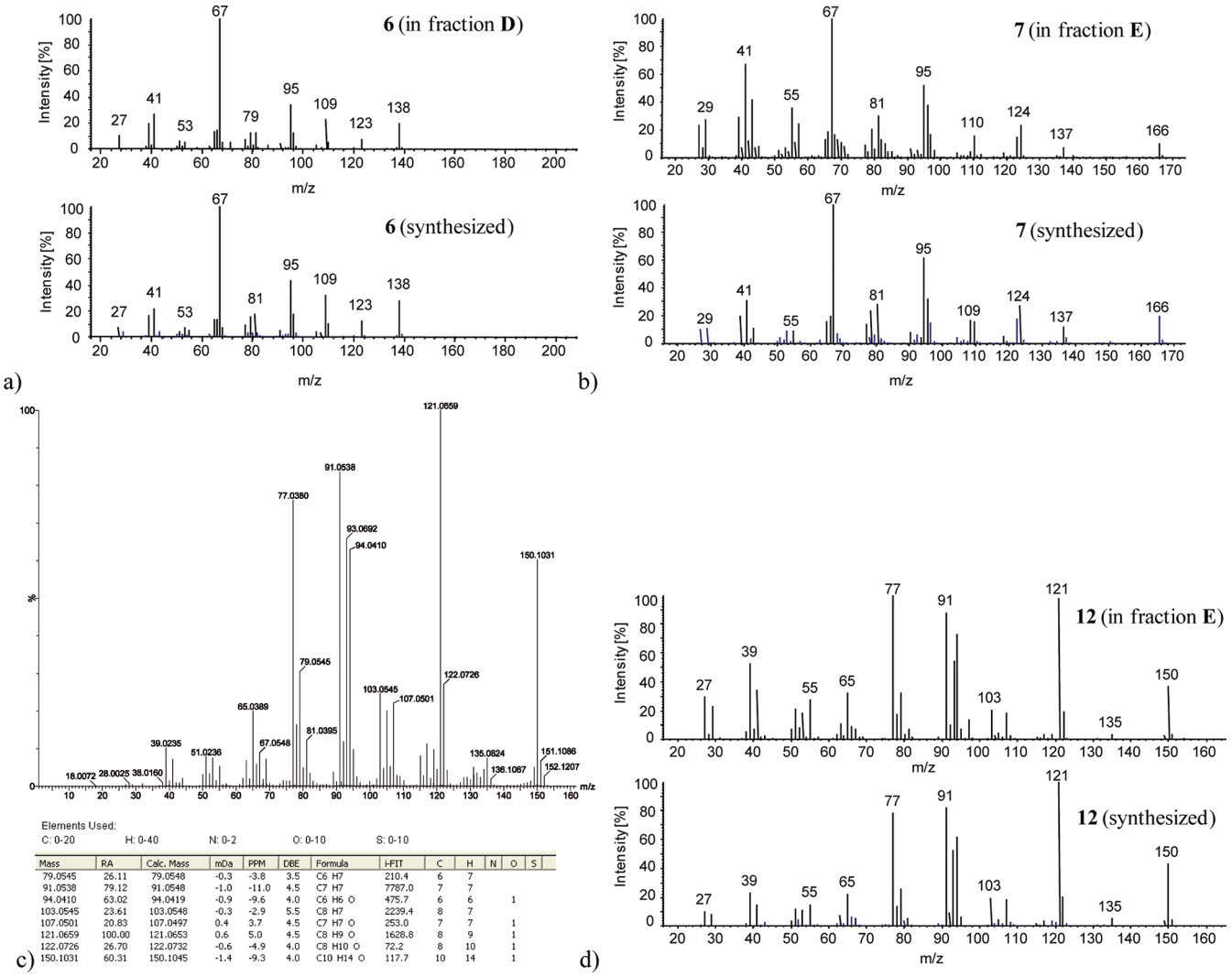


Figure 2. Continued

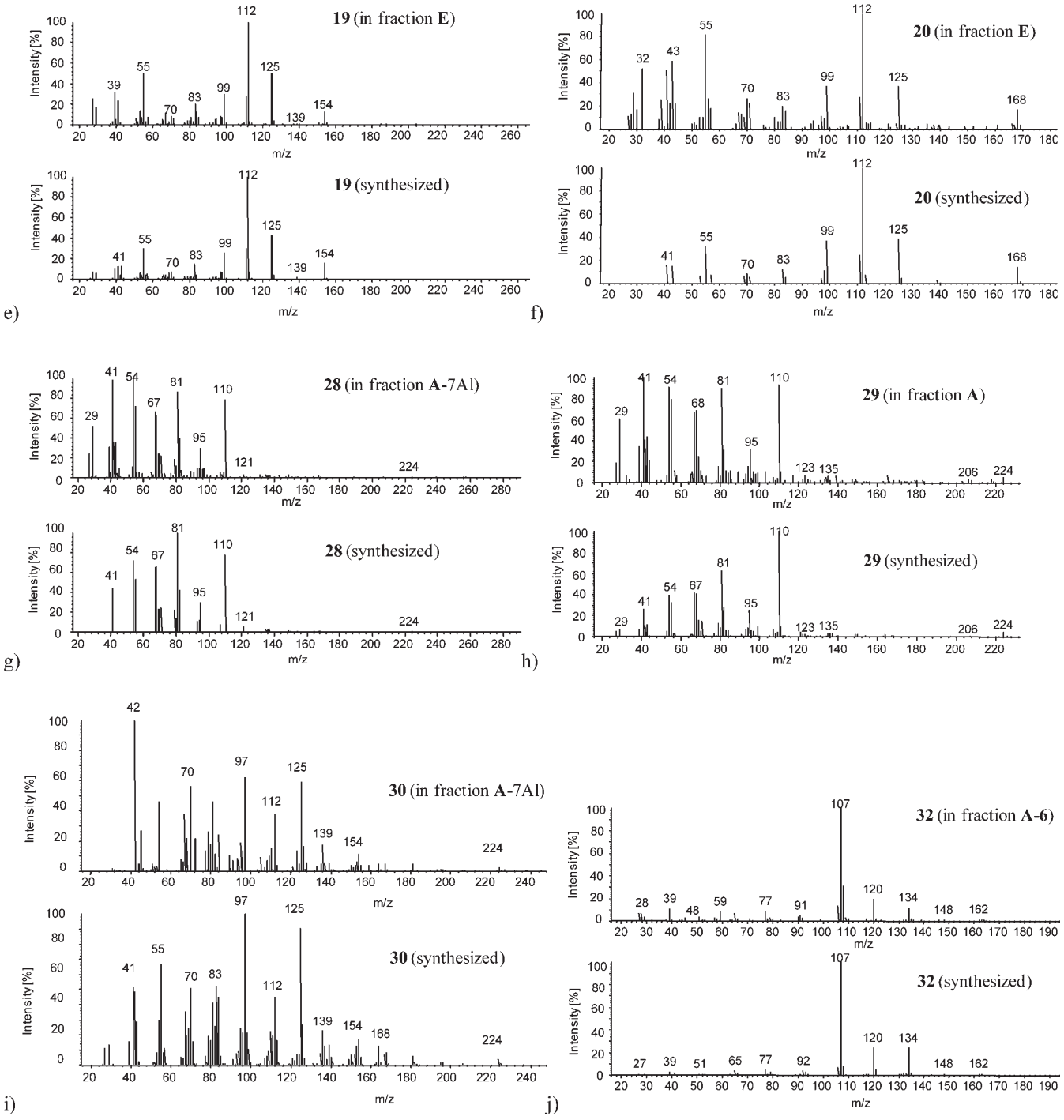


Figure 2. Mass spectra of new molecules identified in chicken and compared with those of the synthetic reference: (a) 5-propyl-1-cyclopentene-1-carbaldehyde **6**; (b) 5-pentyl-1-cyclopentene-1-carbaldehyde **7**; (c) high-resolution GC-TOF-MS obtained on **12** in chicken extract E; (d) (*E*)-3-(5-ethylcyclopent-1-enyl)acrylaldehyde **12**; (e) 3-butyl-2-hydroxy-2-cyclopenten-1-one **19**; (f) 2-hydroxy-3-pentyl-2-cyclopenten-1-one **20**; (g) (8*Z*)-8-tetradecen-5-olide **28**; (h) (8*E*)-8-tetradecen-5-olide **29**; (i) (6*E*)-6-tetradecen-5-olide **30**; (j) 4-methyl-2-pentylpyridine **32**.

phosphine)ferrocene) was added, followed by 3.0 N pentylmagnesium bromide (10 mL, 17.4 mmol, 1.5 equiv). The reaction was allowed to warm to 0 °C until the starting material was consumed. The reaction was quenched with saturated ammonium chloride solution (20 mL) and extracted with ethyl acetate (20 mL). The ethyl acetate layer was extracted with 1 N aqueous HCl (20 mL) to extract the pyridine product from neutral organics. The aqueous acid layer was neutralized with 1 N NaOH and extracted with ethyl acetate (20 mL). The ethyl acetate layer was washed with brine and dried on anhydrous sodium sulfate. The product phase was filtered and concentrated on the rotary evaporator to give 4-methyl-2-pentylpyridine **32** (850 mg, 44.9% yield).

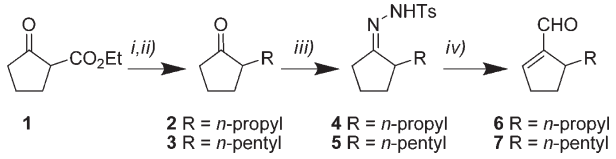
LRI (SWax) 1677 LRI (SPB-1) 1286. In natural extract: LRI (SWax) 1682; LRI (SPB-1) 1294. ^{1}H NMR: 0.89 (t; 3H); 1.34 (m; 4 H); 1.72 (m; 2 H); 2.30 (s; 3 H); 2.72 (dd; 2 H); 6.91 (d; 1 H); 6.96 (s; 1 H); 8.37 (d; 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0 (q); 21.0 (q); 22.6 (t);

Table 1. Linear Retention Indices (on Polar and Nonpolar Columns) and Relative Abundance in the Different Extracts of the New Molecules Identified in the Present Study[a,b]

compd	$LRI_{exp\ SWax}$	$LRI_{exp\ SPB-1}$	A	B	C	D	E	F	$LRI_{ref\ SWax}$	$LRI_{ref\ SPB-1}$
6	1516	1105	_	tr	_	0.32	0.18	_	1518	1106
7	1725	1309	_	_	_	_	0.54	_	1732	1308
12	1855	1299	_	0.69	_	0.85	1.55	2.87	1853	1296
19	2095	1287	tr(A-7Al)	_	_	_	_	_	2097	1288
20	2197	1389	tr	_	_	tr	0.15	_	2195	1390
28	2662	1852	tr(A-7Al)	_	_	_	_	_	2682	1855
29	2691	1871	tr	_	_	_	_	_	2707	1873
30	2744	1896	tr(A-7Al)	_	_	_	_	_	2757	1900
32	1682	1294	tr(A-6)	_	_	_	_	_	1702	1286

[a] A = cooked fat Leybold distillate extract; B = cooked fat Leybold trap extract; C = cooked fat Leybold solid-phase microextraction extract; D = roasted fat (Likens−Nickerson) extract; E = roasted juice hydrodistillate extract; F = roasted skin hydrodistillate extract. The abundances are given in relative percentage of the integration of the total ion current and are therefore not quantitative data. LRI_{exp}: LRI measured in chicken extract; LRI_{ref}: LRI of synthesized product; tr: trace: <0.1%; tr(X-Y): compound identified in the fraction Y after fractionation on SiO_2 of extract X. [b] The linear retention indices of the synthesized references are given in the right columns.

Scheme 1. Synthesis of 5-Propyl-1-cyclopentene-1-carbaldehyde 6 and 5-Pentyl-1-cyclopentene-1-carbaldehyde 7[a]



1 **2** R = *n*-propyl, **3** R = *n*-pentyl; **4** R = *n*-propyl, **5** R = *n*-pentyl; **6** R = *n*-propyl, **7** R = *n*-pentyl

[a] Reagents and conditions: (i) NaH, 1-iodoalcane, THF; (ii) concd aq HCl; (iii) $TsNHNH_2$, EtOH, HCl cat., reflux; (iv) *n*-BuLi, TMEDA−THF, and then DMF.

29.7 (t); 31.7 (t); 38.3 (t); 121.9 (d); 123.6 (d); 147.2 (s); 148.9 (d); 162.3 (s). MS (EI, 70 eV), *m/z* (%): 134(24); 120(25); 108(8); 107(100); 106(7); 77(5); 65(4); 39(4). HR-GC-TOF-MS: 163.1363 ($C_{11}H_{17}N$, +1.2 ppm).

General Procedure for New Compound Evaluation. The new compound was diluted with propylene glycol in order to provide a 10% solution. This solution was then diluted using a 0.3% aqueous solution of NaCl. Three flavorist experts evaluated the taste properties of the new compounds using in-house descriptors.

RESULTS AND DISCUSSION

Preparation and GC/MS Analysis of Aroma Extracts from Different Parts (Skin, Fat, and Juice) of Chicken. To maximize our chance of finding new molecules, we investigated distinct parts of top-quality chicken (Poulet de Bresse AOC from France) obtained under different conditions (cooking or roasting). These distinct parts had to be extracted with techniques appropriate to their nature (solid or liquid, water or oil phase) (Figure 1). Leybold distillation (or molecular distillation, or thin-film distillation) was applied to the cooked fat. During this distillation, the most volatile compounds were captured in a trap at −200 °C and were subsequently analyzed by GC/MS and SPME-GC/MS. Simultaneous distillation extraction (Likens−Nickerson) was applied to the roasted fat. The roasted juice and the roasted skin were both extracted by hydrodistillation under reduced pressure.

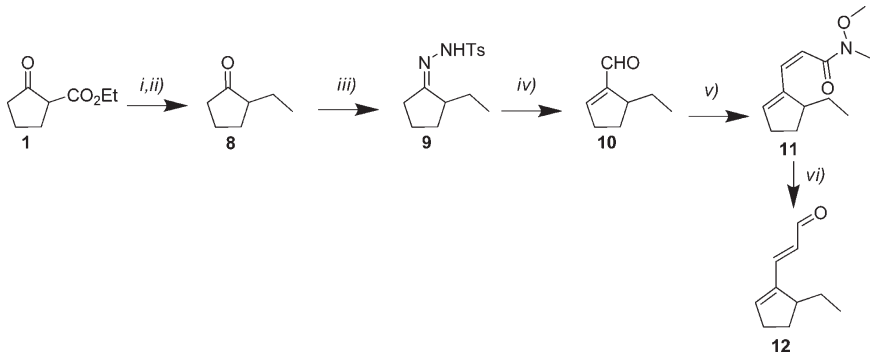
The odor of the resulting extracts was evaluated by expert flavorists, who agreed that the aroma of the resulting extracts reflected the typicity of the starting materials. The evaluations are given in Materials and Methods. In particular, the sweet, creamy character of the cooked abdominal fat was clearly present in extract A, evaluated as "heavy, sweet, fatty, creamy". The roasted skin extract F was "typical of grilled, roasted skin".

The volatile composition of each extract was analyzed by GC/MS. The complete results are given in Supporting Information. Aldehydes (in particular alkanals (*E*)-2-alkenals, dienals) were the major components of the different fractions. Interestingly, some chemical families or molecules tended to be characteristic of some extracts in which they were found in high amounts. In particular, alcohols (e.g., 1-octen-3-ol, 1-hexanol, and (2*Z*)-octenol) and ketones (e.g., 1-hydroxy-2-heptanone and 2-decanone) were found in higher amounts in roasted skin extract F as compared with the other extracts. Phenylacetaldehyde, 1-heptanol, and 4-butanolide were found in higher amounts in cooked fat Leybold trap extract B. A compound that was newly identified as (2*E*)-3-(5-ethyl-1-cyclopentenyl)propenal **12** (see the following) was found in all extracts, with a higher abundance in roasted skin extract F. Even if these differences between extracts can also result from the different cooking procedures or from the extraction method, they likely reflect, at least in part, the typicity of the distinct chicken parts.

Fractionation of cooked fat Leybold distillate (extract A) and roasted skin hydrodistillate (extract F) by chromatography on silica gel enabled us to detect many additional trace components, which may be keys in the aroma of these fractions. The odor evaluations of all resulting fractions are given in Materials and Methods. Many pyrazines were found in trace amounts in roasted skin extract F, resulting from Maillard reactions on the skin during roasting. Compared with roasted juice, which contained a series of γ-lactones (from 4-butanolide to 4-dodecanolide), cooked fat Leybold distillate A contained mainly long-chain δ-lactones ($>C_{12}$), including the previously unknown lactones described hereafter. Trace amounts of pyridines were also detected in extract A after fractionation. Short chain pyridines (C_2 to C_5) were identified in roasted juice extract E, whereas traces of longer chain pyridines (C_4 to C_7) were found in cooked fat extract A.

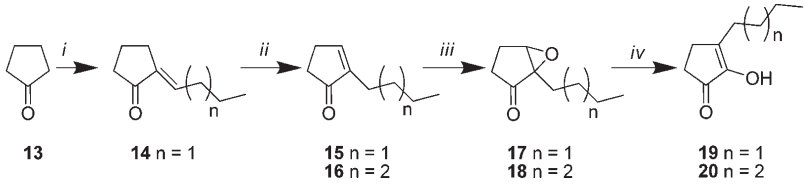
In-depth GC/MS analysis of these extracts and fractions revealed the presence of several unknown molecules, generally present in only trace amounts in several extracts or fractions. The

Scheme 2. Synthesis of (*E*)-3-(5-Ethyl 1-cyclopentenyl)propenal 12[a]



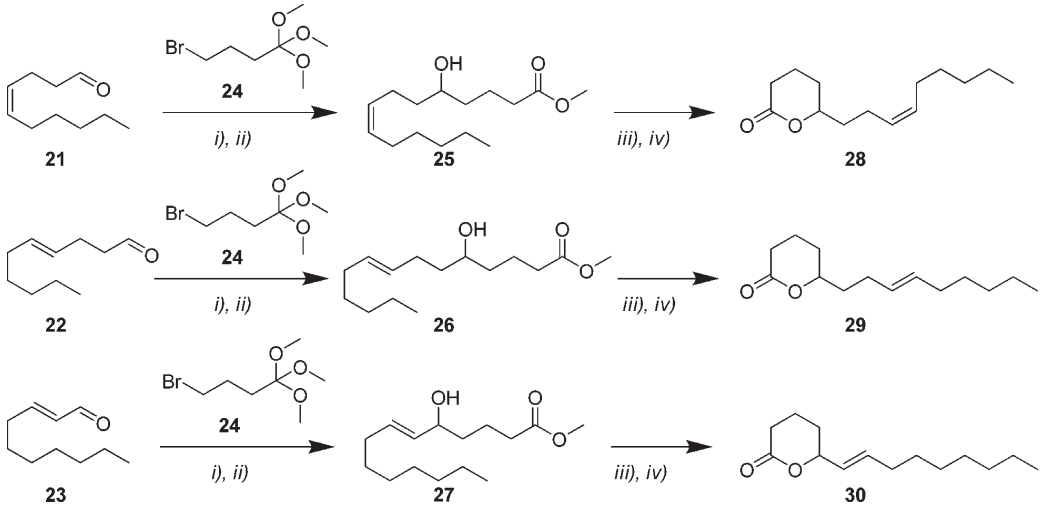
[a] Reagents and conditions: (i) K_2CO_3, EtI, acetone, reflux; (ii) concd aq HCl, reflux 20 h; (iii) $TsNHNH_2$, EtOH, HCl cat., reflux; (iv) *n*-BuLi, TMEDA−THF, and then DMF; (v) diethyl (*N*-methoxy-*N*-methylcarbamoylmethyl) phosphate, MeONa, THF; (vi) $LiAlH_4$, Et_2O.

Scheme 3. Synthesis of 3-Butyl-2-hydroxy-2-cyclopenten-1-one 19 and 2-Hydroxy-3-pentyl-2-cyclopenten-1-one 20[a]



[a] Reagents and conditions: (i) butyraldehyde, pyrrolidine, MTBE; (ii) I_2, 160 °C; (iii) H_2O_2, MeOH; (iv) acetic acid, sulfuric acid.

Scheme 4. Synthesis of the New δ-Lactones (8*Z*)-Tetradecen-5-olide 28, (8*E*)-tTetradecen-5-olide 29, and (6*E*)-Tetradecen-5-olide 30 Identified in Chicken Fat[a]



[a] Reagents and conditions: (i) **24**, t-BuLi, Et_2O (−78 °C); (ii) 5% aq AcOH (0 °C); (iii) KOH, EtOH; (iv) H_2SO_4.

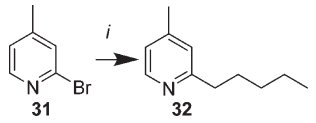
elucidation of their structure was further investigated, as described hereafter.

Identification and Synthesis of New Volatile Compounds. Identification of the structure of unknowns based solely on mass spectra interpretation is challenging. Although many unknowns could be elucidated during this work via mass spectra interpretation, others remained unidentified. Their mass spectra and LRIs are given in Supporting Information. For certain compounds such as **12**, additional microchemical techniques helped in the identification. All newly identified compounds have been synthesized to confirm their occurrence in chicken. Their MS, their LRIs on polar and nonpolar columns, and their relative abundances in the different extracts are given in Figure 2 and Table 1.

*Identification of Cyclic Aldehydes **6**, **7**, and **12** in Roasted Juice.* A series of rarely described cyclic aldehydes, 5-*n*-alkyl-1-cyclopentene-1-carbaldehyde (C_2 to C_5), was identified in roasted juice. In 1993, 5-methyl-, 5-ethyl-, and 5-butyl-1-cyclopentene-1-carbaldehyde were identified for the first time by Werkhoff et al. in

chicken.[4] In our study, two additional members of this chemical class were identified, namely, 5-propyl-1-cyclopentene-1-carbaldehyde **6** and 5-pentyl-1-cyclopentene-1-carbaldehyde **7**. Compounds **6** and **7** were recently tentatively identified by Madruga et al. in goat meat but had not been confirmed by synthesis.[13] In our study, **6** and **7** were prepared from the corresponding 2-alkylcyclopentanones by using the Shapiro reaction (Scheme 1). The comparison of their mass spectra and LRIs (both on polar and nonpolar phase) confirmed their occurrence in chicken.

Scheme 5. Synthesis of 4-Methyl-2-pentylpyridine 32[a]



[a] Reagents: (i) n-$C_5H_{11}MgBr$, $Pd(dppf)_2Cl_2$.

One unknown compound was found in significant amount in all fractions. This unknown had a mass spectrum very similar to that of 2,4,7-decatrienal. All isomers of 2,4,7-decatrienal, obtained as described in ref 14, eluted after the unknown product on the polar GC column. The same spectrum had already been obtained by Schroll et al. during an analysis of hen meat and hypothesized as corresponding to an unspecified isomer of 2,4,7-decatrienal.[15] As given in Figure 2c, high-resolution GC-TOF-MS indicated $C_{10}H_{14}O$ (150.1031, −1.4 mDa) as the molecular formula, therefore containing four double-bond equivalents. Interestingly, the fragment m/z 121.0659 (C_8H_9O, +0.6 mDa) corresponded to a loss of an ethyl group rather than a loss of CHO. The product was purified from the extract F by using NP-HPLC purification, yielding a diluted solution of pure unknown compound on which we performed microchemical reactions to determine the number of double bond(s) and ring(s). The reduction with sodium borohydride gave a product with a molecular ion at m/z 152. Then catalytic hydrogenation gave a

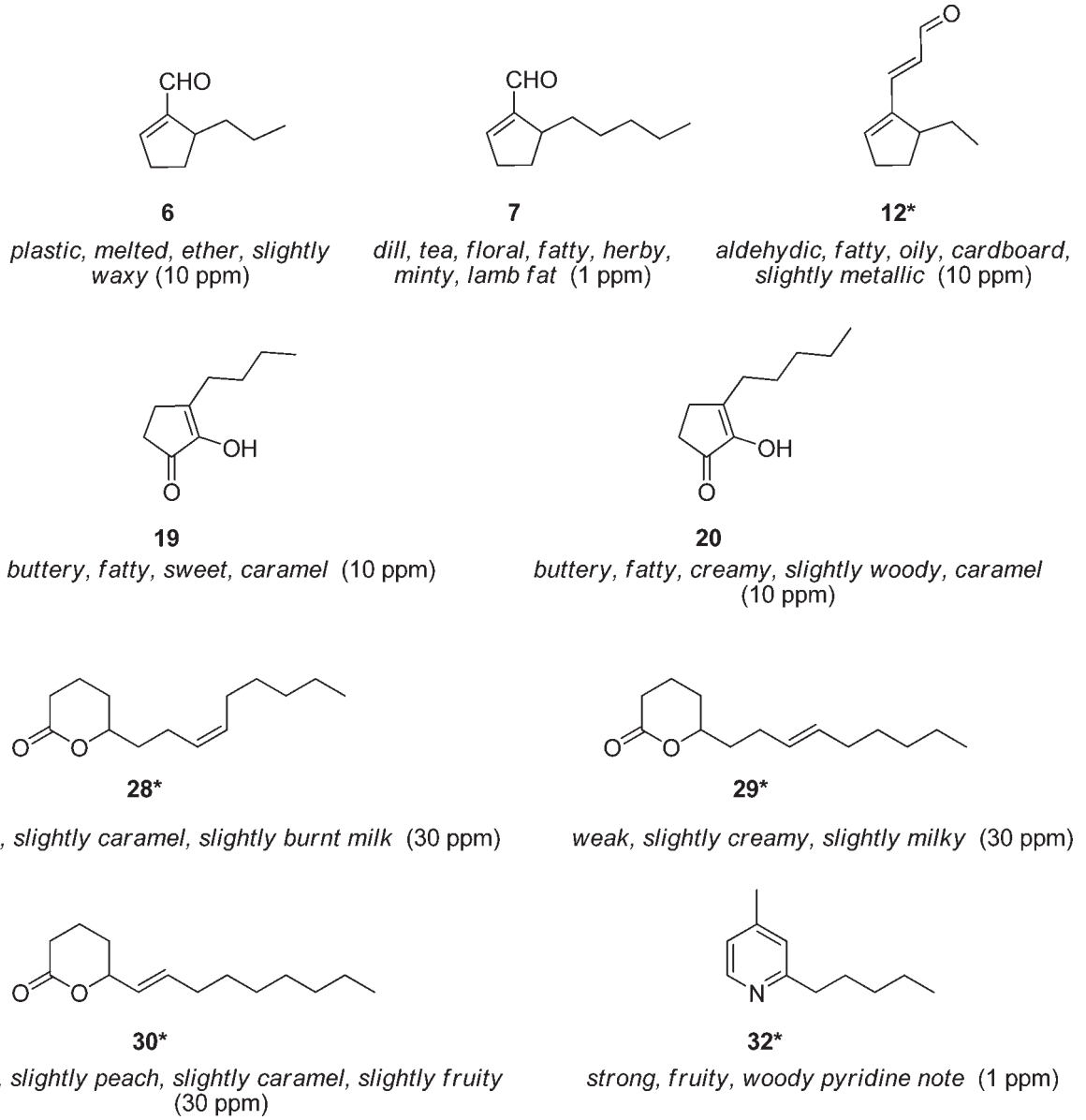


Figure 3. Molecules newly identified in chicken and their flavor description (in salted water 0.3%). *First natural occurrence.

product whose nominal mass had to be determined by negative-CI GC/MS, using ammonia as reagent gas. The nominal mass of m/z 156 so obtained indicated the presence of one ring. By using all the information, the product was identified as penal (*E*)-3-(5-ethylcyclopent-1-enyl)acrylaldehyde **12**. This was confirmed by chemical synthesis (Scheme 2) after comparison of both MS spectra and LRIs. To the best of our knowledge, this new aldehyde has never been identified in a natural product. The formation of such cyclic aldehydes is not yet fully understood. They may come from degradation of either linolenic acid or cyclic fatty acids. Cyclopentyl and cyclohexyl fatty acids are known to form during the frying of fat.[16]

*Identification of New Hydroxyketones **19** and **20** in Cooked Fat.* The analysis of cooked fat revealed a series of uncommon cyclic ketones, namely, 3-alkyl-2-hydroxy-2-cyclopenten-1-ones. 3-Ethyl-2-hydroxy-2-cyclopenten-1-one was found in the cooked fat Leybold trap extract B, and the longer homologues hypothesized as 3-butyl-2-hydroxy-2-cyclopenten-1-one **19** and 2-hydroxy-3-pentyl-2-cyclopenten-1-one **20** were found in the cooked fat distillate A. They were synthesized for confirmation by acid-catalyzed rearrangement of 2,3-epoxy-2-alkylcyclopentanones, obtained by alkaline hydrogen peroxide oxidation of the corresponding 2-alkylcyclopent-2-en-1-ones (Scheme 3).[17] Previously, they were only reported in smoke condensate of cigarettes.[18]

*Identification of New Lactones **28**, **29**, **30** in Cooked Fat.* The fractionation of the cooked fat Leybold distillate extract A by flash chromatography provided 10 fractions that were evaluated on paper strips and are described as given in Materials and Methods. In particular, fraction A-7 had a "lactonic, peachy, creamy" odor. This fraction contained mainly fatty acids (from C_{10} to C_{18}), which rendered the detection of minor components difficult. An alumina treatment enabled us to selectively remove the acids and subsequently detect many minor volatile compounds, in particular δ- and γ-lactones. In particular, three unidentified trace compounds were hypothesized as (8*Z*)-8-tetradecen-5-olide, (8*E*)-8-tetradecen-5-olide, and (6*E*)-6-tetradecen-5-olide on the basis of their MS fragmentation. These three new lactones **28**−**30** were confirmed by synthesis via cyclization of the corresponding 5-hydroxyesters (Scheme 4).[19] Their natural occurrence is reported here for the first time. Their enantiomeric distribution was not investigated in the present study.

*Identification of 2-Pentyl-4-methylpyridine **32** in Cooked Fat.* A series of 2-alkylpyridines (C_4 to C_7) and 3-alkylpyridines (C_2 and C_5) were found in trace amounts in roasted juice, as well as in fractions A-6 and A-7 obtained after fractionation on silica gel of the cooked fat Leybold distillate extract A. Although most of these alkyl pyridines have already been identified by Buttery et al. in the basic fraction of a roasted lamb fat extract,[20] only a few of them have already been reported in chicken.[6,10] Moreover, the taste enhancer properties of 2-pentyl, 2-hexyl, and 2-heptylpyridine were discovered,[21] as described recently.[22] In the present study, 2-pentyl-4-methylpyridine **32** was tentatively identified in fraction A-6 and confirmed by synthesis (Scheme 5).

The sensory evaluation of the synthetic compounds tasted by expert flavorists is given in Figure 3. The quantitation of these novel compounds in the extracts, as well as their odor and flavor thresholds was beyond the scope of this work. However, their flavor descriptors could give some indications of the direction of their contribution. The fatty odor of the cyclic aldehydes **6**, **7**, and **12** may contribute to the typicity of roasted juice. The two cyclic hydroxyketones **19** and **20**, together with 3-ethyl-2-hydroxy-2-cyclopenten-1-one, may contribute to the caramellic note of roasted chicken. Because of their creamy and milky character, the new lactones **28**−**30** may impart creaminess to the overall aroma of cooked fat. Interestingly, 2-pentyl-4-methylpyridine **32** possesses a natural green, spicy-bell pepper odor, which represents a novel aspect of green notes for perfumery applications.[23]

This study gives new insight into the aroma complexity of the poulet de Bresse. The extraction of different parts of chicken obtained under different cooking conditions provided extracts having different molecular compositions. Although the volatiles found in chicken meat have already been widely investigated for many years, our approach allowed us to discover new molecules. The in-depth GC/MS analysis of the separate fractions revealed several trace unknown molecules. MS interpretation and microchemical reactions allowed us to identify nine molecules for the first time in chicken. They were all confirmed by chemical synthesis after comparison of their mass spectra and LRIs. The natural occurrence of five of them is reported here for the first time.

■ ASSOCIATED CONTENT

Supporting Information. Volatile composition of chicken extracts A−F. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

DMF, dimethylformamide; EI, electron ionization; GC/MS, gas chromatograph mass spectrometer; LRIs, linear retention indices; MTBE, methyl *tert*-butyl ether; NP-HPLC, normal phase high-performance liquid chromatography; SPME, solid-phase microextraction; THF, tetrahydrofuran; BuLi, butyllithium; TOF, time-of-flight

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