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Evaluation of Process Parameters Governing the Aroma Generation in Three Hazelnut Cultivars (*Corylus avellana* L.) by Correlating Quantitative Key Odorant Profiling with Sensory Evaluation

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Supporting Information

ABSTRACT: The majority of the world hazelnut crop is roasted, thus developing a unique aroma that depends on the cultivar used and on the roasting conditions applied. Although several studies have investigated the volatile fraction of different cultivars and have correlated the data with overall sensory profiles, studies establishing a correlation between key odorants among the bulk of odorless volatiles and the respective aroma profiles are not yet available. On the basis of recently published stable isotope dilution assays (SIDAs) using comprehensive two-dimensional gas chromatography−time-of-flight mass spectrometry (GC×GC-TOF-MS), differences in concentrations of key odorants in different hazelnut cultivars roasted under defined conditions were monitored and compared with sensory data obtained by projective mapping, aroma profile analysis, and triangle tests. The results showed that the aroma-active compounds 2-acetyl-1-pyrroline, 2-propionyl-1-pyrroline, 5-methyl-(*E*)-2-hepten-4-one, 2,3-diethyl-5-methylpyrazine, 3,5-dimethyl-2-ethylpyrazine, and 2-furfurylthiol are appropriate marker odorants to differentiate the various nut aromas. In particular, the appreciated roasty, nutty aroma of optimally roasted hazelnuts was developed if both 5-methyl-(*E*)-2-hepten-4-one and 3-methyl-4-heptanone were >450 μg/kg, whereas the sum of the two 2-acyl-1-pyrrolines and two pyrazines should not exceed 400 μg/kg to avoid an over-roasted smell. Such a desired aroma can be obtained for each cultivar, but obviously specific roasting times, temperatures, and roasting techniques had to be applied.

KEYWORDS: *hazelnut aroma, key odorant profiling, projective mapping, GC×GC-TOF-MS*

INTRODUCTION

Commercially important hazelnut cultivars are grown in only a few regions of the world, in particular, in Turkey, which produced 71% of the worldwide crop of 430,000 t in 2011, followed by Italy and other countries.[1] About 90% of the world annual crop is shelled, roasted, and refined by confectionery industries to impart the unique aroma to numerous products, such as spreads or chocolate. Different hazelnut cultivars are associated with a certain quality and, hence, are often differentiated by morphological traits,[2] DNA typing,[3] or chemical analysis.[4] However, the aroma is considered to be among the primary determinants of nut quality and should be taken into account when cultivars are selected or when breeding programs are conducted.[5,6]

The evaluation of the aroma quality by a sensory panel is challenging and demands skillful planning of laborious experiments. New statistical tools and novel fingerprinting approaches using comprehensive two-dimensional gas chromatography (GC×GC) have stimulated the investigation of hazelnut volatiles in the past two decades aimed at an objective assessment of the aroma quality. Alasalvar et al.[4,7] evaluated the sensory impact of 18 of the most important commercial hazelnut cultivars from Turkey in two consecutive studies and showed that hardly any significant difference was observable for raw hazelnuts, whereas the aroma of roasted Tombul hazelnuts differed from that of all other processed hazelnuts. However, although the application of E-nose and dynamic headspace analysis data allowed the differentiation of single cultivars, no correlation of odor-active compounds with the overall aroma evaluation was done. Cordero et al.[8,9] and Kiefl et al.[10] recently profiled nuts from Azerbaijan, Chile, Italy, and Turkey by means of GC×GC-MS to identify process-dependent as well as cultivar-specific marker compounds. Although the release of known key odorants during roasting among different cultivars was studied and different profiles were observed, no sensory evaluation was performed in these studies.

By applying the molecular sensory science approach, Burdack-Freitag and Schieberle[11,12] were the first to show that a defined set of aroma-active compounds in their natural concentrations are able to evoke the aroma of raw and pan-roasted Italian 'Tonda Romana' hazelnuts. However, such studies are not currently available either for other cultivars or for the same cultivar at different roasting regimens. Therefore, in particular, the influence of roasting time and temperature on the generation of key odorants is poorly understood. Furthermore, a correlation between the specific odor activity of certain aroma compounds and the overall sensory profile of roasted nuts has not yet been established. A good example for such a correlation is the analysis of off-odors in hazelnuts indicating that, for example, above a certain concentration prenyl ethyl ether caused a metallic, solvent-like off-note.[13] In this study, the critical threshold was determined by sensory

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Table 1. Results Obtained by Application of an Aroma Extract Dilution Analysis on Distillates Isolated from 'Gentile' (G), 'Romana' (R), and Akçakoca (A) Hazelnuts, Each Roasted for 23 min at 160 °C

odorant	odor quality	RI (FFAP)	RI (DB5)	FD factor in		
				G	R	A
3-methylbutanal	malty	943	650	64	16	4
2-methylbutanal	malty	971	667	32	8	4
2,3-butanedione	buttery	986	600	8	8	8
2,3-pentanedione	buttery	1056	700	16	2	4
3-methyl-4-heptanone	fruity, nutty	1138	918	32	64	32
5-methyl-(*Z*)-2-hepten-4-one	nutty, fruity	1183	922	16	32	8
unknown	earthy	1228	nd[a]	8	64	8
5-methyl-(*E*)-2-hepten-4-one	nutty, fruity	1270	966	256	128	128
1-octen-3-one	mushroom-like	1289	970	16	32	64
2-acetyl-1-pyrroline	popcorn-like, roasty	1321	916	2048	256	512
dimethyl trisulfide	sulfury	1352	955	16	8	8
2,3,5-trimethylpyrazine	earthy	1376	990	8	2	<1
2-propionyl-1-pyrroline	popcorn-like, roasty	1405	1017	1024	256	256
2-isopropyl-3-methoxypyrazine	pea-like, green pepper	1414	1087	64	4	4
2-furfuryl mercaptan	coffee-like, sulfury	1421	904	128	256	512
3,5-dimethyl-2-ethylpyrazine	earthy	1440	1077	64	64	64
acetic acid	sour	1447	nd	16	64	8
2,3-diethyl-5-methylpyrazine	earthy, roasty	1470	1151	64	32	128
2-acetyl-3,4,5,6-tetrahydropyridine	popcorn-like, roasty	1538	1038	128	128	128
2-acetylpyridine	popcorn-like, earthy	1564	1046	16	16	64
phenylacetaldehyde	honey, flowery	1623	1033	32	8	8
3-mercapto-3-methyl-1-butanol	meaty	1643	962	32	64	16
2- and 3-methylbutyric acid	sweaty	1667	841	32	8	8
(*E*,*E*)-2,4-nonadienal	fatty, green	1680	1208	8	4	8
unknown	green pepper	1733	nd	32	16	8
(*E*,*E*)-2,4-decadienal	fatty	1783	1304	32	64	32
2-methoxyphenol	smoky, phenolic	1839	1081	16	4	8
trans-4,5-epoxy-(*E*)-2-decenal	metallic	1993	1374	32	32	128
4-methoxybenzaldehyde	anise-like	2017	1246	32	8	4
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like, sweet	2075	1052	2048	2048	1024
4-ethenyl-2-methoxyphenol	clove-like, smoky	2222	1304	128	4	<1
4-hydroxy-3-methoxybenzaldehyde	vanillic, sweet	2591	1389	16	64	64

[a]nd, compound could not be detected.

analysis to indicate the amount of the off-odorant able to adversely change the aroma profile.

However, such concentration effects of single key odorants on the overall aroma of nondeteriorated hazelnuts have not yet been studied. Hence, the aim of this work was to characterize the formation of key odorants from different hazelnut cultivars under different roasting conditions and to establish relationships between odorant concentrations and the overall aroma to finally develop a model for the prediction of optimal processing parameters. The recently developed stable isotope dilution approach in combination with GC×GC-TOF-MS for odorant quantitation was applied, allowing the fast analysis of a larger set of aroma compounds in hazelnut samples in a single analysis.[14]

MATERIALS AND METHODS

Chemicals. Reference compounds and other chemicals were obtained from Sigma-Aldrich (Steinheim, Germany) in the commercially available grade of purity. Details on chemicals and isotopically labeled internal standards used in the quantitative approach are reported in the accompanying paper.[14]

Sample Material. Raw, shelled hazelnuts (*Corylus avellana* L.) (Marchisio, Cuneo, Italy) were hermetically sealed under vacuum in nonpermeable polypropylene/aluminum/polyethylene packages and stored at −20 °C prior to analysis. The following hazelnut samples from different regions in Italy and a blend of hazelnuts from Akçakoca in Turkey were selected: Piedmont, cultivar 'Tonda Gentile delle Langhe' (G); Lazio, cultivar 'Tonda Gentile Romana' (R); Campani, cultivar 'Tonda di Giffoni' (Gi) and a blend of cultivars from Akçakoca (A), respectively, Karafindik and Mincane. Hazelnuts of a uniform dimension (diameter = 12−13 mm) were visually inspected for defects, then roasted at 160 °C in a convection oven for 12, 23, or 30 min and ground with a mill (Moulinette, Solingen, Germany) after freezing in liquid nitrogen. Hazelnuts were also submitted to roasting in an industrial plant at different time/temperature ratios. These commercial samples are denoted A, G, and T without numbers in the respective tables and figures.

Volatile Isolation. Hazelnut powder (10 g) was extracted twice with distilled diethyl ether (total volume = 200 mL) by stirring for 30 min at room temperature. The filtrates were directly used for SAFE distillation,[15] and the distillate was dried over anhydrous sodium sulfate before concentration to 250 μL using Vigreux columns of different sizes.

High-Resolution Gas Chromatography−Olfactometry (HRGC-O). Aroma distillates were separated on an Agilent DB-FFAP column (30 m, 0.32 mm i.d., 0.32 μm) (J&W, Waldbronn, Germany) and on an Agilent DB-5 column (30 m, 0.32 mm i.d., 0.32 μm) (J&W) placed in a Carlo Erba 5160 Mega series gas chromatograph (Hofheim, Germany) equipped with an on-column injector using helium as the carrier gas. The effluent was transferred by a Y-type glass splitter into two deactivated fused silica capillaries (50 cm each, 0.2 mm i.d.) directed either to a sniffing port or into an FID

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detector for simultaneous detection. A constant pressure of 80 kPa was applied. The sample (1 μL) was injected, and the temperature was programmed starting at 40 °C, held for 2 min, then raised at 6 °C/min to 230 °C, and held for 5 min. Retention indices were calculated by cochromatography with a homologous series of *n*-alkanes.[11]

Aroma Extract Dilution Analysis (AEDA). To screen for the most potent aroma-active compounds, an AEDA was applied.[16] For this purpose, the aroma distillate was stepwise diluted 1:1 with diethyl ether, and aroma-active compounds were located by sniffing each dilution (HRGC-O) and by calculating retention indices on two columns of different polarities (Table 1). Evaluation of the dilutions was performed until no odorant could be sniffed in the diluted extract, and the flavor dilution (FD) factor was, thus, denoted for each compound.

Comprehensive Two-Dimensional Gas Chromatography–Time of Flight–Mass Spectrometry (GC×GC-TOF-MS). A Leco Pegasus 4D GC×GC-TOF-MS instrument (St. Joseph, MI, USA) was used consisting of an Agilent GC model 7890A, a dual-stage quad-jet thermal modulator, and a secondary oven coupled to the mass spectrometer providing unit mass resolution. A Thermo Scientific on-column injection port (Dreieich, Germany) was used for cold on-column injection of 1 μL, which was operated by a CTC Analytics GC-PAL autosampler (Zwingen, Switzerland). The column was a 30 m × 0.25 mm i.d., 0.25 μm (Agilent, Waldbronn, Germany), equipped with a 2 m × 0.53 mm i.d. deactivated precolumn in the first dimension and a VF-5-MS column (2 m × 0.15 mm i.d., 0.3 μm) (Varian, Darmstadt, Germany) in the second dimension. A constant head pressure of 250 kPa was applied. The primary oven temperature was programmed as follows: After 2 min at 40 °C, the temperature was raised by 4 °C/min to 230 °C and held for 7 min. The secondary oven started at 80 °C for 2 min, then was raised at 4 °C/min to 130 °C, kept for 12 min isothermally, and then raised at 4 °C/min to 230 °C, and held for 5 min. Mass spectra were acquired within *m/z* 40–250 at a rate of 100 spectra/s. Data were manipulated using GC Image and GC Project (Lincoln, NE, USA). Quantitation was performed as described in the accompanying paper[14] using the 19 isotopically labeled internal standards given in Figure 1.

Sensory Evaluation. Twenty-four panelists (18 women, 6 men, between 24 and 31 years old, with no history of known olfactory disorders or allergies) were recruited for four different sensory experiments. Prior to the evaluation, the panelists discussed aroma properties of various hazelnut samples in three training sessions to develop a common language for aroma description. Sensory analyses were performed at 22 °C in a sensory panel room, equipped with single booths under yellow-red light conditions.

First, to assess similarities and differences among different hazelnut aromas from respective samples, a projective mapping experiment was performed.[17] The panel was not specifically trained for this experiment, but the following instructions were given: "Two samples should be placed very near if they seem identical, and two samples should be placed distant to one another if they seem different to you; this should be done according to your own criteria; do not hesitate to express strongly the differences you perceive by using the most part of the screen (total space)." A maximum of 20 samples including 4–5 control samples were presented at a time. The *X* and *Y* co-ordinates were recorded with Fizz software (Biosystèmes, France), and derived Euclidean distance matrices were analyzed using ALSCAL and INDSCAL multidimensional scaling methods (SPSS 14.0, SPSS Inc., USA).

Second, triangle tests according to DIN ISO 4120 guidelines were performed to differentiate the raw and 23 min roasted hazelnuts, which differed the most, and to determine breakthrough thresholds of single odorants in model mixtures. Either deodorized sunflower oil or two odorant mixtures in sunflower oil were used as the matrix. Details on the composition are given in the respective tables.

Third, fixed-range scaling experiments were carried out to determine dose–response curves for single odorants in odorless sunflower oil (purified by thin-film distillation) covering the concentration range measured in the hazelnut samples. A 20 cm line-marking scale was given with two anchors to define end points by

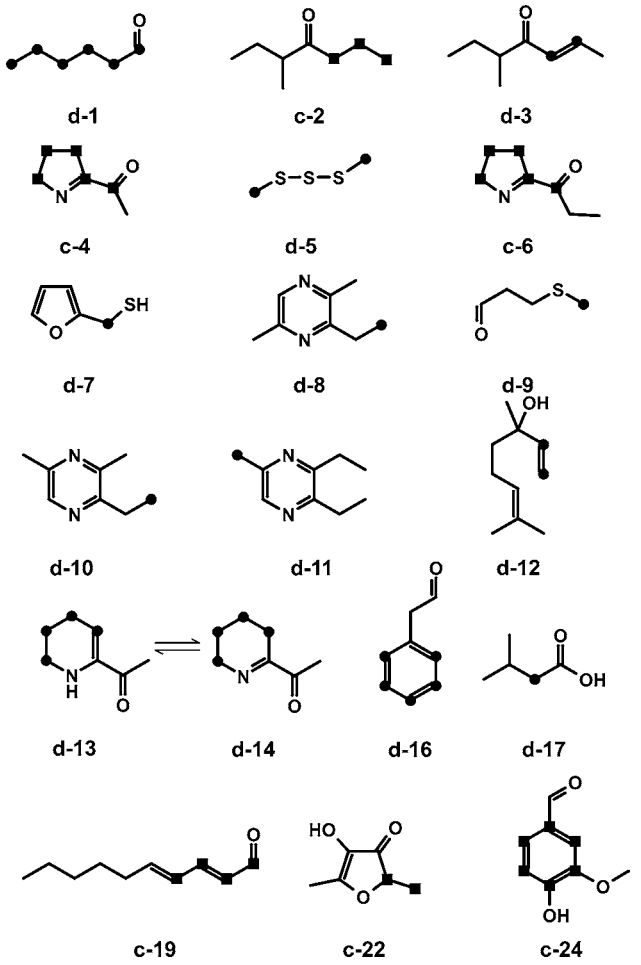

Figure 1. Structures of the labeled internal standards used in the stable isotope dilution assays: deuterium label (●) or ^{13}C label (■).

calibration using the odorless matrix on the left side (no odor) and the odorant solution in the highest concentration on the right side (most intense odor). The concentration of the weakest odor-active reference solution was chosen as 7 times above the odor threshold of the respective reference compound. The trained panelists were asked to evaluate the intensity of the odorant solutions and to assign the perceived differences or similarities in intensity on the 20 cm scale. Data were acquired using FIZZ software (Biosystèmes).

Finally, aroma profile analysis of the nut samples was performed by evaluating the intensities of eight given aroma attributes on a seven-point scale (0; 0.5; 1.0; 1.5; 2.0; 2.5; 3.0) to compare raw and 23 min roasted hazelnuts. All samples were presented in white, nontransparent Teflon vials in random order after coding with unique, three-digit, random numbers.

■ RESULTS AND DISCUSSION

Nuts of the three cultivars, 'Gentile' (G), 'Giffoni' (Gi), and 'Romana' (R), grown in different regions of Italy as well as a blend of different cultivars from Akçakoca (A), Turkey, were selected to evaluate either the overall aroma of the raw nuts or the aroma formed after 23 min of roasting. Differences in the sensory evaluation at significant α levels were observed by pairwise comparison of the overall aromas of processed hazelnuts indicating that distinct aroma properties were developed during roasting of the single cultivars (see the Supporting Information). On the other hand, the aromas of the raw nuts from the different cultivars could hardly be differentiated. These results were in agreement with data

published previously.[4,7] The aroma of roasted 'Gentile' was most discriminant among the other roasted cultivars, probably due to the less coffee-like and roasty odor notes as indicated by the aroma profile analysis (Figure 2B). However, the aroma of

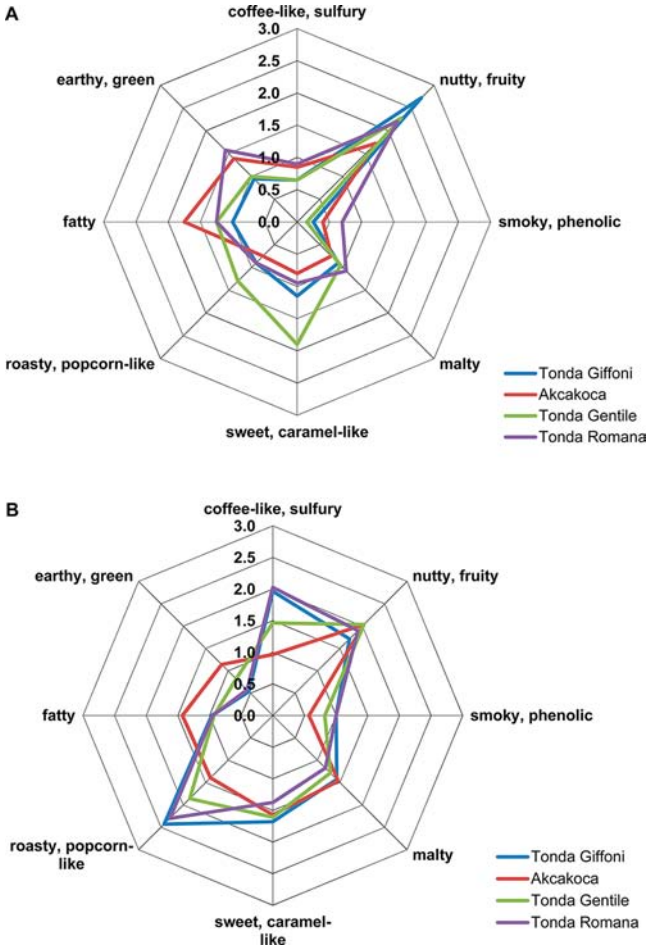

Figure 2. Aroma profiles of raw hazelnuts (A) and hazelnut cultivars roasted for 23 min at 160 °C (B).

the raw as well as the roasted 'Giffoni' nuts was less distinguishable across all, but especially in comparison to 'Romana', and, therefore, these nut samples were not investigated further.

Akçakoca (A), 'Gentile' (G,) and 'Romana' (R) hazelnuts were then used to study the influence of different roasting times on the aroma generation at 12, 23, or 30 min, respectively, resulting in a total of 15 different samples together with the raw nuts and three industrially roasted samples. A projective mapping experiment was applied to evaluate the differences between the overall aromas by comparing all samples in one session.[17,18] Compared to a traditional scaling experiment using one graphical scale for each of the 120 possible pairwise comparisons of 15 samples, the projective mapping provides difference scales by measuring the Euclidean distance between samples placed on a 2D plane according to the dissimilarity or similarity of their aroma, respectively. A consensus perceptual map was built by averaging the individual maps given by 19 of 24 assessors (Figure 3). Five judgments were rejected, because one or more of the four control samples, labeled "c", was placed far from the respective, identical sample (i.e., minimum half of the distance of the plane). Considering the variation between

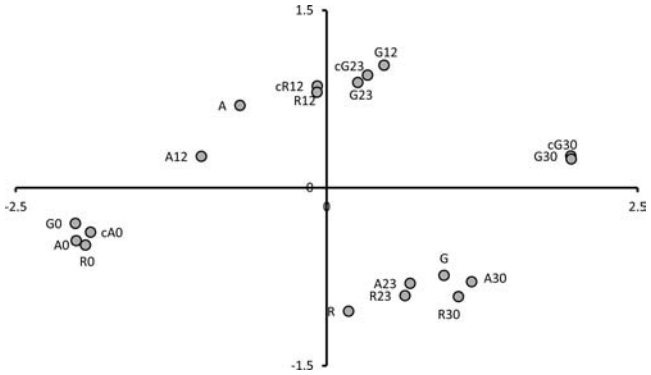

Figure 3. Consensus perceptual map of the raw (0) as well as 12, 23, and 30 min roasted and industrially roasted (no number) hazelnut cultivars Akçakoca (A), 'Gentile' (G), and 'Romana' (R). Duplicate samples are presented as controls (c). Aroma perception in the first dimension (*x*-axis) correlates with roasting degree and in the second dimension (*y*-axis) with the hedonic value.

individual maps represented by the distance between the control samples, three main clusters could be defined: raw hazelnuts on the left (G0, A0, R0, cA0), a middle group in the upper part (A, G12, R12, G23), and the over-roasted samples on the bottom right corner (A23, R23, A30, G30, R30, G, R).

Interestingly, the perceptual map showed that the samples did not cluster according to roasting times. For example, 'Gentile' 12 (G12) as well as G23 both belonged to the middle group occupying the highest scale of the second dimension, whereas 'Romana' 23 (R23) tended to the over-roasted group. All oven-roasted 'Gentile' samples were placed along the positive second dimension, indicating that they less rapidly turn to over-roasted as compared to 'Romana' or Akçakoca hazelnuts. Thus, it should be easier to process 'Gentile' hazelnuts because they are more tolerant toward roasting time. Akçakoca 12 (A12) was placed between raw and the middle group, indicating that these nuts need a longer roasting time to develop the desired aroma. The experiment was repeated using a quadratic plane and leaving out the laboratory-roasted Akçakoca hazelnuts to check the robustness of the method (see the Supporting Information). To finalize the projective mapping experiments, the data were discussed with the panel, revealing that the assessors intuitively ordered the samples along a first dimension indicating the degree of roasting and along a second dimension describing the hedonic scale. However, the arrangement of samples in the second "hedonic" dimension was less uniform (see the Supporting Information). Some assessors used both dimensions equally in arranging the samples (1D < 0.8, 2D > 0.2), and the other half preferred to arrange them along one dimension (1D > 0.8, 2D < 0.2). Half readily differentiated between "good" and "poor" hazelnut aroma, whereas others primarily evaluated the roasting degree. In agreement with the aroma profiles (Figure 2), which were acquired before the projective mapping experiments, the aroma of the middle group (represented by, e.g., 'Gentile' 23; Figure 3) was described as moderately roasty-nutty, the over-roasted group, being less appreciated (represented by, e.g., 'Romana' 23; Figure 3), was evaluated as coffee-like and sulfury, and, finally, nuts of the unroasted group were assigned as green, nutty. These data suggested that roasted hazelnuts should fall within the middle group (Figure 3; top left) to be considered as optimally roasted.

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Table 2. Retention Indices and Concentrations (Micrograms per Kilogram) of 24 Key Odorants in Roasted Hazelnuts As Influenced by Roasting Time[a]

				'Tonda Romana' (R)				'Tonda Gentile' (G)				Akcakoca (A)				industrially roasted		
no.	compound	1D RI (FFAP)	2D t_R (s) (VF-5)	0 min	12 min	23 min	30 min	0 min	12 min	23 min	30 min	0 min	12 min	23 min	30 min	A	G	R
1	hexanal	1070	1.72	772	852	986	1491	109	219	249	480	2310	1823	1699	1637	891	449	492
2	3-methyl-4-heptanone	1134	2.47	121	132	82	88	109	118	123	98	80	58	78	74	110	50	117
3	5-methyl-(*E*)-2-hepten-4-one	1282	2.16	9	398	408	386	7	542	495	539	6	140	270	218	388	474	461
4	2-acetyl-1-pyrroline	1329	1.75	≤2	27	53	85	≤2	≤2	55	71	≤2	9	28	44	≤2	18	19
5	dimethyl trisulfide	1368	2.00	≤3	≤3	4	5	≤3	≤3	≤3	≤3	≤3	≤3	≤3	≤3	≤3	6	≤3
6	2-propionyl-1-pyrroline	1403	2.09	≤2	13	33	56	≤2	2	24	38	≤2	≤2	11	27	≤2	20	17
7	2-furfuryl mercaptan	1429	1.61	≤3	≤3	≤3	5	≤3	≤3	≤3	4	≤3	≤3	≤3	≤3	≤3	≤3	≤3
8	3,6-dimethyl-2-ethylpyrazine	1436	2.46	3	128	465	536	3	79	367	620	3	39	203	262	95	360	142
9	3-(methylthio)propionaldehyde	1443	1.59	3	112	112	29	3	68	80	25	3	75	84	38	32	52	71
10	3,5-dimethyl-2-ethylpyrazine	1452	2.52	≤4	7	83	96	≤4	≤4	38	53	≤4	≤4	36	75	≤4	≤4	≤4
11	2,3-diethyl-5-methylpyrazine	1484	3.16	4	44	133	146	4	29	102	175	4	13	52	69	43	113	50
12	3,7-dimethylocta-1,6-dien-3-ol	1527	2.64	≤5	≤5	≤5	≤5	≤5	≤5	≤5	≤5	≤5	≤5	≤5	≤5	18	≤5	≤5
13	2-acetyl-1,4,5,6-tetrahydropyridine	1554	3.32	55	55	275	341	55	62	263	316	55	55	97	144	104	261	187
14	2-acetyl-3,4,5,6-tetrahydropyridine	1554	2.33	44	44	221	300	44	44	159	211	44	44	44	98	90	184	133
15	2-acetylpyridine	1588	2.25	3	17	41	58	3	7	24	43	3	8	27	39	20	32	30
16	2-phenylacetaldehyde	1637	2.36	8	1340	1142	653	8	1508	1197	693	8	737	1036	632	362	474	258
17	3-methylbutanoic acid	1659	1.35	35	70	187	102	27	72	91	117	13	51	65	72	44	78	88
18	(*E*,*E*)-2,4-nonadienal	1683	4.17	9	≤5	≤5	≤5	≤5	≤5	≤5	≤5	43	38	11	≤5	≤5	≤5	≤5
19	(*E*,*E*)-2,4-decadienal	1783	5.54	4	85	200	347	4	47	122	185	10	121	179	135	74	79	86
20	2-methoxyphenol	1839	2.33	≤6	≤6	11	14	≤6	9	8	12	≤6	≤6	≤6	7	≤6	7	7
21	4-methoxybenzaldehyde	2017	3.17	3	13	19	27	3	6	15	18	3	5	14	17	9	22	16
22	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	2030	1.75	24	1457	2311	2403	17	927	1814	2402	17	425	1029	1117	841	1796	1558
23	4-ethenyl-2-methoxyphenol	2193	2.79	7	140	202	174	7	97	125	172	1	38	52	59	38	103	141
24	4-hydroxy-3-methoxybenzaldehyde	2579	2.30	64	112	176	193	103	150	149	152	3	116	165	195	147	121	179

[a]Quantitated by stable isotope dilution assays according to ref 14, RSD < 20%. The concentrations of 2-isopropyl-3-methoxypyrazine (LOQ, 4 μg/kg) and 3-mercapto-3-methyl-1-butanol (LOQ, 3 μg/kg) were below their quantitation limit (LOQ) in each sample.

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Table 3. Odor Activity Values (OAV > 1) of Raw (0) and Differently Roasted (12, 23, and 30 min) as well as Industrially Roasted Hazelnuts of Akçakoca (A), 'Tonda Gentile' (G), and 'Tonda Romana' (R) Hazelnuts[a]

		'Tonda Romana' (R)				'Tonda Gentile' (G)				Akçakoca (A)				industrially roasted		
no.	compound	0 min	12 min	23 min	30 min	0 min	12 min	23 min	30 min	0 min	12 min	23 min	30 min	A	G	R
1	hexanal	3	3	4	5	<1	1	1	2	8	7	6	6	3	2	2
2	3-methyl-4-heptanone	141	154	95	103	126	137	143	114	93	67	91	86	127	58	136
3	5-methyl-(*E*)-2-hepten-4-one	2	105	107	102	2	143	130	142	2	37	71	57	102	125	121
4	2-acetyl-1-pyrroline	24	290	579	922	24	24	599	776	24	100	308	474	24	192	203
5	dimethyl trisulfide	1	1	2	2	1	1	1	1	1	1	1	1	1	3	1
6	2-propionyl-1-pyrroline	22	134	334	563	22	22	243	384	22	22	113	269	22	196	174
7	2-furfuryl mercaptan	8	8	8	13	8	8	8	11	8	8	8	8	8	8	8
8	3,6-dimethyl-2-ethylpyrazine	<1	1	3	3	<1	<1	2	4	<1	<1	1	2	1	2	1
9	3-(methylthio)propionaldehyde	15	623	620	161	15	380	442	138	15	419	464	209	179	291	392
10	3,5-dimethyl-2-ethylpyrazine	1	2	25	28	1	1	11	16	1	1	11	22	1	1	1
11	2,3-diethyl-5-methylpyrazine	9	88	266	293	9	57	204	350	9	25	103	137	87	227	100
12	3,7-dimethylocta-1,6-dien-3-ol	12	12	12	12	12	12	12	12	12	12	12	12	45	12	12
13	2-acetyl-1,4,5,6-tetrahydropyridine	46	46	229	284	46	52	219	263	46	46	81	120	86	218	155
14	2-acetyl-3,4,5,6-tetrahydropridine	36	36	184	250	36	36	132	176	36	36	36	82	75	153	111
16	2-phenylacetaldehyde	<1	54	46	26	0	60	48	28	<1	29	41	25	14	19	10
17	3-methylbutanoic acid	2	3	8	5	1	3	4	5	1	2	3	3	2	4	4
18	(*E*,*E*)-2,4-nonadienal	6	3	3	3	3	3	3	3	29	25	7	3	3	3	3
19	(*E*,*E*)-2,4-decadienal	<1	1	1	2	<1	<1	1	1	<1	1	1	1	<1	<1	1
22	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	1	63	100	104	1	40	79	104	1	18	45	49	37	78	68
24	4-hydroxy-3-methoxybenzaldehyde	<1	3	4	3	<1	2	2	3	<1	1	1	1	1	2	3

[a]Quantitative data (μg/kg) are given in Table 2.

The industrially roasted 'Romana' and 'Gentile' hazelnuts (G and R, respectively, in Figure 3) were classified as over-roasted, although these were specifically processed by the manufacturer to obtain a good aroma. In contrast, only industrially roasted Akçakoca hazelnuts clustered in the "roasty, nutty" group, although the respective oven-roasted Akçakoca nuts did not. Obviously, the industrial roasting conditions initiate different time courses of aroma formation compared to oven-roasting.

Next, it should be verified that these descriptive sensory data do correlate with the quantitative data of the key odorants. To confirm the key odorants previously identified in roasted hazelnuts,[12] first, an AEDA was applied to distillates from nuts of the three cultivars, all roasted at 160 °C for 23 min. The results (Table 1) clearly showed that qualitatively the same set of odorants was detectable across all cultivars. The average difference between the corresponding FD factors amounted to not more than two dilution steps. Considering a quite high standard deviation for such sniffing experiments, these differences are, however, not significant.[19] However, the concentration differences evoking the different nut aroma signatures must, thus, range within these dilution steps, which can, however, be proven only by quantitative analyses. In this view, for future research on hazelnuts, sniffing of one dilution of each distinct-smelling aroma extract should be sufficient to locate aroma-active compounds and to subsequently subject them to quantitative analysis.

Using the newly developed approach to quantitate key odorants by a SIDA in combination with GC×GC-TOF-MS,[14] the concentrations of 24 odor-active compounds were determined in the 15 nut samples. The results (Table 2) showed clear differences between and as well as within the three different cultivars roasted for different times. For example, the concentrations of compounds **3**, **4**, **6**, **8**−**11**, and **13**−**16** increased up to 100 times from raw (0) to 30 min roasted nuts and up to 10 times after roasting times of 12 and 30 min. However, **9** and **16** showed a decrease after 23 min. The pairwise comparison of concentrations from equally processed Akçakoca, 'Gentile', and 'Romana' samples showed concentration differences by a factor of 2 and higher in about one-third of all possible combinations (Table 2). These differences in the overall aromas should be reflected by a calculation of the odor activity values (ratio of concentration to odor threshold), and the data obtained (Table 3) clearly indicated the importance of, in particular, odorants **3**, **4**, **6**, **9**−**11**, **13**, **14**, **16**, and **22** for the roasted nut aroma. However, no clear correlation to the sensory data (Figure 2) could be derived at this point.

Compared to the above-mentioned compounds, 3-methyl-4-heptanone (**2**) stayed at nearly the same concentration level in raw and roasted hazelnuts, whereas the nutty-smelling 5-methyl-(*E*)-2-hepten-4-one increased in concentration within the first 12 min of roasting. However, the nutty, fruity note decreased in the roasted nuts compared to the raw nuts (Figure 2). Therefore, it may be hypothesized that the other key odorants may influence the impact of the nutty odor note. Sensory model mixtures were, therefore, prepared to study such effects using a reduced number of relevant odorants to simplify the interpretation of possible odorant interactions.[20,21] The roasty, popcorn-like-smelling compounds **4** and **6** were selected because they proved to be the most relevant odorants of roasted 'Romana' hazelnuts according to omission experiments performed previously.[12] The earthy-smelling pyrazines **8**, **10**, and **11** belonged to the second most important class of odorants according to their OAV values (Table 3), compounds **2** and **3** showed the highest OAVs causing a nutty and fruity smell (Table 3), and compound **7** proved to be the principle

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giving a burnt note to longer roasted hazelnuts already at low amounts.[14]

To show which concentration ratios may change the overall aroma profile of such model mixtures, the nutty-smelling compound **3** was mixed at various concentration levels in sunflower oil (matrix I) as well as in the odor-active matrix II (Table 4). The triangle test data show that concentration differences greater than a factor 2 could be orthonasally distinguished, and this ability is not limited by the presence of additional odorants as long as these do not fully cover the respective aroma attribute. These data (Table 4) supported the hypothesis that the small differences in the concentration of compound **3** measured within roasted hazelnuts could not be differentiated orthonasally, whereas 209 μg/kg in roasted Akçakoca could be differentiated from 397 and 525 μg/kg, respectively, present in 'Romana' and 'Gentile' hazelnuts (Table 2).

Table 4. Detection of an Increase in the Concentration of 5-Methyl-(*E*)-2-hepten-4-one (3) Dissolved either in Sunflower Oil (Matrix I) or in Matrix II Containing Seven Additional Odorants

		no. of correct answers (n = 16)		level of significance[a] (α)	
3 (μg/kg)		matrix I[b]	matrix II[c]	matrix I[b]	matrix II[c]
209	397	10	11	0.05	0.01
209	525	11	14	0.01	0.001
397	525	6	8	>0.05	>0.05
525	2500	15	15	0.001	0.001
209	2500	14	15	0.001	0.001
2500	5000	11	12	0.01	0.001
2500	10000	14	12	0.001	0.001

[a]According to DIN ISO 4120 guidelines. [b]Odorless sunflower oil. [c]A mixture of odorants **2**, **4**, **6**, **7**, **8**, **10**, and **11** in concentrations present in 'Tonda Romana' roasted for 23 min in odorless sunflower oil.

Furthermore, dose–response curves for 5-methyl-(*E*)-2-hepten-4-one, 2-acetyl-1-pyrroline, and 2,3-diethyl-5-methylpyrazine in sunflower oil showed a linear response of the concentration and scaling intensity starting with concentrations 7 times above the respective odor thresholds, and even at the milligrams per kilogram level no saturation effect was observed (Figure 4). However, a steady response to the increasing concentrations of, for example, 5-methyl-(*E*)-2-hepten-4-one, was just perceivable above 100 μg/kg, being 26 times its odor threshold. This effect was even more pronounced in odorous matrices (Table 5). When mixed with compounds **4**, **6**, **8**, **10**, and **11** in a model matrix containing the six odorants as present in G12, compound **3** was not detected below 147 μg/kg and the detection threshold increased to 255 μg/kg in a model matrix containing higher concentrations of **4**, **6**, **7**, **8**, **10**, and **11** simulating the aroma of 'Romana' (23 min) (Table 5). These data showed that in the presence of other odorants several hundred micrograms per kilogram of compound **3** are needed to detect a nutty odor note. Therefore, the high concentrations of 5-methyl-(*E*)-2-hepten-4-one measured in 'Gentile' and 'Romana' hazelnuts, on the one hand, and the low the concentrations of roasty aroma compounds, on the other hand, may intensify the distinct smell of the middle group hazelnuts (Figure 3).

In contrast, 3-methyl-4-heptanone showed recognition and detection thresholds in roasted hazelnut aroma matrices far above the measured concentrations (Table 5). Thus, it can be assumed that compound **2** does not contribute much to the nutty aroma of roasted hazelnuts, although the structural similarity to compound **3** and an OAV >100 (Table 3) suggested the opposite.

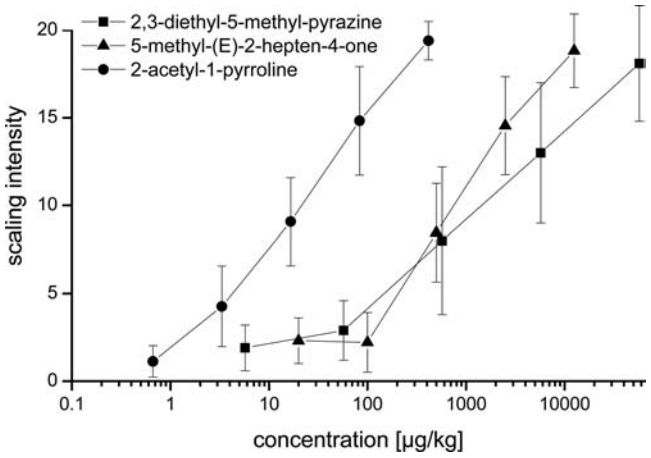

Figure 4. Dose–response curves of important hazelnut odorants across their natural concentration range correlated with the orthonasal perception of respective intensities.

Table 5. Orthonasal Recognition Thresholds (RT) and Detection Thresholds (DT) of 5-Methyl-(*E*)-2-hepten-4-one (3) and 3-Methyl-4-heptanone (2) in an Oil Matrix Containing Additional Odorants

	matrix I[a]		matrix II[b]	
odorant	DT (μg/kg)	RT (μg/kg)	DT (μg/kg)	RT (μg/kg)
3	65	147	137	255
2	294	3049	1428	6468

[a]Odorant mix displaying the concentrations of the selected odorants in 'Tonda Gentile' (12 min roasted; **4**, **6**, **8**, **10**, and **11**). [b]Odorant mix displaying the concentrations of the selected odorants in 'Tonda Romana' (23 min roasted, **4**, **6**, **7**, **8**, **10**, and **11**).

The sensory experiments have shown that compounds **4**, **6**, **7**, **8**, **10**, and **11** are able to suppress the perception of 5-methyl-(*E*)-2-hepten-4-one depending on their concentration ratios generated during roasting. Similar effects have previously been described for an artificial odorant mixture, indicating that even for an expert panel the identification of a single odorant in a mixture is limited to a maximum of three or four compounds.[20,21]

According to our data (Table 3), several odorants with high OAVs are suggested to significantly contribute to hazelnut aroma, but just a few prominent odor qualities could be perceived in the overall aroma (Figure 2B). A roasty, earthy to coffee-like, sulfury aroma was developed during roasting of raw hazelnuts, whereas the nutty odor note was only breaking through in the early stage of roasting. To develop a model for the prediction of a desired hazelnut aroma, a 2D plot of the concentrations of **2** and **3** versus the concentrations of **4**, **6**, **7**, **8**, **10**, and **11** using the data from the 15 hazelnut samples (Table 2) was constructed (Figure 5). This is useful to define the middle group aromas on the basis of their odorant concentrations and to predict the formation of a desired nutty and roasty aroma by interpreting the instrumental data.

The plot clearly suggests the respective concentration levels for the selected compounds in correlation to the hedonic

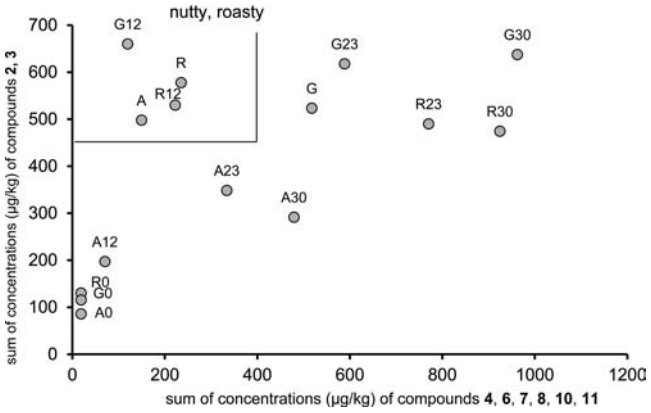

Figure 5. Two-dimensional concentration plot for modeling the generation of a nutty, roasty hazelnut aroma.

acceptance of the hazelnut samples. The two-dimensional concentration plot shows that the breakthrough threshold of **2** and **3** might be 450 μg/kg in the hazelnut samples, whereas concentrations of the roasty, earthy aroma compounds >400 μg/kg might suppress this odor note (Figure 5). To support the importance of these quantitative considerations, model mixtures containing the natural concentrations of compounds **2**, **3**, **4**, **6**, **7**, **8**, **10**, and **11** in each nut sample were prepared in odorless sunflower oil, and the mixtures underwent the projective mapping approach as performed previously for the nut samples. The results showed (see Supporting Information) that the panelists classified the model mixtures in a similar pattern as found for the nut samples. This again supports the hypothesis that the eight key odorants are critical to reconstruct the perceptual map of hazelnut aromas: in particular, compound **2** is responsible for the weak nutty aroma of raw hazelnuts, but the specific composition of compounds **3**, **4**, **6**, **8**, **10**, and **11** generates a nutty, roasty aroma, whereas a high concentration of the roasty coffee-like-smelling odorants including **7** dominates the aroma profile in over-roasted hazelnuts.

In conclusion, the experiments showed that roasted hazelnuts showing an appreciated nutty, roasty aroma can be obtained for each of the hazelnut cultivars selected depending on roasting time, temperature, and roasting technique applied. Distinct concentration ratios of key odorants, such as **2**, **3**, **6**, **7**, **8**, **10**, and **11**, proved to be essential to characterize the various aroma signatures. Dose–response data and the breakthrough thresholds suggest that odorant concentrations far above their odor threshold are needed to suppress or to generate distinct odor notes in complex odorant mixtures. The aroma formation is, thus, driven by thermally induced reactions to produce roasty, earthy-smelling key odorants and by the degradation of other precursors to give nutty odor notes.[11,12,22] Thus, in the future, the quantitative analysis of these precursors could help to predict the aroma potential of raw hazelnuts without roasting and sensory evaluation.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures and table. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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