Characterization of the Aroma Signature of Styrian Pumpkin Seed Oil (*Cucurbita pepo* subsp. *pepo* var. *Styriaca*) by Molecular Sensory Science

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ABSTRACT: Application of the aroma extract dilution analysis on a distillate prepared from an authentic Styrian pumpkin seed oil followed by identification experiments led to the characterization of 47 odor-active compounds in the flavor dilution (FD) factor range of 8−8192 among which 2-acetyl-1-pyrroline (roasty, popcorn-like), 2-propionyl-1-pyrroline (roasty, popcorn-like), 2-methoxy-4-vinylphenol (clove-like), and phenylacetaldehyde (honey-like) showed the highest FD factors. Among the set of key odorants, 2-propionyl-1-pyrroline and another 20 odorants were identified for the first time as constituents of pumpkin seed oil. To evaluate the aroma contribution in more detail, 31 aroma compounds showing the highest FD factors were quantitated by means of stable isotope dilution assays. On the basis of the quantitative data and odor thresholds determined in sunflower oil, odor activity values (OAV; ratio of concentration to odor threshold) were calculated, and 26 aroma compounds were found to have an OAV above 1. Among them, methanethiol (sulfury), 2-methylbutanal (malty), 3-methylbutanal (malty), and 2,3-diethyl-5-methylpyrazine (roasted potato) reached the highest OAVs. Sensory evaluation of an aroma recombinate prepared by mixing the 31 key odorants in the concentrations as determined in the oil revealed that the aroma of Styrian pumpkin seed oil could be closely mimicked. Quantitation of 11 key odorants in three commercial pumpkin seed oil revealed clear differences in the concentrations of distinct odorants, which were correlated with the overall aroma profile of the oils.

KEYWORDS: *Styrian pumpkin seed oil, aroma extract dilution analysis, stable isotope dilution assay, aroma recombinate, methanethiol, 2-acetylpyrroline, 2-propionyl-1-pyrroline*

■ INTRODUCTION

The oil prepared from the roasted seeds of Styrian pumpkin (*Cucurbita pepo* subsp. *pepo* var. *Styriaca*, Cucurbitaceae) is a culinary specialty of Styria, a southeastern province of Austria. Due to its characteristic intense roasty, nutty aroma, it is well appreciated as food flavoring, and has been approved by the commission of the European Union as a product of "Protected Designation of Origin (PDO)". Usually, the oil is produced in family-owned oil mills by processing small batches of <100 kg of dry-roasted pumpkin seeds.[1] After crushing, the seeds are roasted for up to 60 min at temperatures of ∼130 °C and then pressed at elevated temperatures. For the production of 1 L of oil, an average of 2.5 kg of seeds is required corresponding to 30−40 pumpkins.[2] Today, Styrian pumpkin seed oil is no longer considered as a "poor man's oil" but is ranked as a high-priced gourmet product and, thus, several studies aimed at identifying chemical markers for its origin have been performed.[3] However, although the unique aroma is certainly an important attribute of the oil quality, only a few studies have analyzed its volatile fraction and, in particular, data on the key aroma compounds causing the typical aroma of Styrian pumpkin seed oil are scarcely available.

Nikiforov et al.[4] were the first to analyze the volatiles of Styrian pumpkin seed oil. They identified several alkylpyrazines as major compounds, such as 2,6-dimethylpyrazine, 2-ethyl-5-methylpyrazine, methylpyrazine, trimethylpyrazine, and 2-ethyl-3,6-dimethylpyrazine. Buchbauer et al.[5] additionally identified 2-ethyl-3,5-dimethylpyrazine and 2-isobutyl-3-methylpyrazine

and quantitated 2,6-dimethylpyrazine and methylpyrazine, which were postulated as character impact compounds in different Styrian pumpkin seed oils.[5]

The volatiles of roasted Styrian pumpkin seeds were later analyzed by Siegmund and Murkovic.[1] Several Strecker aldehydes, such as methylpropanal, 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, and 3-(methylthio)-propanal, as well as methylpyrazine, ethylpyrazine, 2,5-dimethylpyrazine, and 2-ethyl-3,6-dimethylpyrazine were identified and quantitated. These authors indicated that, in particular, roasting temperatures >100 °C are necessary to obtain the characteristic aroma of the oil.

It is well-accepted in the literature[6] that a volatile must be present above its odor threshold to contribute to the odor of a given food, and further the odorant must be detectable by an odorant receptor in the human nose. A concept addressing these prerequisites to identify the key odorants out of the bulk of odorless volatiles is the molecular sensory science approach involving gas chromatography−olfactometry (GC-O), odor activity values, and aroma recombinates.[6] Matsui et al.[7] were the first to apply GC-O on a pumpkin seed oil. As a result, the authors suggested 2-acetyl-1-pyrroline, (*E,E*)-2,4-decadienal, 2,3-diethyl-5-methylpyrazine, dimethyl trisulfide, 2-ethyl-3,5-

dimethylpyrazine, 3-methylbutanoic acid, and (E,E)-2,4-nonadienal as important odorants in the commercial oil. However, the importance of each odorant was not reliably established, because quite large dilution steps (1:10) were used in the GC-O method applied to find the key contributors to the overall aroma (aroma extract dilution analysis (AEDA)). Furthermore, no quantitation was performed, and also a pumpkin seed oil prepared from coated seeds of the pumpkin species *Telfairia occidentalis* was used.

The literature survey shows that, up to now, no reliable data on the key odorants in Styrian pumpkin seed oil are available. Quantitations of selected volatiles were performed either by analyzing peak areas of FID signals or without using suitable internal standards. Therefore, the aim of this study was (i) to identify the key aroma compounds of an authentic Styrian pumpkin seed oil by combining an analytical with a sensory approach (AEDA), (ii) to quantitate the most important odorants, and (iii) to determine their relative importance to the overall flavor by means of aroma recombination studies. By comparing the results obtained with those of three commercial pumpkin seed oils, the data should also serve as a first hint to find quality markers for pumpkin seed oils.

■ MATERIALS AND METHODS

Pumpkin Seed Oil. The handcrafted Styrian pumpkin seed oil (oil A) used for the study was obtained from a family business in Styria. The seeds used for the oil were grown in Styria (St. Josef, Styria, Austria) and, after roasting, pressed with an oil mill (Wildbach, Styria, Austria). The oil was stored at −25 °C prior to use. The commercial oils B−D were purchased in a specialty shop.

Chemicals. *Reference Odorants.* The following reference odorants were obtained from the sources given in parentheses: benzaldehyde, 2,3-butanedione, 2,3-diethyl-5-methylpyrazine, 2,6-dimethylpyrazine, dimethyl trisulfide, 2-ethyl-3,5-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, ethyl 2-methylbutanoate, 2-ethyl-5-methylpyrazine, 2-ethyl-6-methylpyrazine, ethylpyrazine, 2-furanmethanethiol, hexanal, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 4-hydroxy-3-methoxybenzaldehyde, indole, 2-isopropyl-3-methoxypyrazine, methanethiol, 2-methoxy-4-vinylphenol, 4-methylacetophenone, 2-methylbutanoic acid, 3-methylbutanoic acid, 4-methylphenol, methylpropanal, 3-(methylthio)propanal, (E,E)-2,4-nonadienal, (E,Z)-2,4-nonadienal, γ-nonalactone, (E)-2-nonenal, octanal, 2,3-pentanedione, α-pinene, phenylacetaldehyde, 2-phenylethanol, 3-phenylpropionic acid, propionic acid, and trimethylpyrazine (Sigma-Aldrich, Taufkirchen, Germany); acetic acid, 2-methylbutanal, and 3-methylbutanal (Merck, Darmstadt, Germany); 1-hexen-3-one and 1-octen-3-one (Lancaster, Muehlheim am Main, Germany); 2-methoxyphenol (Serva, Heidelberg, Germany); 2-ethyl-3-methylpyrazine (Acros Organics, Geel, Belgium). (E)-β-Damascenone and (Z)-4-heptenal were gifts from Symrise (Holzminden, Germany).

The following reference odorants were synthesized according to literature methods: 2-acetyl-1-pyrroline,[8] 2-methoxy-5-vinylphenol,[9] and 2-propionyl-1-pyrroline.[10]

Isotopically Labeled Internal Standards. The following internal standards were synthesized according to literature methods: $[^2H_{2−6}]$-2-acetyl-1-pyrroline;[11] $[^{13}C_4]$-2,3-butanedione;[12] $[^2H_3]$-2,3-diethyl-5-methylpyrazine, $[^2H_3]$-2-ethyl-3,5-dimethylpyrazine, $[^2H_3]$-2-ethyl-3,6-dimethylpyrazine, and $[^2H_3]$-2-methoxyphenol;[13] $[^2H_3]$-2,6-dimethylpyrazine and $[^{13}C_2]$-2,3-pentanedione;[14] $[^2H_6]$-dimethyl trisulfide and $[^2H_6]$-methylpropanal;[15] $[^2H_3]$-ethyl 2-methylbutanoate,[16] $[^2H_2]$-2-furanmethanethiol; and $[^2H_3]$-3-(methylthio)propanal;[17] $[^2H_{2−4}]$-hexanal, $[^2H_3]$-4-hydroxy-3-methoxybenzaldehyde, and $[^2H_2]$-(E,E)-2,4-nonadienal;[18] $[^{13}C_2]$-4-hydroxy-2,5-dimethyl-3(2H)-furanone;[19] $[^2H_{10}]$-3-methylbutanoic acid;[20] $[^2H_3]$-2-methoxy-4-vinylphenol,[9] $[^2H_2]$-2-methylbutanal, $[^2H_2]$-(E)-2-nonenal, and $[^2H_{2−4}]$-1-octen-3-one;[21] $[^2H_2]$-3-methylbutanal;[22] $[^2H_2]$-γ-nonalactone;[23]

$[^2H_4]$-octanal;[24] $[^{13}C_2]$-phenylacetaldehyde;[25] $[^2H_{2−4}]$-2-propionyl-1-pyrroline;[26] and $[^2H_3]$-trimethylpyrazine.[27]

$[^2H_3]$-Methanethiol was synthesized prior to use, and its concentration was determined as described earlier.[20]

$[^{13}C_2]$-Acetic acid and $[^2H_{6−7}]$-4-methylphenol were purchased from Sigma-Aldrich.

Isolation of the Volatiles. Diethyl ether (300 mL) was added to the pumpkin seed oil (oil A; 25 mL), and the mixture was vigorously stirred at room temperature for 1 h. The volatiles were then isolated by means of the solvent-assisted flavor evaporation (SAFE) technique.[28] The distillate obtained was treated with aqueous sodium carbonate (0.5 M, 3 × 100 mL) to obtain a neutral/basic and an acidic fraction.[27] Both fractions were concentrated at 40 °C to 250 μL using a Vigreux column (50 cm × 1 cm) followed by microdistillation.[27] For the identification experiments, the neutral/basic fraction was separated according to polarity using silica gel chromatography.[10]

High-Resolution Gas Chromatography−Olfactometry (HRGC-O). This was performed by means of a Thermo Fisher Scientific Trace Ultra gas chromatograph (Dreieich, Germany) using the following fused silica capillaries: FFAP (free fatty acid phase; 30 m × 0.32 mm, 0.25 μm film thickness) (Phenomenex, Torrance, CA, USA) and DB-5 (30 m × 0.25 mm, 0.25 μm film thickness) (Agilent Technologies, Santa Clara, CA, USA). The samples were applied by the cold-on column technique at 40 °C and separated using helium as the carrier gas. For the FFAP capillary, the initial temperature of 40 °C was held for 2 min and then raised at 6 °C/min to 230 °C. For the DB-5 capillary, the initial temperature of 40 °C was held for 2 min and then raised at 6 °C/min to 240 °C. For HRGC-O, the effluent was split 1:1 at the end of the capillary by means of a Y-type splitter using two deactivated fused silica capillaries (50 cm × 0.25 mm i.d.). One part was directed to the flame ionization detector (FID) held at 250 °C, and the other part to a heated, self-made sniffing port made of alumina (200 °C). Calculation of linear retention indices (RI) was done by using a series of *n*-alkanes as described previously.[10]

Aroma Extract Dilution Analysis. In a first experiment, the volatiles present in the undiluted fractions (AF and NBF) were evaluated by four sniffers to eliminate potential gaps in detecting odor-active regions. Then, the flavor dilution (FD) factors of the odor-active compounds were determined by diluting the extract stepwise 1:1 (v/v) with diethyl ether and by analyzing each dilution by HRGC-O. Therefore, besides the original extract, 13 dilutions had to be sniffed (2 to 8192). By definition, the FD factor obtained for each odorant in the AEDA is equal to the highest dilution in which the odorant can be perceived at the sniffing port. The AEDA results obtained by three panelists differing by not more than two FD factors were averaged.

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

Two-Dimensional High-Resolution Gas Chromatography−Olfactometry−Mass Spectrometry (TD-HRGC-O-MS). To locate the odor-active trace components and to obtain unequivocal mass spectra, in some cases application of TD-HRGC-O-MS was necessary using the Moving Column Stream Switching system (MCSS) (Fisons Instruments, Mainz, Germany). The first gas chromatograph, a Mega 2 series (Fisons Instruments), housing an FFAP capillary, was connected to the MCSS system, leading the effluent to an FID and the first sniffing port by means of a Y-type splitter. Heart-cuts were then led by a heated transfer line onto the column in the second gas chromatograph, a Varian 3800 (Varian, Darmstadt, Germany). The end of this capillary was coupled to an ion trap mass spectrometer Saturn 2000 (Varian) and a second sniffing port via a Y-type splitter to allow a simultaneous sensory detection while mass spectra were recorded.

Static Headspace−Olfactometry (SH-O). Pumpkin seed oil (oil A; 15 g) was filled into headspace vials (120 mL) and sealed with a

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

| odorant | labeled internal standard | mass trace[a] (m/z) | | RF[b] |
		analyte	internal standard	
2-acetyl-1-pyrroline	$[^2H_{2-6}]$-2-acetyl-1-pyrroline	112	114–118[c]	0.71
2,3-butanedione	$[^{13}C_4]$-2,3-butanedione	87	91	1.00
2,3-diethyl-5-methylpyrazine	$[^2H_3]$-2,3-diethyl-5-methylpyrazine	151	154	1.00
2,6-dimethylpyrazine	$[^2H_3]$-2,6-dimethylpyrazine	109	112	0.94
dimethyl trisulfide	$[^2H_6]$-dimethyl trisulfide	127	133	0.99
acetic acid	$[^{13}C_2]$-acetic acid	61	63	0.94
2-ethyl-3,5-dimethylpyrazine	$[^2H_3]$-2-ethyl-3,5-dimethylpyrazine	137	140	0.97
2-ethyl-3,6-dimethylpyrazine	$[^2H_3]$-2-ethyl-3,6-dimethylpyrazine	137	140	0.93
ethyl 2-methylbutanoate	$[^2H_3]$-ethyl 2-methylbutanoate	131	134	0.97
2-ethyl-5-methylpyrazine	$[^2H_3]$-trimethylpyrazine	123	126	0.87
2-ethyl-6-methylpyrazine	$[^2H_3]$-trimethylpyrazine	123	126	0.77
ethylpyrazine	$[^2H_3]$-2,6-dimethylpyrazine	109	112	0.97
2-furanmethanethiol	$[^2H_2]$-2-furanmethanethiol	115	117	0.95
hexanal	$[^2H_{2-4}]$-hexanal	101	103–105[c]	1.07
4-hydroxy-2,5-dimethyl-3(2H)-furanone	$[^{13}C_2]$-4-hydroxy-2,5-dimethyl-3(2H)-furanone	129	131	1.00
4-hydroxy-3-methoxybenzaldehyde	$[^2H_3]$-4-hydroxy-3-methoxybenzaldehyde	153	156	0.98
methanethiol	$[^2H_3]$-methanethiol	49	52	0.71
2-methoxyphenol	$[^2H_3]$-2-methoxyphenol	125	128	0.97
2-methoxy-4-vinylphenol	$[^2H_3]$-2-methoxy-4-vinylphenol	151	154	0.94
2-methylbutanal	$[^2H_3]$-2-methylbutanal	87	90	0.92
3-methylbutanal	$[^2H_3]$-3-methylbutanal	87	90	0.90
2- and 3-methylbutanoic acid[d]	$[^2H_{10}]$-3-methylbutanoic acid	103	113	0.93
4-methylphenol	$[^2H_{6-7}]$-4-methylphenol	109	115–116[c]	0.89
methylpropanal	$[^2H_6]$-methylpropanal	73	79	0.78
3-(methylthio)propanal	$[^2H_3]$-3-(methylthio)propanal	105	108	0.94
(E,E)-2,4-nonadienal	$[^2H_2]$-(E,E)-2,4-nonadienal	139	141	1.00
γ-nonalactone	$[^2H_2]$-γ-nonalactone	157	159	0.78
(E)-2-nonenal	$[^2H_2]$-(E)-2-nonenal	141	143	0.70
octanal	$[^2H_4]$-octanal	129	133	1.00
1-octen-3-one	$[^2H_{2-4}]$-1-octen-3-one	127	129–131[c]	0.60
2,3-pentanedione	$[^{13}C_2]$-2,3-pentanedione	101	103	0.87
phenylacetaldehyde	$[^{13}C_2]$-phenylacetaldehyde	121	123	0.90
2-propionyl-1-pyrroline	$[^2H_{2-4}]$-2-propionyl-1-pyrroline	126	128–130[c]	0.68
trimethylpyrazine	$[^2H_3]$-trimethylpyrazine	123	126	0.96

[a]Mass trace (MS-CI) used for peak area calculation of analyte and standard, respectively. [b]MS response factor. [c]Internal standard was used as a mixture of isotopologues. [d]Differentiation of 2-and 3-methylbutanoic acid was performed as described in ref 23.

septum. The samples were equilibrated at 28 °C for 30 min. Six vessels were prepared, and decreasing headspace volumes (20, 10, 5, 2.5, 1, and 0.5 mL) were withdrawn with a gastight syringe. The samples were subsequently analyzed as follows: Starting with the largest volume (20 mL), this was injected into a Trace Ultra gas chromatograph (Thermo Fisher Scientific, Dreieich, Germany), equipped with a model 915 cold-trap unit (Thermo Fisher Scientific). During the injection, air was released through a pressure control valve while the aroma compound was cryofocused on an uncoated fused-silica capillary (i.d. = 0.53 mm) held at −150 °C. Then, the cold-trap was heated to 250 °C for 5 min (heating rate = 15 °C/min), and the volatiles were transferred onto the DB-5 capillary column. An oven temperature of 0 °C was held for 3 min, and then the temperature was raised at 6 °C/min to 60 °C and further at 15 °C/min to 240 °C. Helium was used as the carrier gas.

At the end of the column, the effluent was transferred to an FID, a sniffing port, and an ion trap mass spectrometer Saturn 2100 T (Varian, Darmstadt, Germany). Mass spectra were generated in the electron impact mode (MS-EI) at 70 eV and in the chemical ionization mode (MS-CI) with an ionization energy of 105 eV and methanol as the reagent gas. The GC-O was done by three panelists.

Separation of 2-Ethyl-3-methylpyrazine and Trimethylpyrazine. Because 2-ethyl-3-methylpyrazine and trimethylpyrazine coeluted on both the FFAP and DB-5 GC columns, another stationary phase was used to separate both compounds. As previously reported,[27] the PTA-5 capillary column (30 m × 0.32 mm, 0.5 μm film thickness) (Supelco, Bellefonte, PA, USA) was able to successfully separate both pyrazines. For the PTA-5 capillary, the initial temperature of 40 °C was held for 2 min and then raised at 4 °C/min to 160 °C and finally at 20 °C/min to 230 °C.

Determination of the Concentrations of Stable Isotope-Labeled Internal Standards. Because most of the syntheses were performed at a microscale level, common purification procedures, such as distillation or crystallization, could not be applied. To determine the exact concentrations, the following approach was used: First, a solution containing defined amounts of the respective unlabeled compound and methyl octanoate as a reference standard were analyzed by HRGC-FID to calculate a response factor. Then, a defined amount of methyl octanoate was added to a defined volume of the solution containing the labeled compound, and the resulting mixture was analyzed again by HRGC-FID. The concentration of the labeled compound was calculated from the peak area using the FID response factor determined for the unlabeled compound.

Quantitation by Stable Isotope Dilution Assays (SIDA). Pumpkin seed oil (1–250 g; depending on the amounts of the target compounds estimated in preliminary experiments) was dissolved in

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diethyl ether, and defined amounts of the isotopically labeled standards in dichloromethane were added to the solution. After equilibration for 30 min, the isolation of the volatiles was performed as described above.

High-Resolution Gas Chromatography–Mass Spectrometry (HRGC-MS). The following compounds were present in higher amounts and could be analyzed by one-dimensional HRGC-MS: acetic acid, 2,6-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-6-methylpyrazine, ethylpyrazine, hexanal, 2- and 3-methylbutanoic acid, and trimethylpyrazine. For this purpose, a Varian 431 gas chromatograph, equipped with an FFAP capillary column (30 m × 0.25 mm, 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA), was coupled to an ion trap mass spectrometer Varian 2000. Mass spectra were recorded in the chemical ionization (CI) mode with methanol as the reagent gas.

Two-Dimensional High-Resolution Gas Chromatography–Mass Spectrometry (TD-HRGC-MS). Quantitation of the remaining compounds (except methanethiol and methylpropanal) was performed by means of a TD-HRGC/MS system using the FFAP column (30 m × 0.32 mm, 0.25 μm film thickness) in the first dimension and an OV-1701 column (30 m × 0.32 mm, 0.25 μm film thickness) (all J&W Scientific) in the second dimension. The TD-HRGC-MS system consisted of a Trace 2000 series gas chromatograph (Thermo Quest, Eglsbach, Germany) coupled to a Varian GC 3800 by means of an uncoated and deactivated fused silica transfer line (0.32 mm i.d.) held at 250 °C and, finally, to an ion trap mass spectrometer Varian Saturn 2000. Heart-cuts were done by means of the moving capillary stream switching system (see above). Mass spectra were recorded in the CI mode with methanol as the reagent gas.

Quantitation of Methanethiol and Methylpropanal. Methanethiol and methylpropanal were determined by SIDAs in headspace samples. For the quantitation of methanethiol, the oil (10 g) was filled in headspace vials (120 mL) and sealed with an airtight septum. A defined volume of the labeled compound was injected into the headspace vials with a gastight syringe. After equilibration (28 °C, 30 min) with continuous shaking, aliquots of the headspace were withdrawn and analyzed by means of static headspace GC-MS as described above. The same approach was used for the quantitation of methylpropanal, but the labeled methylpropanal was added first to the oil (5 g), and then the vials were sealed with an airtight septum.

Determination of MS Calibration Factors. For each compound, a calibration factor was calculated by analyzing mixtures of defined amounts of the labeled and unlabeled compound in five different mass ratios (1:5, 1:3, 1:1, 3:1, 5:1). The MS response factors calculated are summarized in Table 1.

Determination of Odor Thresholds. Odor thresholds were determined in sunflower oil following a recently published protocol.[29]

Aroma Profile Analysis. The sensory evaluation of the pumpkin seed oils and the recombinate was performed by a sensory panel consisting of 20 panelists. The panelists were regularly trained in orthonasal odor perception as previously described.[29] Characteristic aroma descriptors, determined in preliminary sensory experiments, were used for the evaluation. Each descriptor was represented by the odor of a reference compound dissolved in sunflower oil at a concentration 100-fold above the respective odor threshold. The following compounds given in parentheses were chosen for the odor attributes evaluated: roasty, popcorn-like (2-acetyl-1-pyrroline); smoky, clove-like (2-methoxy-4-vinylphenol); earthy, roasted potato-like (2,3-diethyl-5-methylpyrazine); fatty ((E,E)-2,4-nonadienal); caramel-like (4-hydroxy-2,5-dimethyl-3(2H)-furanone); malty (3-methylbutanal); sweaty (3-methylbutanoic acid); green (hexanal); cooked potato-like (3-(methylthio)propanal); sulfury (methanethiol); and buttery (2,3-pentanedione).

The recombinate and the pumpkin seed oil were evaluated by rating the intensities of the 11 descriptors on the basis of a seven-point scale from 0 (not perceivable) over 0.5, 1.0, 1.5, 2.0, 2.5, to 3 (strongly perceivable). The single judgments of the panelists were averaged.

Aroma Recombination. For aroma recombination, deodorized sunflower oil was used as the matrix. All aroma compounds showing odor activity values (OAVs) > 1 were dissolved in the oil at concentration levels equal to those determined in pumpkin seed oil A.

The similarity of the recombinate on a scale from 0 to 3 was judged in comparison to the oil by the trained sensory panel.

Recombinate Extended with Four Alkylpyrazines. A triangle test[29] was carried out to evaluate if four pyrazines, which had shown only low FD factors but high concentrations, have an influence on the overall aroma of the Styrian pumpkin seed oil. Ethylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, and 2-ethyl-5-methylpyrazine were added in the concentrations analyzed in the pumpkin seed oil to the recombinate (aroma model I) to obtain aroma model II. Aroma model I was evaluated in comparison to II in a triangle test, and the assessors were asked to identify the sample that differed from the other two samples. The statistical significance was calculated on the basis of § 64 LFGB method L 00.90-7 appendix.[1]

■ RESULTS AND DISCUSSION

Characterization of Key Odorants in Styrian Authentic Pumpkin Seed Oil A. The volatile fraction from Styrian pumpkin seed oil A was isolated by solvent extraction, followed by high-vacuum distillation. A sniffing test on a strip of filter paper confirmed that the aroma distillate fully represented the aroma of the pumpkin seed oil. Application of the AEDA to the distillate revealed 53 odor-active areas in the FD factor range of 8−8192. Among them, a compound with a roasty, popcorn-like odor showed the highest intensity (**15**) (Figure 1), followed by
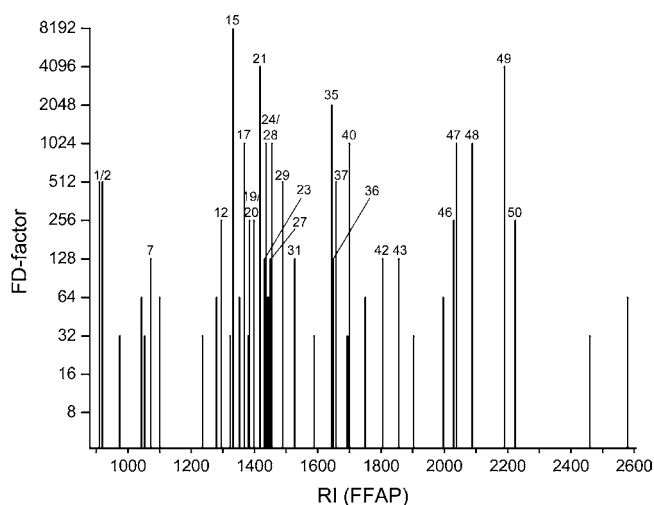

Figure 1. Flavor dilution (FD) chromatogram of the volatile fraction of an aroma distillate of Styrian pumpkin seed oil A (compounds showing FD ≥ 32 are given). Numbers correspond to Table 2.

three compounds with a roasty, popcorn-like note (**21**), a spicy, clove-like odor (**49**), and a honey-like aroma (**35**). To identify the compounds responsible for the perceived odors, first, the retention indices of the odor-active areas were determined on two different GC stationary phases by GC-O experiments. A comparison of odor quality and retention indices of the orthonasally detected compounds with data of ∼1000 food odorants available in an in-house database then suggested structures for each odorant. For unequivocal identification, the neutral/basic fraction was fractionated on silica gel using pentane/diethyl ether mixtures of increasing polarity. In the single fractions obtained, the odorants were detected by GC-O and their mass spectra were recorded using either HRGC-MS or TD-HRGC-O-MS. The structure was finally confirmed by comparing the analytical and sensory attributes with those of the respective reference compounds. For the identification of some odorants, a fractionation by TD-GC was necessary because several trace odorants coeluted with other volatiles by

present in high amounts. Acidic compounds were identified directly in the respective fractions because no coelution occurred.

Following this procedure, **15** was identified as 2-acetyl-1-pyrroline (Figure 2), **21** as 2-propionyl-1-pyrroline, and **35** as
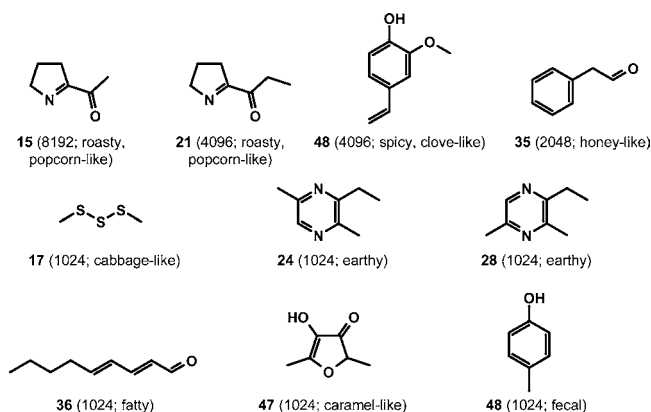

Figure 2. Structures of the most odor-active volatiles identified in Styrian pumpkin seed oil A (numbers and FD factors refer to Table 2).

phenylacetaldehyde. Further compounds with high FD factors were characterized as 2-methoxy-4-vinylphenol (**48**), dimethyl trisulfide (**17**), 2-ethyl-3,5-dimethyl- and 2-ethyl-3,6-dimethyl-pyrazine (**24** and **28**), and, additionally (*E,E*)-2,4-nonadienal (**36**), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**47**), and 4-methylphenol (**48**). For the identification of compound **20** (Figure 1), with a retention index of 1398 on the FFAP capillary and of 1004 on the DB-5 capillary, a third stationary phase had to be used. Because both trimethylpyrazine and 2-ethyl-3-methylpyrazine were identified and could, thus, be responsible for the earthy note detected, these were separated on a less polar stationary phase. After separation, trimethylpyrazine was identified as the earthy-smelling odorant, whereas 2-ethyl-3-methylpyrazine was sensorially not detectable.

Altogether, 47 of the 53 odorants detected by AEDA could be identified (Table 2). Among them, 21 are reported in this study for the first time not only as key odorants but even as volatile constituents of Styrian pumpkin seed oil. In particular, 2-propionyl-1-pyrroline, (*E,E*)-2,4-nonadienal, and 4-methyl-phenol are worth mentioning due to their high FD factors (Table 2).

The procedure used for the isolation of the volatiles may discriminate highly volatile aroma compounds with low boiling points. Therefore, GC-O was applied on static headspace samples using a series of decreasing volumes above the pumpkin seed oil in a GC-O experiment. Two compounds, the sulfury-smelling methanethiol and the malty-smelling methylpropanal (Table 3), were identified as further odorants in the seed oil. Methanethiol, which was still perceived in a low headspace volume of 1 mL, was also characterized for the first time as a volatile constituent of pumpkin seed oil.

Quantitation of the Odorants. Because the AEDA is a screening method to select aroma active compounds from the bulk of odorless volatiles, quantitative data are required to more precisely evaluate the contribution of the odorants to the oil. Thus, 31 odorants identified with the highest FD factors during AEDA (Table 2) were quantitated by means of SIDAs. The quantitative data are mean values measured in several batches of the same oil.

The results of the quantitative measurements (Table 4) revealed acetic acid (sour), 2-methylbutanal (malty), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (caramel), 3-methylbu-tanal (malty), and methylpropanal (malty) with the highest concentrations. In addition, further compounds were found in high concentrations, for example, 2-methylbutanoic acid, 2-methoxy-4-vinylphenol, trimethylpyrazine, 2-ethyl-3,6-dimeth-yl-pyrazine, 3-methylbutanoic acid, and hexanal. Some odorants, however, were present only in trace amounts, such as 1-octen-3-one, 2-acetyl-1-pyrroline, (*E,E*)-2,4-nonadienal, 2-methoxyphenol, and 2-furanmethanethiol.

Calculation of Odor Activity Values. To get a deeper insight into the contribution of the quantitated aroma compounds to the overall aroma of the seed oil, OAVs, defined as the ratio of concentration to odor thresholds, were calculated for each odorant. For the six newly determined thresholds, sunflower oil was chosen as an appropriate matrix. Of the 31 aroma compounds quantitated, 5 compounds, namely, 1-octen-3-one, γ-nonalactone, 2-methoxyphenol, octanal, and (*E*)-2-nonenal, had an OAV below 1 and are, therefore, assumed to be of minor importance for the overall pumpkin seed oil aroma. Although these odorants were detected during the AEDA, it must be taken into account that during GC-O the complete amount of a volatile is volatilized, whereas the concentration above a given matrix depends on its volatility, that is, physicochemical parameters.

The remaining 26 odorants showed OAVs ranging between 2 and 2316 (Table 5). Among them, the sulfury-smelling methanethiol showed the highest OAV, followed by the malty-smelling 3-methylbutanal and 2-methylbutanal. In addition, high OAVs were also calculated for 2,3-diethyl-5-methylpyrazine, methylpropanal, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, and 3-(methylthio)propanal. With quite high OAVs also the roasty, popcorn-like-smelling odorants 2-propionyl-1-pyrroline and 2-acetyl-1-pyrroline are suggested as key contributors to the roasty note of the pumpkin seed oil on the basis of OAV calculations.

Aroma Recombination. Because it is difficult to predict the overall aroma quality of a complex mixture of aroma compounds, aroma recombination experiments are a useful tool to verify the results on the receptor/perception level and, also, to ensure that all key odorants were detected. For this purpose, all odorants with OAVs > 1 were added to sunflower oil in the concentrations determined, resulting in recombinate I. A comparison of aroma profiles of the recombinate (Figure 3A) and the Styrian pumpkin seed oil (Figure 3B) showed a good similarity in the intensity of the attributes evaluated. In the Styrian pumpkin seed oil as well in the recombinate the roasty, popcorn-like odor attribute was perceived with the highest intensity. In addition, also all other aroma descriptions were evaluated to be nearly similar. These data confirmed that the key aroma compounds were successfully identified and quantitated, and it became obvious that the characteristic aroma of Styrian pumpkin seed oil is caused by a unique mixture of 26 aroma-active compounds in their "natural" concentrations, the aroma signature of the oil.

Alkylpyrazines. In the literature it is often discussed that the entire group of alkylpyrazines contribute to the overall aroma of roasted oilseeds or nuts, respectively. Nikiforov et al.[4] postulated several alkylpyrazines as dominating aroma compounds of Styrian pumpkin seed oil, among them, for example, 2,6-dimethylpyrazine, methylpyrazine, and 2-ethyl-5-methylpyrazine. In this study, these pyrazines were not perceived with

Table 2. Most Odor-Active Compounds (FD ≥ 8) Identified in an Aroma Distillate Prepared from Styrian Pumpkin Seed Oil (Oil A)

no.	odorant[b]	odor quality[c]	RI on[a] FFAP	RI on[a] DB-5	FD[d]	earlier reported[e] as volatile constituent
1	3-methylbutanal	malty	910	650	512	1, 7
2	2-methylbutanal	malty	920	656	512	1, 7
3	2,3-butanedione	buttery	974	590	32	1, 7
4	α-pinene	pine needle-like	1007	935	16	
5	ethyl 2-methylbutanoate	fruity	1043	850	64	7
6	2,3-pentanedione	buttery	1053	705	32	
7	hexanal	green	1073	802	128	1, 7
8	1-hexen-3-one	metallic	1100	772	64	
9	unknown	sweet	1169	nd[g]	8	
10	(Z)-4-heptenal	fishy	1236	901	32	7
11	octanal	citrus-like, green	1280	1011	64	7
12	1-octen-3-one	mushroom-like	1295	978	256	
13	ethylpyrazine	roasty, buttery	1316	915	8	1
14	2,6-dimethylpyrazine	roasty	1323	911	32	4
15	2-acetyl-1-pyrroline	roasty, popcorn-like	1332	922	8192	7
16	2,3-dimethyl-5-methoxypyrazine	roasty, sweet	1352	917	64	
17	dimethyl trisulfide	cabbage-like	1368	970	1024	1, 7
18	2-ethyl-5-methylpyrazine	nutty	1380	996	32	1, 4, 7
19	2-ethyl-6-methylpyrazine	nutty	1384	1000	256	1
20	trimethylpyrazine	earthy	1398	1004	256	4
21	2-propionyl-1-pyrroline	roasty, popcorn-like	1418	1025	4096	
22	2-isopropyl-3-methoxypyrazine[f]	pea-like, earthy	1425	1094	16	
23	2-furanmethanethiol	sulfury, burnt	1432	913	128	
24	2-ethyl-3,6-dimethylpyrazine	earthy	1436	1071	1024	1, 4, 7
25	2,3-diethylpyrazine	earthy	1442	1089	64	
26	acetic acid	vinegar-like	1445	612	64	
27	3-(methylthio)propanal	cooked potato-like	1450	905	128	1
28	2-ethyl-3,5-dimethylpyrazine	earthy	1455	1084	1024	5, 7
29	2,3-diethyl-5-methylpyrazine	earthy	1489	1165	512	7
30	benzaldehyde	bitter almond-like	1499	959	8	1, 7
31	(E)-2-nonenal	fatty	1527	1159	128	
32	propionic acid	sweaty	1535	835	16	
33	unknown	fatty	1588	nd	32	
34	unknown	fatty	1610	nd	8	
35	phenylacetaldehyde	honey-like	1644	1041	2048	1, 7
36	(E,Z)-2,4-nonadienal[f]	fatty	1649	1191	128	
37	2- and 3-methylbutanoic acid	sweaty	1658	870	512	7
38	unknown	fatty	1673	nd	8	
39	unknown	honey-like	1693	nd	32	
40	(E,E)-2,4-nonadienal	fatty	1700	1216	1024	
41	4-methylacetophenone	sweet	1750	1200	64	
42	(E)-β-damascenone	cooked apple-like	1805	1389	128	7
43	2-methoxyphenol	smoky, sweet	1856	1089	128	
44	2-phenylethanol	flowery	1903	1115	32	1, 4
45	unknown	sweet	1996	nd	64	
46	γ-nonalactone	peach-like	2030	1363	256	
47	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel-like	2038	1070	1024	7
48	4-methylphenol	fecal	2088	1077	1024	
49	2-methoxy-4-vinylphenol	spicy, clove-like	2190	1315	4096	7
50	2-methoxy-5-vinylphenol[f]	clove-like	2224	1353	256	
51	indole	fecal	2460	1299	32	
52	4-hydroxy-3-methoxybenzaldehyde	vanilla-like	2579	1409	64	7
53	3-phenylpropionic acid	flowery	2621	1389	8	

[a]Retention index. [b]The compound was identified by comparing its mass spectra (MS-EI, MS-CI), retention indices on capillaries FFAP and DB-5, and the odor quality and the odor intensity perceived during sniffing with data of reference compounds. [c]Odor quality perceived at the sniffing port. [d]Flavor dilution factor determined by AEDA on capillary FFAP. [e]The compound was earlier reported as a volatile compound in pumpkin seed oil or pumpkin seeds in the given reference. .[f]No unequivocal mass spectrum could be obtained. Identification is based on the remaining criteria given in footnote. [g]nd, not determined.

Table 3. Important Odorants Identified in the Headspace of Pumpkin Seed Oil A

odorant	odor quality	RI[a] on DB-5	vol[b] (mL)	earlier reported[c]
methanethiol	sulfury	355	1	
methylpropanal	malty	486	1	1, 7
2,3-butanedione	buttery	515	20	1
3-methylbutanal	malty	628	1	1, 7
2-methylbutanal	malty	640	5	1, 7

[a]Retention index. [b]Smallest headspace volume in which the odorant was perceived. [c]The compound was earlier reported as a volatile compound in pumpkin seed oil or pumpkin seeds in the given reference.

Table 4. Concentrations of 31 Important Aroma Compounds in the Styrian Pumpkin Seed Oil

aroma compound	concn[a] (μg/kg)	n[b]
acetic acid	32950	4
2-methylbutanal	5923	3
4-hydroxy-2,5-dimethyl-3(2H)-furanone	4762[c]	4
3-methylbutanal	4741	3
methylpropanal	4274	5
2-methylbutanoic acid	1996[c]	6
2-methoxy-4-vinylphenol	1592	5
trimethylpyrazine	863	5
2-ethyl-3,6-dimethylpyrazine	754	3
3-methylbutanoic acid	515[c]	6
hexanal	490	3
4-hydroxy-3-methoxybenzaldehyde	320[c]	3
2,3-butanedione	221	4
2,3-diethyl-5-methylpyrazine	169	4
phenylacetaldehyde	146	4
methanethiol	139[d]	5
4-methylphenol	113	3
2-ethyl-3,5-dimethylpyrazine	112	3
γ-nonalactone	105[c]	5
octanal	41.5[c]	3
3-(methylthio)propanal	21.7[c]	3
(E)-2-nonenal	21.2[c]	4
dimethyl trisulfide	19.0	4
2-propionyl-1-pyrroline	14.4[d]	4
ethyl-2-methylbutanal	11.3	4
2,3-pentanedione	10.7	4
1-octen-3-one	8.2	4
2-acetyl-1-pyrroline	7.5[c]	5
(E,E)-2,4-nonadienal	6.8[c]	3
2-methoxyphenol	6.4	7
2-furanmethanethiol	2.2	3

[a]Mean value differing by not more than ±10%. [b]Number of different bottles of the same oil analyzed. [c]Mean value differing not more than ±20%. [d]Mean value differing not more than ±30%.

high FD factors (Table 2), and methylpyrazine was even not detected, although 2,6-dimethylpyrazine, ethylpyrazine, 2-ethyl-5-methylpyrazine, and 2-ethyl-6-methylpyrazine had shown the highest FID peak areas in the volatile fraction (data not shown). These four pyrazines were, therefore, quantitated by means of SIDA, to evaluate if they might contribute to the overall aroma. Ethylpyrazine, 2,6-dimethylpyrazine, and 2-ethyl-6-methylpyrazine were quantitated with the highest amounts (Table 6), but the odor thresholds of all these pyrazines are in the milligrams per kilogram range. For example, the odor

Table 5. Orthonasal Odor Thresholds (OTs) and Odor Activity Values (OAVs) of 31 Aroma Compounds in Seed Oil A

aroma compound	OT[a] (μg/kg)	OAV[b]	ref[c]
methanethiol	0.06	2316	30
3-methylbutanal	5.4	878	31
2-methylbutanal	10	592	32
2,3-diethyl-5-methylpyrazine	0.5	338	32
methylpropanal	15	285	d
4-hydroxy-2,5-dimethyl-3(2H)-furanone	25	190	33
2-propionyl-1-pyrroline	0.1	131	34
3-(methylthio)propanal	0.2	109	31
acetic acid	350	94	d
2-acetyl-1-pyrroline	0.09	83	35
4-methylphenol	2.3	49	d
2-ethyl 3,5-dimethylpyrazine	2.9	39	34
2,3-pentanedione	0.3	37	34
2-methoxy-4-vinylphenol	50	32	9
3-methylbutanoic acid	22	23	36
2,3-butanedione	10	22	31
2-methylbutanoic acid	101	20	d
ethyl 2-methylbutanoate	0.7	16	36
2-ethyl-3,6-dimethylpyrazine	76	10	d
dimethyl trisulfide	2.5	8	30
phenylacetaldehyde	22	7	33
2-furanmethanethiol	0.4	6	35
(E,E)-2,4-nonadienal	1.5	5	34
trimethylpyrazine	290	3	33
hexanal	296	2	21
4-hydroxy-3-methoxybenzaldehyde	181	2	37
1-octen-3-one	10	<1	21
octanal	56	<1	36
γ-nonalactone	148	<1	32
2-methoxyphenol	16	<1	36
(E)-2-nonenal	140	<1	d

[a]Odor threshold determined in sunflower oil. [b]Odor activity values were calculated by dividing the concentration (Table 4) by the respective odor threshold. [c]Literature data for odor thresholds. [d]Newly determined odor threshold.

thresholds of ethylpyrazine were newly determined in sunflower oil to be 19000 μg/kg and for 2,6-dimethylpyrazine to be 7700 μg/kg. Thus, it could be assumed that the four pyrazines do not contribute to the overall aroma of Styrian pumpkin seed oil. However, possible additive effects with other, more odor-active pyrazines could not be excluded. Therefore, an additional sensory experiment was carried out. Aroma recombinate I was spiked with the four pyrazines in the concentrations listed in Table 6, resulting in recombinate II, which was evaluated in a triangle test in comparison to recombinate I without the four pyrazines added.

The results showed that the sensory panel was not able to detect a difference between recombinates I and II, because only 7 of 17 panelists were able to correctly identify the differing mixture. Thus, additive effects of these pyrazines could be ruled out and, furthermore, it could be confirmed that the four pyrazines do not contribute to the overall aroma of the pumpkin seed oil.

Analysis of Commercial Styrian Pumpkin Seed Oils. To compare the results obtained for the handcrafted seed oil A with commercial Styrian pumpkin seed oils, three oils (B−D) were bought in a specialty shop. For comparison, 11 key aroma
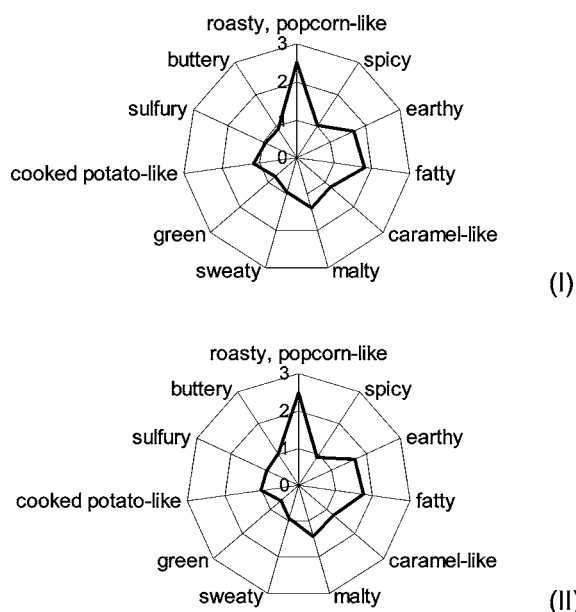
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Figure 3. Aroma profile analyses of the handcrafted Styrian pumpkin seed oil A (I) and of the aroma recombinate (II).

Table 6. Concentrations of Four Alkylpyrazines That Showed Low or No FD Factors in the Volatile Fraction of Pumpkin Seed Oil A

pyrazine	concn[a] (μg/kg)	OT (μg/kg)	OAV
ethyl-	6159	19000	<1
2,6-dimethyl-	4438	7700	<1
2-ethyl-6-methyl-	1383	1500[b]	<1
2-ethyl-5-methyl-	205	100[b]	2

[a]Mean value of quadruplicates, differing not more than ±10%. [b]Taken from ref 38.

compounds characterized as key odorants in the handcrafted oil A were quantitated in oils B–D (Table 7).

The results showed that in the three oils B–D as in the handcrafted pumpkin seed oil A, 2- and 3-methylbutanal followed by 2,3-diethyl-5-methylpyrazine and 4-hydroxy-2,5-dimethyl-3(2H)-furanone showed the highest OAVs. However, clear differences in the concentrations/OAVs could be observed, and in particular the lowest amounts of 2,3-diethyl-

5-methylpyrazine, 2-propionyl-1-pyrroline, 2-acetyl-1-pyrroline or 3-(methylthio)propanal were measured in oil D. These data were in correlation with the results of aroma profile analyses of the oils (Figure 4). In the aroma profile of oil D, in particular, the roasty, popcorn-like odor attribute was rated low in oils C and D compared to oils A and B. The same was true for the caramel-like odor, which was also rated low in oil D, which was correlated with the lower amounts of, for example, the caramel-like-smelling 4-hydroxy-2,5-dimethyl-3(2H)-furanone. On the other hand, the clove-like-smelling 2-methoxy-4-vinylphenol was higher in oil D as well as in oil C.

Overall, the results show that the typical aroma of Styrian pumpkin seed oil consists of a unique mixture of aroma-active compounds in defined concentrations. In particular, compounds derived from carbohydrate/amino acid reactions, such as 2-acetyl-1-pyrroline, 2-propionyl-1-pyrroline, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone, and the aroma-active Strecker aldehydes 2-methylbutanal, 3-methylbutanal, 3-(methylthio)-propanal, and phenylacetaldehyde contribute to the aroma of Styrian pumpkin seed oil. These odorants are undoubtedly generated from their precursors by the roasting step during the production of the oil. To confirm the effect of the roasting step, it would, however, be necessary to evaluate which odorants are just transferred from the raw pumpkin seeds to the oil and which aroma-active compounds are formed from distinct aroma precursors occurring in the raw seeds. Such studies aimed at clarifying the role of aroma precursors present in authentic raw Styrian pumpkin seed are underway.

In previous studies on roasted peanuts[27,39] nearly the same set of key odorants was identified, but their concentrations differed greatly from those of the pumpkin seed oil. However, our data support the idea that the different aroma impressions of roasted food samples are caused by quantitative rather than by qualitative differences in the respective key odorants. Furthermore, with these results on the aroma signature of Styrian pumpkin seed oil at hand, and a following determination of the concentration ranges of the key odorants in a larger set of pumpkin seed oils, such data would undoubtedly allow the assessment of the authenticity of this specialty oil.

Table 7. Concentrations and Odor Activity Values[a] of 11 Key Odorants in 3 Commercial Styrian Pumpkin Seed Oils B–D[v]

	A		B		C		D	
odorant	concn (μg/kg)	OAV	concn (μg/kg)	OAV	concn (μg/kg)	OAV	concn (μg/kg)	OAV
3-methylbutanal	4741	878	3369	624	3335	618	3715	689
2-methylbutanal	5923	592	3784	378	3383	338	3611	361
2,3-diethyl-5-methylpyrazine	169	338	88	176	60	120	55	110
4-hydroxy-2,5-dimethyl-3(2H)-furanone	4762	190	4327	173	3533	141	2536	101
2-propionyl-1-pyrroline	14	131	9.9	90	4.8	44	1.9	17
3-(methylthio)propanal	22	109	14	71	15	75	6.7	34
2-acetyl-1-pyrroline	7.5	83	4.4	49	3.9	43	1.8	20
2-ethyl-3,5-dimethylpyrazine	112	39	178	63	122	43	129	45
2-methoxy-4-vinylphenol	1592	32	1491	30	2138	43	2089	42
2-ethyl-3,6-dimethylpyrazine	754	10	937	12	583	8	557	7
phenylacetaldehyde	146	7	96	4	103	5	94	4

[a]Odor activity values (ratio of concentration to odor thresholds) were calculated on the basis of the odor thresholds given in Table 5. [v]Data for the authentic oil A are given for comparison. Data are mean values of triplicates differing by not more than ±10%.
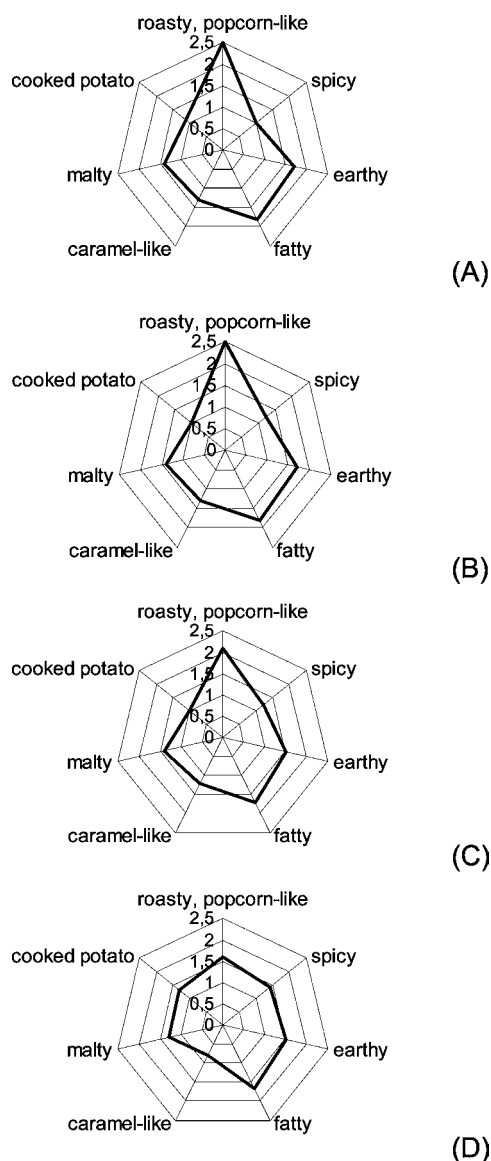
Figure 4. Aroma profile analyses of the handcrafted Styrian pumpkin seed oil (A) and of three commercial Styrian pumpkin seed oils (B–D).

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Siegmund, B.; Murkovic, M. Changes in chemical composition of pumpkin seeds during the roasting process for production of pumpkin seed oil (part 2: volatile compounds). *Food Chem.* **2004**, *84*, 367–374.

(2) Fruhwirth, G.; Hermetter, A. Seeds and oil of the Styrian oil pumpkin: components and biological activities. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 1128–1140.

(3) Joebstl, D.; Bandoniene, D.; Meisel, T.; Chatzistathis, S. Identification of the geographical origin of pumpkin seed oil by use of rare earth elements and discriminant analysis. *Food Chem.* **2010**, *123*, 1303–1309.

(4) Nikiforov, A.; Knapp, M.; Buchbauer, G.; Jirovetz, L. Determination of the dominating odor components (character impact compounds) of Styrian pumpkin seed oil (in German). *Nutrition* **1996**, *20*, 643–644.

(5) Buchbauer, G.; Boucek, B.; Nikiforov, A. On the aroma of Austrian pumpkin seed oil: correlation of analytical data with olfactoric characteristics. *Nutrition* **1998**, *22*, 246–249.

(6) Schieberle, P.; Hofmann, T. Mapping the combinatorial code of food flavors by means of molecular sensory science approach. In *Chemical and Functional Properties of Food Components Series. Food Flavors. Chemical, Sensory and Technological Properties*; Jelen, H., Ed.; CRC Press: Boca Raton, FL, 2012; pp 413–438, ISBN 978-1-4398-1491-8.

(7) Matsui, T.; Guth, H.; Grosch, W. A comparative study of potent odorants in peanut, hazelnut, and pumpkin seed oils on the basis of aroma extract dilution analysis (AEDA) and gas chromatography-olfactometry of headspace samples (GCOH). *Lipid* **1998**, *2*, 51–56.

(8) Hofmann, T.; Schieberle, P. New and convenient syntheses of the important roasty, popcorn-like smelling food aroma compounds 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine from their corresponding cyclic α-amino-acids. *J. Agric. Food Chem.* **1998**, *46*, 616–619.

(9) Scheidig, C.; Czerny, M.; Schieberle, P. Changes in key odorants of raw coffee beans during storage under defined conditions. *J. Agric. Food Chem.* **2007**, *55*, 5768–5775.

(10) Schieberle, P. Primary odorants of popcorn. *J. Agric. Food Chem.* **1991**, *39*, 1141–1144.

(11) Schieberle, P.; Grosch, W. Quantitative analysis of aroma compounds in wheat and rye bread crusts using a stable isotope dilution assay. *J. Agric. Food Chem.* **1987**, *35*, 252–257.

(12) Schieberle, P.; Hofmann, T. Evaluation of the character impact odorants in fresh strawberry juice by quantitative measurements and sensory studies on model mixtures. *J. Agric. Food Chem.* **1997**, *45*, 227–232.

(13) Cerny, C.; Grosch, W. Quantification of character-impact odour compounds of roasted beef. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 417–422.

(14) Mayer, F.; Czerny, M.; Grosch, W. Influence of provenance and roast degree on the composition of potent odorants in Arabica coffee. *Eur. Food Res. Technol.* **1999**, *209*, 242–250.

(15) Milo, C.; Grosch, W. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. *J. Agric. Food Chem.* **1996**, *44*, 2366–2371.

(16) Guth, H.; Grosch, W. Quantitation of potent odorants of virgin olive oil by stable-isotope dilution assays. *J. Am. Oil Chem. Soc.* **1993**, *70*, 513–518.

(17) Sen, A.; Grosch, W. Synthesis of six deuterated sulfur containing odorants to be used as internal standards in quantification assays. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 541–547.

(18) Guth, H.; Grosch, W. Odorants of extrusion products of oat meal – changes during storage (in German). *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 22–28.

(19) Sen, A.; Schieberle, P.; Grosch, W. Quantitative determination of 2,5-dimethyl-4-hydroxy-3-(2H)-furanone and its methyl ether using a stable isotope dilution assay. *Lebensm. Wiss. Technol.* **1991**, *24*, 364–369.

(20) Guth, H.; Grosch, W. Identification of the character impact odorants of stewed beef juice by instrumental analyses and sensory studies. *J. Agric. Food Chem.* **1994**, *42*, 2862–2866.

(21) Guth, H.; Grosch, W. Deterioration of soya-bean oil: quantification of primary flavour compounds using a stable isotope dilution assay. *Lebensm. Wiss. Technol.* **1990**, *23*, 513–522.

(22) Schieberle, P.; Grosch, W. Changes in the concentrations of potent crust odorants during storage of white bread. *Flavour Fragrance J.* **1992**, *7*, 213–218.

(23) Poisson, L.; Schieberle, P. Characterization of the key aroma compounds in an American bourbon whisky by quantitative measurements, aroma recombination, and omission studies. *J. Agric. Food Chem.* **2008**, *56*, 5820–5826.

(24) Blekas, G.; Guth, H. Evaluation and quantification of potent odorants of Greek virgin olive oils. *Dev. Food Sci.* **1995**, *37A*, 419–427.

(25) Schuh, C.; Schieberle, P. Characterization of the key aroma compounds in the beverage prepared from Darjeeling black tea: quantitative differences between tea leaves. *J. Agric. Food Chem.* **2006**, *54*, 916−924.

(26) Schieberle, P. Quantitation of important roast-smelling odorants in popcorn by stable isotope dilution assays and model studies on flavor formation during popping. *J. Agric. Food Chem.* **1995**, *43*, 2442−2448.

(27) Chetschik, I.; Granvogl, M.; Schieberle, P. Quantitation of key peanut aroma compounds in raw peanuts and pan-roasted peanut meal. Aroma reconstruction and comparison with commercial peanut products. *J. Agric. Food Chem.* **2010**, *58*, 11018−11026.

(28) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation, a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237−241.

(29) Czerny, M.; Christlbauer, M.; Fischer, A.; Granvogl, M.; Hammer, M.; Hartl, C.; Moran-Hernandez, N.; Schieberle, P. Re-investigation on odour thresholds of key food aroma compounds and developments of an aroma language based on odour qualities of defined aqueous odorant solutions. *Eur. Food Res. Technol.* **2008**, *228*, 265−273.

(30) Kubickova, J.; Grosch, W. Quantification of potent odorants in Camembert cheese and calculations of their odour activity values. *Int. Dairy J.* **1998**, *8*, 17−23.

(31) Preininger, M.; Grosch, W. Evaluation of key odorants of the neutral volatiles of Emmentaler cheese by the calculation of odour activity values. *Lebensm. Wiss. Technol.* **1994**, *27*, 237−244.

(32) Wagner, R.; Grosch, W. Key odorants of french fries. *J. Agric. Food Chem.* **1998**, *75*, 1385−1392.

(33) Frauendorfer, F.; Schieberle, P. Identification of the key aroma compounds in cocoa powder based on molecular sensory correlations. *J. Agric. Food Chem.* **2006**, *54*, 5521−5529.

(34) Burdack-Freitag, A. Formation of important odorants in hazelnuts during the roasting process (in German). Ph.D. thesis, Technical University of Munich, Munich, Germany, 2007.

(35) Schieberle, P. Studies on the flavour of roasted white sesame seeds. In *Progress in Flavour Precoursers Studies*; Schreier, P., Winterhalter, P., Eds.;Allured Publishing: Carol Stream, IL, 1993; pp 343−360.

(36) Reiners, J.; Grosch, W. Odorants of virgin olive oils with different flavor profiles. *J. Agric. Food Chem.* **1998**, *46*, 2754−2763.

(37) Pfnür, P. Investigations of the aroma of chocolate (in German). Ph.D. thesis, Technical University of Munich, Munich, Germany, 1998.

(38) Guadagni, D. G.; Buttery, R. G.; Turnbaugh, J. G. Odour thresholds and similarity ratings of some potato chip components. *J. Sci. Food Agric.* **1972**, *23*, 1435−1444.

(39) Chetschik, I.; Granvogl, M.; Schieberle, P. Comparison of the key aroma compounds in organically grown, raw West-African peanuts (*Arachis hypogea*) and in ground, pan-roasted meal produced thereof. *J. Agric. Food Chem.* **2008**, *56*, 10237−10243.