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Identification of Characterizing Aroma Components of Roasted Chicory "Coffee" Brews

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Supporting Information

ABSTRACT: The roasted and ground root of the chicory plant (*Cichorium intybus*), often referred to as chicory coffee, has served as a coffee surrogate for well over 2 centuries and is still in common use today. Volatile components of roasted chicory brews were identified by direct solvent extraction and solvent-assisted flavor evaporation (SAFE) combined with gas chromatography−olfactometry (GC−O), aroma extract dilution analysis (AEDA), and gas chromatography−mass spectrometry (GC−MS). A total of 46 compounds were quantitated by stable isotope dilution analysis (SIDA) and internal standard methods, and odor-activity values (OAVs) were calculated. On the basis of the combined results of AEDA and OAVs, rotundone was considered to be the most potent odorant in roasted chicory. On the basis of their high OAVs, additional predominant odorants included 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon), 2-methylpropanal, 3-methylbutanal, 2,3-dihydro-5-hydroxy-6-methyl-4*H*-pyran-4-one (dihydromaltol), 1-octen-3-one, 2-ethyl-3,5-dimethylpyrazine, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF), and 3-hydroxy-2-methyl-4-pyrone (maltol). Rotundone, with its distinctive aromatic woody, peppery, and "chicory-like" note was also detected in five different commercial ground roasted chicory products. The compound is believed to an important, distinguishing, and characterizing odorant in roasted chicory aroma. Collectively, a group of caramel- and sweet-smelling odorants, including dihydromaltol, cyclotene, maltol, HDMF, and sotolon, are also thought to be important aroma contributors to roasted chicory aroma.

KEYWORDS: *roasted chicory, aroma, flavor, aroma extract dilution analysis, stable isotope dilution analysis*

INTRODUCTION

Chicory (*Cichorium intybus*) is a perennial herb of the Asteraceae family. Historically, chicory was grown by ancient Egyptians for medicinal purposes. Today, chicory is cultivated throughout the world, including Europe, North Africa, and parts of Asia. Starting from sowing until harvesting, the specifications of every stage of growth and development are well-established, and the quality can be assessed by strict examination.[1]

The roots and leaves of chicory are multifunctional. The roots are an excellent source of inulin, a type of gum used in the food industry. Mature roots are often dried and roasted to serve as a coffee surrogate or additive, while young and tender roots are boiled and eaten as a vegetable. The leaves can also be consumed as a vegetable or used as forage for ruminant livestock.[2]

Chicory "coffee" is a beverage with a bitter and slightly sweet taste as well as a spicy/peppery and sweet/caramel aroma. Furthermore, pure chicory coffee contains no caffeine, which means that chicory coffee is naturally caffeine-free.[3] The first time chicory was used as a coffee substitute or mixed with coffee is not certain. Chicory coffee became popular in the Napoleonic era (ca. 1808) because of a major coffee shortage. In the United States, the practice of consuming chicory coffee began in Louisiana when a Union naval blockade cut off the port of New Orleans and, subsequently, created a coffee shortage.[4]

Despite its desirable "coffee-like" flavor, little research is available on the specific components responsible for the characteristic aroma and taste of roasted chicory. A sensory study compared the perceived taste and flavor attributes of roasted chicory to those of coffee and noted that chicory contained more caramel-like and sweet aroma attributes than coffee.[5] The first mention of any chicory flavor compound was made by Tonsbeek et al.,[6] who reported that beef broth contained a compound with a "roasted chicory-like aroma character", which was subsequently identified as 4-hydroxy-5-methyl-3(2*H*)-furanone. In the first investigation of the volatile components of roasted chicory root, 35 compounds were identified by gas chromatography−mass spectrometry (GC−MS) and it was reported that acetophenone was a characteristic volatile component.[7] In a later study, roasted chicory root oil components were isolated by column chromatography and analyzed by mass spectrometry (MS) and various spectroscopic methods [infrared (IR), ultraviolet (UV), and nuclear magnetic resonance (NMR)].[8] The following major volatile components of the roasted root were identified: vanillin, 5-hydromethyl-2-furfural, 2-acetylpyrrole, furfural, phenylacetic acid, and others.

The aroma-active components of roasted chicory aroma were first reported by Baek and Cadwallader.[9] Volatile

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compounds of roasted chicory root were isolated by two different methods, simultaneous distillation−solvent extraction (SDE) and dynamic headspace analysis (DHA), and then analyzed by GC−MS and gas chromatography−olfactometry (GC−O). Many volatile constituents were identified; specifically, a number of pyrazines and furans were found in high abundance. The aroma-active compounds identified by GC−O included 2-ethyl-3,5-dimethylpyrzine, 2,3-butanedione, 1-octen-3-one, 3-methylbutanal, and one unknown compound with a chicory and burnt sugar-like note.[9] In 2008, volatile compounds isolated by hydrodistillation or air-dried were determined for raw (unroasted) chicory roots from Lithuania, with octane, nonadecane, 2-pentadecanone, and hexadecane reported to be typical volatile constituents of raw chicory roots.[10] A more recent study on a cereal coffee brew and its roasted ingredients (including chicory) reported 30 aroma-active compounds based on results of GC−O.[11] This study identified some compounds not previously reported, including various sulfur compounds and methoxypyrazines, among others.

None of the above-mentioned studies has provided a comprehensive qualitative and quantitative analysis of the aroma components of roasted chicory. Especially of interest are the identities of compounds responsible for specific characteristic aroma notes, such as sweet, caramel-like notes and especially the spicy, peppery, aromatic woody "chicory-like" note. Therefore, the objective of this study was to identify and quantitate the odor-important compounds responsible for the characteristic aroma of aqueous brews prepared from roasted and ground chicory root. This study is the first to comprehensively evaluate the aroma components of pure roasted chicory "coffee" brews by combined use of GC−O and aroma extract dilution analysis (AEDA) and exact quantitation of selected odorants by stable isotope dilution analysis (SIDA).

MATERIALS AND METHODS

Roasted Chicory. Pure roasted chicory products evaluated in this study were obtained from commercial sources, as indicated in Table 1,

Table 1. Chicory Products Evaluated/Analyzed in the Study

code	product description	company
S1	100% chicory (ground), medium−dark roast	imported by Community Coffee, Baton Rouge, LA, U.S.A.
S2	100% roasted chicory root granules; origin, India	imported from France; New River Gourmet, Shawsville, VA, U.S.A.
S3	#45 roasted chicory root granules, *Cichorium intybus*; origin, India	Atlantic Spice Co., North Truro, MA, U.S.A.
S4	roasted chicory root fine powder, *Cichorium intybus* 100% organic, certified organic by Intertek; origin, India	distributed by Blue Lily Organic LLC, Phoenix, AZ, U.S.A.
S5	Leroux instant chicory, 100% chicory; origin, France	imported by Crossings Fine Foods, Champlain, NY, U.S.A.; manufactured by Leroux, Orchies, France

and were stored at room temperature until analyzed. The mention of brand names is not for advertisement or endorsement purposes and does not imply any research contract or sponsorship.

Chemicals. Reagent-grade dichloromethane (CH_2Cl_2), diethyl ether [anhydrous, containing 100 ppm of butylated hydroxytoluene (BHT)], pentane, hydrochloric acid (HCl, concentrated), sulfuric acid (H_2SO_4, concentrated), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), sodium chloride (NaCl), and sodium sulfate (Na_2SO_4, granular, anhydrous) were purchased from Fisher Scientific (Fairlawn, NJ, U.S.A.). Odorless water was prepared by boiling deionized−distilled water in an open 4 L Erlenmeyer glass flask until the volume was reduced by one-third.

Authentic reference standards used for identification and quantitation for compounds listed in Tables 3−6 and including *n*-alkane standards (C_7−C_{30}) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, U.S.A.), except as otherwise noted: (*Z*)-4-heptenal (**9**) (Alfa Aesar, Ward Hill, MA, U.S.A.), 1-octen-3-one (**11**) and 4-vinylguaiacol (**48**) (Lancaster, Windham, NH, U.S.A.), and dimethyltrisulfide (**14**) (Columbia, Brunswick, OH, U.S.A.). The following compounds were synthesized using published methods: (*Z*)-1,5-octadien-3-one (**13**),[12] *trans*-4,5-epoxy-(*E*)-2-decenal (**43**),[13] dihydromaltol (**37**),[14] and rotundone (**50**).[15]

Stable Isotopically Labeled Standards. The following labeled compounds listed in Table 2 and shown in Figure 1 were obtained from commercial sources: 3-methyl-[$^2H_2$]-butanal (**I-4**), [$^2H_3$]-guaiacol (**I-38**), and [$^2H_3$]-*p*-cresol (**I-45**) (CDN Isotopes, Pointe-Claire, Quebec, Canada) and [$^2H_6$]-dimethyl sulfide (**I-1**), [$^2H_5$]-acetic acid (**I-17**), [$^2H_5$]-propionic acid (**I-22**), [$^2H_7$]-butanoic acid (**I-29**), and phenylacetic acid (**I-57**) (Sigma-Aldrich).

The following compounds were synthesized according to published procedures: [$^2H_4$]-hexanal (**I-7**),[16] [$^2H_4$]-octanal (**I-10**), [$^2H_4$]-nonanal (**I-15**), and [$^2H_4$]-decanal (**I-20**),[17] [$^2H_{2-3}$]-1-octen-3-one (**I-11**) and [$^2H_{2-3}$]-1-octen-3-ol (**I-60**),[12] [$^2H_5$]-3-ethyl-2,5-dimethylpyrazine (**I-16**),[18] [$^2H_2$]-2-methylpropanoic acid (**I-26**),[19] [$^{13}C_2$]-phenylacetaldehyde (**I-30**),[20] [$^2H_2$]-3-methylbutanoic acid (**I-31**),[21] [$^2H_2$]-2,4-decadienal (**I-34**) and [$^2H_2$]-(*E*)-2-octenal (**I-59**),[22] [$^2H_4$]-*β*-damascenone (**I-35**) and [$^2H_3$]-*β*-ionone (**I-40**),[23] [$^2H_3$]-eugenol (**I-42**), [$^2H_3$]-(*E*)-isoeugenol (**I-53**), and [$^2H_3$]-(*Z*)-isoeugenol (**I-61**),[24] [$^{13}C_2$]-4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) (**I-44**),[25] [$^2H_5$]-ethyl (*E*)-2-cinnamate (**I-47**),[26] [$^{13}C_2$]-3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (sotolone) (**I-49**),[27] [$^2H_3$]-methylindole (**I-55**),[28] and [$^2H_3$]-vanillin (**I-56**).[29]

Detailed procedures used to synthesize the following compounds are provided in the Supporting Information: 2-methyl-[2,3-$^2H_2$]-propanal (**I-2**), 2-methyl-[3,4-$^2H_2$]-butanal (**I-3**), [1,2-$^{13}C_2$]-2,3-pentanedione (**I-6**), $^2H_3$-maltol (**I-41**), $^2H_4$-rotundone (**I-50**), [$^2H_{2-4}$]-indole (**I-54**), [5,5,6,6-$^2H_4$-hexanoic acid (**I-62**), [3,3,4,4-$^2H_4$]-heptanoic acid (**I-63**), [3,3,4,4-$^2H_4$]-octanoic acid (**I-64**), [3,3,4,4-$^2H_4$]-nonanoic acid (**I-65**), and [3,3,4,4-$^2H_4$]-decanoic acid (**I-66**).

Certain isotopically labeled (deuterated) standards used in this study (e.g., **I-35**, **I-40**, **I-41**, and **I-50**) have the potential to undergo deuterium−hydrogen exchange under certain conditions. Compounds **I-35** and **I-40** were previously determined to be highly stable in low pH solution,[24] as was the case for chicory brews, which had a pH of 3.6. Our own stability studies demonstrated that **I-41** and **I-50** were stable (no proton exchange occurred) for up to 24 h in a 0.1 M citrate buffer at pH 3.6 (Supporting Information).

Static Headspace Solid-Phase Microextraction (HS-SPME)−GC−O. Chicory coffee brews were prepared from each of the five commercial roasted chicory products as follows: Boiling deodorized water (≈99+ °C; 300 mL) was added to 30 g of chicory powder in a 500 mL beaker. The beaker was covered with aluminum foil and stirred with a polytetrafluoroethylene (PTFE)-coated stir bar for 5 min. The suspension was filtered (0.2−0.5 mm, nylon mess) into a clean 500 mL beaker being cooled in an ice−water bath. Aliquots (10 mL) of each brew were placed in separate 40 mL headspace vials, capped with PTFE-lined silicon septa, and stored at −70 °C prior to analysis. For analysis, vials were transferred to a 60 °C water bath and incubated for 10 min and then a clean/preconditioned SPME fiber [1 cm, carboxen (CAR)/divinylbenzene (DVB)/polydimethylsiloxane (PDMS), Sigma-Aldrich] was inserted through the septum and exposed to the headspace of the vial for 40 min.

GC−O was performed using 6890N GC (Agilent Technologies, Inc., Palo Alto, CA, U.S.A.) equipped with a split/splitless injector, a flame ionization detector (FID, 250 °C), and a sniff port (DATU Technology Transfer, Geneva, NY, U.S.A.). Volatile compounds were desorbed from the SPME fiber by hot splitless injection (260 °C; 4 min valve delay). Separations were performed using a RTX-Wax column (15 m length × 0.54 mm inner diameter × 1 μm film

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Table 2. Stable Isotopically Labeled Standards, Selected Ions (m/z), and Response Factors Used in Stable Isotope Dilution Analysis

number[a]	compound	ion[b]	number[c]	labeled internal standard	ion[b]	R^2 [d]	R_f [e]
1	dimethyl sulfide	62	**I-1**	[$^2H_6$]-dimethyl sulfide	68	1.00	1.10
2	2-methylpropanal	72	**I-2**	[$^2H_2$]-2-methylpropanal	74	1.00	0.597
3	2-methylbutanal	86	**I-3**	[$^2H_2$]-2-methylbutanal	88	0.99+	0.518
4	3-methylbutanal	86	**I-4**	[$^2H_2$]-3-methylbutanal	88	0.99+	0.580
5	2,3-butanedione	86	**I-4**[f]	[$^2H_2$]-3-methylbutanal	88	0.99+	0.0720
6	2,3-pentanedione	100	**I-6**	[$^{13}C_2$-2,3-pentanedione	102	0.99+	0.692
7	hexanal	72	**I-7**	[$^2H_4$]-hexanal	76	0.99+	0.156
10	octanal	110	**I-10**	[$^2H_4$]-octanal	114	0.99+	0.490
11	1-octen-3-one	70	**I-11**	[$^2H_{2-3}$]-1-octen-3-one	73	0.99+	1.14
15	nonanal	114	**I-15**	[$^2H_4$]-nonanal	116	0.99	0.440
16	3-ethyl-2,5-dimethylpyrazine	135	**I-16**	[$^2H_5$]-3-ethyl-2,5-dimethylpyrazine	141	0.99+	0.680
17	acetic acid	60	**I-17**	[$^2H_3$]-acetic acid	63	0.99+	0.504
18	2-ethyl-3,5-dimethylpyrazine	135	**I-16**[f]	[$^2H_5$]-3-ethyl-2,5-dimethylpyrazine	141	0.99+	0.680
19	2,3-diethyl-5-methylpyrazine	150	**I-16**[f]	[$^2H_5$]-3-ethyl-2,5-dimethylpyrazine	141	0.99+	0.680
20	decanal	128	**I-20**	[$^2H_4$]-decanal	130	0.99+	1.92
22	propionic acid	74	**I-22**	[$^2H_5$]-propionic acid	79	1.00	0.789
26	2-methylpropanoic acid	73	**I-26**	[$^2H_2$]-2-methylpropanoic acid	75	1.00	1.06
29	butanoic acid	60	**I-29**	[$^2H_7$]-butanoic acid	63	0.91	1.65
30	phenylacetaldehyde	120	**I-30**	[$^{13}C_2$]-phenylacetaldehyde	122	0.99+	0.844
31	3-methylbutanoic acid	87	**I-31**	[$^2H_2$]-3-methylbutanoic acid	89	0.99+	0.860
34	(*E*,*E*)-2,4-decadienal	152	**I-34**	[$^2H_2$]-(*E*,*E*)-2,4-decadienal	154	0.99	0.827
35	(*E*)-*β*-damascenone	190	**I-35**	[$^2H_4$]-(*E*)-*β*-damascenone	194	1.00	0.721
36	3-methyl-1,2-cyclo-pentanedione (cylcotene)	112	**I-41**[f]	[$^2H_3$]-maltol	129	0.98	1.05
37	dihydromaltol	128	**I-41**[f]	[$^2H_3$]-maltol	129	0.99+	1.38
38	guaiacol	124	**I-38**	[$^2H_3$]-guaiacol	127	0.99+	0.922
40	*β*-ionone	177	**I-40**	[$^2H_3$]-*β*-ionone	180	1.00	0.559
41	maltol	126	**I-41**	[$^2H_3$]-maltol	129	0.99+	0.986
42	eugenol	164	**I-42**	[$^2H_3$]-eugenol	167	0.99+	0.907
45	*p*-cresol	108	**I-45**	[$^2H_3$]-*p*-cresol	111	0.99+	0.869
46	*m*-cresol	108	**I-45**[f]	[$^2H_3$]-*p*-cresol	111	0.99+	1.11
47	ethyl (*E*)-cinnamate	176	**I-47**	[$^2H_5$]-ethyl (*E*)-cinnamate	181	0.99+	1.09
50	rotundone	218	**I-50**	[$^2H_4$]-rotundone	206	0.99+	0.998
53	(*E*)-isoeugenol	164	**I-53**	[$^2H_3$]-(*E*)-isoeugenol	167	0.99+	1.02
54	indole	117	**I-54**	[$^2H_4$]-indole	121	1.00	0.451
55	3-methylindole (skatole)	131	**I-55**	[$^2H_3$]-3-methylindole	134	1.00	0.909
56	vanillin	152	**I-56**	[$^2H_3$]-vanillin	155	1.00	0.915
57	phenylacetic acid	136	**I-57**	[$^{13}C_2$]-phenylacetic acid	138	1.00	0.925
58	trimethylpyrazine	122	**I-16**[f]	[$^2H_5$]-3-ethyl-2,5-dimethylpyrazine	141	0.99+	0.680
59	(*E*)-2-octenal	97	**I-59**	[$^2H_2$]-(*E*)-2-octenal	99	0.99	0.890
60	1-octen-3-ol	57	**I-60**	[$^2H_{2-3}$]-1-octen-3-ol	60	0.99+	0.743
61	(*Z*)-isoeugenol	164	**I-61**	[$^2H_3$]-(*Z*)-isoeugenol	167	0.99+	1.02
62	hexanoic acid	87	**I-62**	[$^2H_4$]-hexanoic acid	91	0.99+	0.356
63	heptanoic acid	73	**I-63**	[$^2H_4$]-heptanoic acid	75	0.99+	1.07
64	octanoic acid	73	**I-64**	[$^2H_4$]-octanoic acid	75	1.00	1.06
65	nonanoic acid	73	**I-65**	[$^2H_4$]-nonanoic acid	75	0.99+	0.988
66	decanoic acid	143	**I-66**	[$^2H_4$]-decanoic acid	145	0.99+	0.903

[a]Numbers corresponded to those in Tables 3−6. [b]Selected ion used in selective ion monitoring−GC−MS. [c]The letter "**I**" indicates isotopically labeled compound. [d]Coefficient of determination for the calibration plot. [e]Response factor. [f]Isotope was not available; a structurally similar compound was used as the internal standard.

thickness, Restek Bellefonte, PA, U.S.A.). Helium was used as the carrier gas at a constant flow rate of 5 mL/min. The oven temperature was programmed from 35 to 225 °C at 10 °C/min with initial and final hold times of 5 and 30 min, respectively. GC−O was conducted by two experienced panelists, and post-peak odor intensity scores were estimated on the basis of consensus agreement, where + = very weak, ++ = medium, and +++ = strong.

Preparation of Aroma Extracts. Roasted chicory brew (250 mL) prepared as described earlier was divided equally between two 250 mL Teflon FEP centrifuge bottles (Thermo Fisher Scientific, Waltham, MA, U.S.A.), and CH_2Cl_2 (50 mL) was added to each bottle. The bottles were sealed with Teflon FEP caps, shaken at 200 rpm (DS-500 orbital shaker, VWR International, Radnor, PA, U.S.A.) for 30 min and then centrifuged at 3500 rpm for 15 min (IEC HN-SII centrifuge, Damon/IEC Division, Needham, MA, U.S.A.). After the solvent layer was collected, the extraction was repeated 2 more times as above. The three CH_2Cl_2 extracts were combined, concentrated to 50 mL using a Vigreux column (45 °C), and stored at −20 °C until subjected to solvent-assisted flavor evaporation (SAFE) as described previously.[19]

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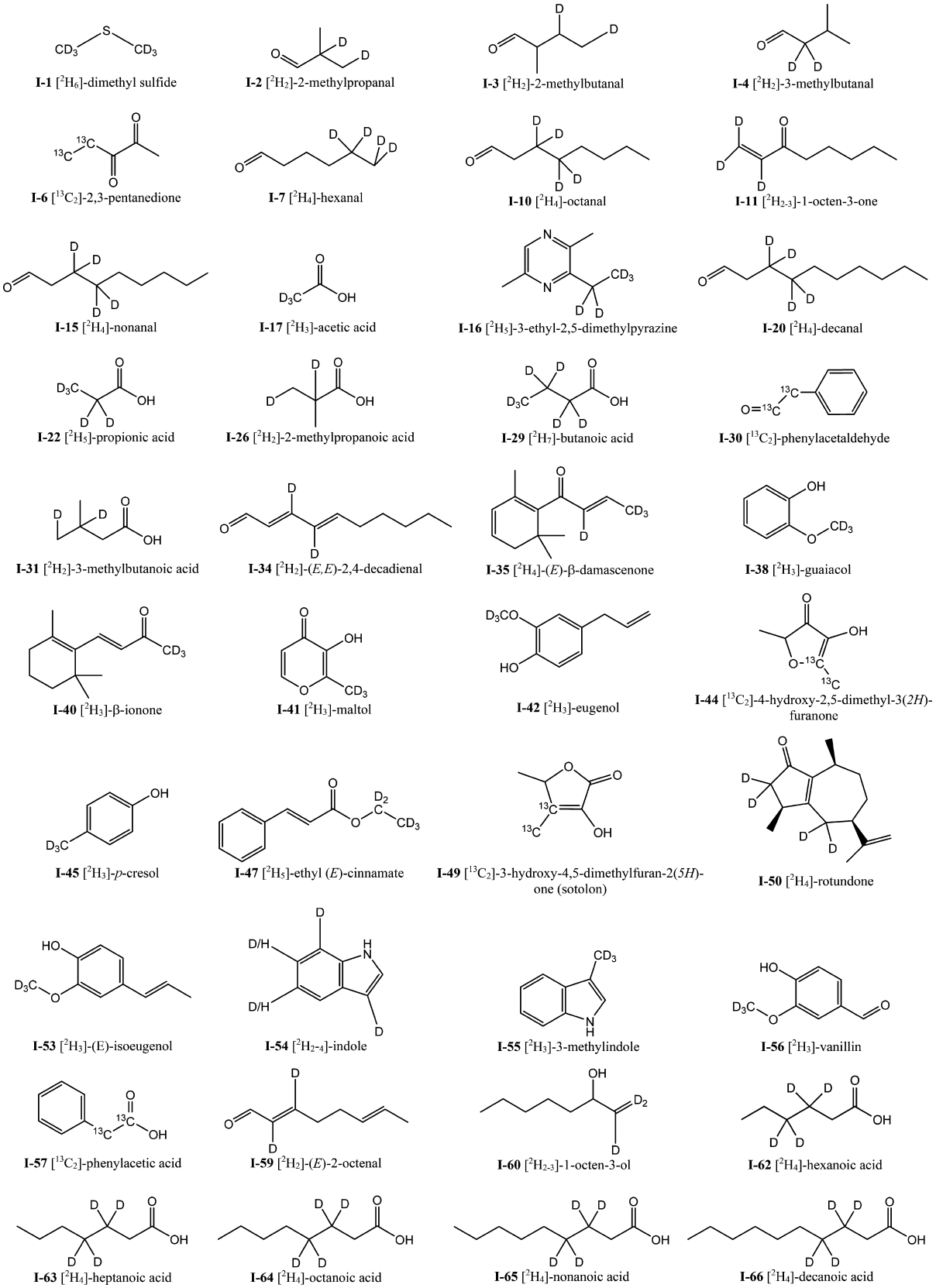

Figure 1. Chemical structures of isotopically labeled standards used in stable isotope dilution analysis. The letter "**I**" means isotopically labeled compound followed by the numbers that correspond to those in Table 2.

Fractionation of Aroma Extracts. The SAFE aroma extract from above was subjected to compound class fractionation. First, the extract was washed with aqueous sodium carbonate ($NaCO_3$) (5%, w/v; 3 × 10 mL) to separate the acidic compounds (aqueous phase) from the neutral/basic compounds (CH_2Cl_2 phase). The aqueous phase was acidified with aqueous 4 N HCl to pH 2 and extracted with

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diethyl ether (3 × 10 mL) to yield the acidic (A) fraction. The neutral/basic fraction from above was extracted with aqueous 0.1 N HCl (3 × 10 mL) to separate the neutral (N) compounds (CH_2Cl_2 phase) from the basic (B) compounds (aqueous phase). The aqueous phase was made alkaline (pH 9) with aqueous 1 N NaOH, and then the basic volatiles were extracted into CH_2Cl_2 (3 × 10 mL). Each fraction from above was washed with aqueous saturated NaCl solution (2 × 10 mL), condensed to 5 mL using a Vigreux column (45 °C), and then dried over anhydrous Na_2SO_4 (5 g). Extracts were further concentrated to 1 mL using a gentle N_2 stream and stored at −20 °C until analysis.

GC−O and AEDA. The GC−O system used for analysis of aroma extracts and dilutions (1 μL injection) consisted of 6890 GC (Agilent Technologies, Inc.) equipped with an FID, a DATU sniff port, and a cool on-column injector (+3 °C oven tracking). Separations were performed using either a RTX-Wax column (15 m length × 0.54 mm inner diameter × 1 μm film thickness, Restek) or a RTX-5 column (15 m length × 0.53 mm inner diameter × 1 μm film thickness, Restek). Helium was used as the carrier gas at a constant flow rate of 5 mL/min. The oven temperature was programmed from 35 to 225 °C at 10 °C/min with initial and final hold times of 5 and 30 min, respectively.

For AEDA, serial dilutions (1:3, 1:9, 1:27, v/v, etc.) were prepared for each aroma extract fraction in CH_2Cl_2. GC−O was performed by two experienced panelists. A flavor dilution (FD) factor is the highest dilution in which an odorant is last detected by GC−O; i.e., if an odorant was detected in the 1:27 dilution but not the 1:81 dilution, it would be assigned a FD of 27. The results of one panelist were used to determine the FD factor for each odorant.

GC−MS. A 6890N GC/5973N mass selective detector (MSD) (Agilent Technologies, Inc.) system was used to perform GC−MS analyses. Each aroma extract fraction was injected (2 μL) into a CIS4 inlet (Gerstel, Germany) using the cold splitless mode (initial temperature, −50 °C; ramp, 12 °C/s to 260 °C; and splitless valve delay, 1.1 min) into a Stabilwax column (30 m × 0.32 mm × 0.25 μm film thickness, Restek). Helium was used as the carrier gas at a constant rate of 1 mL/min. The oven temperature was programmed from 40 to 225 °C at a ramp of 6 °C/min with initial and final hold times of 5 and 30 min, respectively. MSD conditions were as follows: ion source temperature was 200 °C, ionization mode was electron impact (EI), electron energy was 70 eV, and full scan range (m/z 35−300).

Compound Identification. Compound identifications were initially assigned on the basis of comparing the EI mass spectra of peaks against those in the National Institute of Standards and Technology (NIST) 2008 Mass Spectral Library. A compound was considered to be positively identified when its retention indices (RIs) on both polar and nonpolar columns, its odor characteristic, and its EI mass spectrum matched those of an authentic reference standard. Whenever a mass spectrum and/or authentic reference standard were unavailable for a compound, its RI values were compared to literature values and/or online databases (Flavornet and Pherobase) to aid in its tentative identification. RIs were calculated by analysis of a series of *n*-alkanes under the same experimental conditions as aroma extracts.[19]

Quantitation of Rotundone. *GC−FID Calibration of Stock Solutions.* GC−FID calibration was performed to determine the exact concentration of stock solutions of rotundone and [$^2H_4$]-rotundone. Standardization of rotundone (**50**) and [$^2H_4$]-rotundone (**I-50**) solutions was conducted using 6890 GC (Agilent Technologies, Inc.) equipped with a split/splitless injector and FID. An unknown solution (50 μL) of either a rotundone or [$^2H_4$]-rotundone (in ether) was mixed with 50 μL of a known solution of nootkatone (10.4 μg/mL in ether) (Sigma-Aldrich), which served as the internal standard. The mixture was injected (2 μL) in the hot split mode (250 °C; 1:10 split ratio), and separations were performed using a RTX-5 column (15 m × 0.32 mm × 0.5 μm film thickness, Restek). The oven temperature was programmed from 100 to 250 °C at 10 °C min with initial and final hold times of 0.5 and 10 min, respectively. Concentrations of rotundone and [$^2H_4$]-rotundone were determined from their peak area ratios relative to nootkatone (i.e., rotundone versus nootkatone) assuming that the compounds had the same GC−FID response factors.

Determination of the GC−MS Response Factor. The following approximate mass ratios of rotundone and [$^2H_4$]-rotundone were prepared in ether: 1:10, 2:10, 5:10, 10:10, 10:5, 10:2, and 10:1. Analyses were conducted using the previously described GC−MS system. A total of 2 μL of each solution was injected into a CIS-4 inlet (Gerstel) in the hot splitless mode (1.10 min valve delay). Separations were performed using a Stabilwax column (30.0 m length × 0.25 mm inner diameter × 0.25 μm film thickness, Restek). The oven temperature was programmed as follows: initial temperature of 40 °C (5 min hold) and ramp rate of 10 °C/min to a final temperature of 225 °C (35 min hold time). Helium was used as the carrier gas at a constant flow of 1.0 mL/min. The MSD conditions were as follows: capillary direct interface temperature, 260 °C; ionization energy, 70 eV; mass range, m/z 35−300; electron multiplier voltage (autotune), +200 V; and scan rate, 5.27 scans/s. Data acquisition was performed using simultaneous full scan (m/z 35−300) and selective ion monitoring (SIM) (dwell time, 50 ms; ions monitored, m/z 175, 203, 206, 218, 221, and 222). The response factor was determined as the inverse of the slop of a plot of mass ratio versus area ratio of labeled to unlabeled compounds.

Isolation/Extraction. Chicory brews were prepared as previously described. Each brew (approximately 250 mL) was spiked with 200 μL of a solution containing 37.6 μg/mL [$^2H_4$]-rotundone. After mixing, the brew was extracted as described earlier, except that ethyl ether was used instead of CH_2Cl_2. The extract was concentrated to 5 mL by distillation using a Vigreux column (45 °C) and then concentrated to 500 μL using a gentle stream of N_2 gas. The extract was passed through a bed (35 g) of silica gel (Merck grade, 9385; pore size, 60 Å; Sigma-Aldrich) slurry packed with 100 mL of pentane in a glass column (2.5 cm inner diameter × 50 cm length). The following elution solvents (50 mL each) were sequentially used (gravity fed) to elute the target compound: pentane/ether ratio (v/v) = 98:2, 95:5, 90:10, 80:20, 70:30, and 60:40. Fractions (10 mL) were collected. Rotundone eluted between the 70:30 and 60:40 elution solvents. The fractions containing rotundone were pooled and concentrated to about 1 mL by evaporation in the fume hood and then further concentrated to 0.5 mL using a gentle stream of N_2 gas just before analysis. Triplicate extractions were performed.

GC−MS Analysis. GC−MS analyses were conducted as described above, except that the oven temperature was held at 40 °C for 5 min, ramped to 65 °C at 2 °C/min, then ramped to 225 °C at 3 °C/min, and held at 225 °C for 60 min. The MSD conditions were as follows: capillary direct interface temperature, 260 °C; ionization energy, 70 eV; mass range, m/z 35−300; electron multiplier voltage (autotune), +200 V; and scan rate, 5.27 scans/s. Data acquisition was performed using simultaneous full scan (m/z 35−300) and selective ion monitoring (SIM) (dwell time, 50 ms; ions monitored, m/z 175, 203, 206, 218, 121, and 222).

Quantitation of HDMF and Sotolon. Chicory brew was prepared as previously described. Chicory brew (100 mL) was spiked with 50 μL of $^{13}C_2$-HDMF (**I-44**; 0.234 μg/μL in ether) and 10 μL of $^{13}C_2$-sotolone (**I-49**; 0.284 μg/μL in ether). The extraction, SAFE, fractionation, and concentration steps were conducted as described earlier. GC conditions: GC−MS analyses were conducted as described above, except that extracts were injected (2 μL) in the cold splitless mode (initial temperature, −50 °C; ramp, 12 °C/s to 260 °C; and splitless valve delay, 1.1 min). The oven temperature was held at 40 °C for 5 min, ramped to 225 °C at 6 °C/min, and held at 225 °C for 60 min. The MSD conditions were as follows: capillary direct interface temperature, 260 °C; ionization energy, 70 eV; mass range, m/z 33−300; electron multiplier voltage (autotune), +200 V; and scan rate, 5.27 scans/s. Data acquisition was performed using simultaneous full scan (m/z 33−300) and selective ion monitoring (SIM) (dwell time, 50 ms; ions monitored, m/z 43, 45, 83, 85, 128, and 130).

Quantitative Analysis by SPME−GC−MS−SIDA. Chicory brew was prepared as previous described. A 4 mL aliquot of the brew and 1 g of NaCl (conditioned/cleaned at 150 °C for 2 h prior to use) were

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Table 3. SPME−GC−O Comparison of Roasted Chicory Brews Prepared from Five Different Commercial Products

				relative odor intensity[a]				
number[b]	compound	RI[c] (Wax)	odor property[d]	S1[e]	S2	S3	S4	S5
2	2-methylpropanal	<900	chocolate, malty	nd	+	++	+++	+
3/4	2-/3-methylbutanal	919	chocolate, malty	++	++	+++	+++	+++
5	2,3-butanedione	982	buttery, cream cheese	+	++	+	++	+
7	hexanal	1083	leaves, green, grass	+	nd	++	++	nd
	unknown	1189	sour, earthy	++	++	+	nd	++
8	1,8-cineol (eucalyptol)	1201	minty, eucalyptus	++	+	++	++	nd
9	(*Z*)-4-heptenal	1248	dried fish	+++	+++	++	+++	+++
11	1-octen-3-one	1302	mushroom	+++	+++	+++	+++	+++
14	dimethyl trisulfide	1378	cabbage, green	+++	+	+++	+	+++
15	nonanal	1398	soapy, pungent	nd	+	+	+++	nd
	unknown	1408	mushroom, green	++	+++	+	++	nd
	unknown	1533	vitamin	++	+++	+	+++	+++
	unknown	1439	meaty	+	+++	+++	+++	nd
17	acetic acid	1450	vinegar	++	+	+++	+++	+
	methional	1453	potato fries	+	+++	++	nd	+
18	2-ethyl-3,5-dimethylpyrazine	1465	earthy	+	+	+	++	++
23	(*E*)-2-nonenal	1533	stale, cucumber	+++	++	+++	+++	+++
25	linalool	1553	floral, lavender	+	+++	+++	nd	nd
27	(*E*,*Z*)-2,6-nonadienal	1585	melon, cucumber	+++	++	+++	+++	+
28	2-acetylpyrazine	1601	rice, popcorn	+	+	nd	+	nd
29	butanoic acid	1630	cheesy	++	++	++	+	nd
30	phenylacetaldehyde	1643	green, rosy	++	nd	++	++	+
31	3-methylbutanoic acid	1669	cheesy, sweaty	+	+	+	+	++
36	cyclotene	1840	maple, sweet	+	nd	+	+	+
37	dihydromaltol	1860	caramel	+++	+++	++	+	++
	unknown	1826	musty	+	nd	++	++	++
41	maltol	1945	caramel	++	+++	+++	++	+
43	*trans*-4,5-epoxy-(*E*)-2-decenal	2002	metallic, unripe	+++	+++	+	++	+
42	eugenol	2015	spicy, cloves	+	++	++	++	+
45	*p*-cresol	2072	animal stable, fecal	++	++	++	++	+++
	unknown	2122	seasoning	nd	++	+	++	
49	sotolon	2162	seasoning, spicy	+++	++	+	++	++
	unknown	2175	woody, smoke	+++	+	+	++	+++
50	rotundone	2266	woody, aromatic	+++	+++	+++	+++	+++
56	vanillin	2560	vanilla	++	++	++	++	+

[a]Post peak odor intensity: nd, not detected; +, weak; ++, medium; and +++, strong. [b]Numbers correspond to those in Tables 2 and 4−6. [c]Retention index was determined on a RTX-Wax column. [d]Odor quality was determined by panelists during GC−O. [e]Product code (see Table 1).

transferred to a 20 mL headspace vial. The vial was sealed with a PTFE-lined silicon septum equipped screw cap. Prior to analysis, the vial was spiked with a known volume of one or more of the isotope internal standard solutions (see Table S1 of the Supporting Information). Vials were analyzed by SPME−GC−MS using a CombiPal autosampler (CTC Analytics) coupled to a 6890N/5973N GC−MS system (Agilent Technologies, Inc.). Vials were pre-incubated at 60 °C for 10 min, and then a SPME fiber (2 cm, CAR/DVB/PDMS, Sigma-Aldrich) was inserted through the septum and exposed for 30 min to the headspace of the vial.

Volatile compounds were desorbed from the SPME fiber into the GC−MS system by hot splitless injection (260 °C, 4 min valve delay). Separations were performed using a Stabilwax GC column (30 m × 0.32 mm × 0.25 μm film thickness, Restek). Helium was used as the carrier gas at a constant rate of 1 mL/min. The oven temperature was programmed from 40 to 225 °C at 4 °C/min with initial and final hold times of 5 and 30 min, respectively.

The MSD conditions were as follows: capillary direct interface temperature, 260 °C; ionization energy, 70 eV; mass range, m/z 35−300; electron multiplier voltage (autotune), +200 V; and scan rate, 5.27 scans/s. Data acquisition was performed using the simultaneous full scan (m/z 35−300) and selective ion monitoring (SIM) (dwell time, 50 ms; ions monitored are indicated in Table 2).

The concentration for a target compound was determined using the following equation:

$$\text{concentration}_{\text{target}}\ (\mu\text{g/L}) = (\text{area}_{\text{target}}/\text{area}_{\text{IS}}) \times R_f \times \text{vol}_{\text{IS}}\ (\mu\text{L}) \times \text{concentration}_{\text{IS}}\ (\mu\text{g}/\mu\text{L}) \div \text{vol}_{\text{sample}}\ (\text{L})$$

For determination of response factors (R_f), solutions of the following approximate mass ratios of unlabeled to labeled compounds were prepared in ether: 1:10, 2:10, 5:10, 10:10, 10:5, 10:2, and 10:1 and analyzed as above, except that injections were performed in the hot split mode (260 °C; 1:50 split ratio). Response factors were determined as the inverse of the slope of a plot of mass ratio versus area ratio for the unlabeled versus labeled compounds.

For 3-methyl-1,2-cyclopentanedione (cyclotene) (**36**), dihydromaltol (**37**), and maltol (**41**) the calibration curves were determined by SPME−GC−MS as described earlier. The response factor of these three compounds was determined against $^2H_3$-maltol (**I-41**) in an aqueous 0.1 M citric acid buffer with a pH (3.6) close to that of roasted chicory brew. It was important to use a low pH matrix when using $^2H_3$-maltol as the internal standard to minimize any potential hydrogen−deuterium exchange from occurring during the analysis.

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Table 4. Predominant Neutral and Basic Odorants in Roasted Chicory Brew Determined by AEDA

		RI[a]				FD factor[b]	
number[c]	compound	RTX-Wax	RTX-5	odor property[d]	fraction[e]	Wax	RTX-5
3/4	2-/3-methylbutanal[f]	919	<800	chocolate, malty	N	3	<3
5	2,3-butanedione[f]	992	<600	buttery, cream cheese	N	<3	<3
6	2,3-pentanedione[f]	1071	g	buttery	N	<3	g
7	hexanal[f]	1088	800	green, fruit	N	27	3
8	1,8-cineol (eucalyptol)	1207	g	minty, eucalyptus oil	N	<3	g
9	(*Z*)-4-heptenal[h]	1251	873	dry fish	N	3	<3
10	octanal[f]	1295	1004	citrusy, orange	N	3	<3
11	1-octen-3-one[f]	1302	981	mushroom	N	9	27
12	2-acetyl-1-pyrroline[h]	1347	925	popcorn	B	3	<3
13	(*Z*)-1,5-octadien-3-one[h]	1370	986	earthy, metallic	N	27	3
14	dimethyltrisulfide[f]	1390	969	sulfurous, cabbage	N	3	3
15	nonanal[f]	1398	1099	orange peel	N	3	<3
16	3-ethyl-2,5-dimethylpyrazine[f]	1431	1077	earthy, nutty	B	27	9
18	2-ethyl-3,5-dimethylpyrazine[f]	1453	1086	earthy, nutty	B	81	81
19	2,3-diethyl-5-methylpyrazine[h]	1493	1160	nutty	B	<3	<3
20	decanal[f]	1495	1205	lemony, soapy	N	<3	<3
21	(*Z*)-2-nonenal[f]	1510	1143	green, hay	N	<3	<3
23	(*E*)-2-nonenal[f]	1549	1164	hay	N	9	3
24	unknown	1539	1078	vitamin capsule	N	9	3
25	linalool[f]	1558	1107	floral, lavender	N	27	27
27	(*E*,*Z*)-2,6-nonadienal[h]	1593	1154	cucumber	N	3	3
28	2-acetylpyridine[h]	1610	1020	rice, popcorn	N	3	3
30	phenylacetaldehyde[f]	1652	1044	green, rose	N	9	3
32	(*E*,*E*)-2,4-nonadienal[f]	1698	1214	fatty, fried	N	27	9
33	methyl salicylate[h]	1745	1252	minty	N	<3	<3
34	(*E*,*E*)-2,4-decadienal[f]	1815	1316	fatty, fried	N	3	3
35	(*E*)-*β*-damascenone[f]	1828	1384	floral, applesauce	N	3	<3
38	2-methoxyphenol (guaiacol)[f]	1883	1093	smoky	N	243	81
39	2-phenylethanol[f]	1915	1114	rose, wine-like	N	3	<3
40	*β*-ionone[f]	1955	1493	floral, lavender	N	3	<3
42	eugenol[f]	2005	1321	spicy, cloves	N	9	9
43	*trans*-4,5-epoxy-(*E*)-2-decenal[h]	2016	1384	unripe, green, metallic	N	81	9
47	ethyl (*E*)-cinnamate[f]	2139	1469	fruity, spicy	N	9	3
48	4-vinylguaiacol[f]	2219	1313	spicy, smoky, cloves	N	27	9
50	rotundone[f]	2279	1707	incense, peppery	N	243	81
51	unknown	2305	g	woody, aromatic	N	<3	g
53	(*E*)-isoeugneol[f]	2390	1456	spicy, cloves	N	3	<3
54	indole[f]	2450	1290	fecal, mothballs	N	<3	<3
55	3-methylindole (skatole)[f]	2496	1389	fecal, mothballs	N	<3	<3

[a]Retention index. [b]Flavor dilution (FD) factor. [c]Numbers correspond to those in Tables 2, 3, and 6. [d]Odor quality was determined by GC−O. [e]Fraction in which the odorant was detected, neutral (N) and basic (B). [f]Positively identified compound. [g]Not detected. [h]Tentatively identified compound.

Different approximate mass ratios (10:1, 5:1, 1:1, 1:5, and 1:10) of the compounds were spiked in 4 mL of the buffer prior to analysis. Response factors were determined as the inverse of the slope of a plot of mass ratio versus area ratio for the unlabeled compounds against $^{2}H_{3}$-maltol.

Odor Detection Threshold Determination. The sensory testing protocols were approved by the Institutional Review Board of the University of Illinois at Urbana−Champaign (IRB 17658). The best estimate threshold (BET) of rotundone was determined using the ASTM ascending forced-choice method of limits method E679-04.[30] Rotundone standard solution was determined to be free of any odor impurities by analysis of a 100 mg/L solution by GC−O as previously described.[24] The threshold was determined orthonasally in a matrix consisting of an instant coffee aqueous suspension. Individual samples of coffee matrix were made by suspending 0.33 g of Folgers instant coffee crystals (Folger Coffee Company, Cincinnati, OH, U.S.A.) in 40 mL of odor-free water in 125 mL PTFE sniff bottles. Stock solutions of rotundone (prepared in ether) were used to deliver the appropriate amount of the compound to 125 mL Teflon squeeze bottles as previously described.[24] Panelists (n = 32) were given these concentrations (test solutions) in a series along with two matrix blanks containing the same volume of ether added to the each spiked matrix. A series of 11 ascending concentrations was tested. The individual BET was calculated as the geometric mean of the last concentration with an incorrect response and the first concentration with a correct response. The group BET was calculated as the geometric mean of the individual BETs.

Sensory Addition Study. A sensory addition study was conducted to determine if the addition of rotundone to regular coffee would cause panelists to rate the perceived aroma of the rotundone-spiked coffee as more chicory-like compared to the unspiked regular coffee. Regular (instant) coffee matrix and chicory brews were prepared as described above. Rotundone-spiked coffee consisted of regular coffee spiked with rotundone to a final concentration of 34 ppb. Samples (40 mL) were presented to panelists in 125 mL Teflon squeeze bottles, as described above.

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Table 5. Predominant Acidic Odorants in Roasted Chicory Brew Determined by AEDA

number[b]	compound	RI[a] RTX-Wax	RI[a] RTX-5	odor property[c]	FD factor[d]
17	acetic acid[e]	1451	NA[f]	vinegar	3
22	propionic acid[e]	1532	NA	Swiss cheese	<3
26	2-methylpropanoic acid[e]	1588	NA	cheesy, sweaty	3
29	butanoic acid[e]	1625	NA	cheesy, fecal	3
31	3-methylbutanoic acid[e]	1664	NA	Swiss cheese, sweaty	27
36	3-methyl-1,2-cyclopentanedione (cyclotene)[e]	1848	1026	caramel, maple syrup	3
37	2,3-dihydro-5-hydroxy-6-methyl-4*H*-pyran-4-one (dihydromaltol)[e]	1862	1104	caramel, burnt sugar	243
41	3-hydroxy-2-methyl-4-pyrone (maltol)[e]	1955	1104	caramel, burnt sugar	81
44	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF)[e]	2047	1056	caramel, burnt sugar	243
45	*p*-cresol[e]	2077	1079	animal stable, bandage	27
46	*m*-cresol[e]	2095	g	phenolic, medical	<3
49	3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (sotolon)[e]	2219	1120	spicy, curry	729
52	unknown	2373	g	roasted sweet potato	<3
56	vanillin[e]	2560	1412	vanilla	81
57	phenylacetic acid[e]	2574	ND	rosy, honey	<3

[a]Retention index. [b]Numbers correspond to those in Tables 2−4 and 6. [c]Odor quality was determined by GC−O. [d]Flavor dilution (FD) factor was determined on a RTX-Wax column. [e]Positively identified compound. [f]Not available as a result of being unable to accurately determine RI because of the poor/shifting peak shape. [g]Not detected.

Panelists were prescreened using a triangle difference test[24] to determine if they were able to discriminate the rotundone-spiked coffee from the regular coffee. This was an important step because some people are insensitive to the odor of rotundone.[31] From an initial 30 panelists, 23 successfully passed the triangle difference test and were selected to compare the overall similarity of the perceived aroma of three different samples consisting of either chicory brew, unspiked coffee matrix, or coffee matrix spiked with 34 ppb of rotundone. Samples were rated using a 10 point similarity scale, where 1 = not similar and 10 = very similar to a reference consisting of chicory brew. Average rating scores among panelists were analyzed by one-way analysis of variance and least significant differences using Microsoft Excel 2016.

RESULTS AND DISCUSSION

Five commercial brands of roasted chicory were purchased from U.S. suppliers, representing both roasted ground chicory (S1, S2, S3, and S4) and instant roasted chicory granules (S5) (Table 1). A preliminary study was conducted to compare the perceived aromas of the five products. Hot water extracts/infusions (brews) were prepared from all five brands, and the aromas were evaluated by a few lab members familiar with the flavor of chicory coffee. All brews and corresponding aroma extracts possessed typical chicory-like aromas. Four were considered to be similar to one another with respect to aroma, while one product, Leroux instant chicory (S5), was clearly different from the other four.

HS-SPME−GC−O Comparison of Roasted Chicory Products. Brews prepared from the five brands of roasted chicory were compared using HS-SPME−GC−O. As shown in Table 3, all brews had complex aroma profiles and shared a number of the same aroma compounds; especially, sweet/caramel (**36**, **37**, **41**, and **49**) and spicy/peppery (**50**) notes were present in all five products. On the basis of these results, one representative product (S1) was chosen for further detailed analyses. This brand was considered to have a representative aroma, and furthermore, this is the same brand that was studied in our previous study[9] and is a typical New-Orleans-style chicory coffee.

Comprehensive Aroma Analysis by GC−O and AEDA. An aroma extract was prepared from roasted chicory coffee brew using exhaustive solvent extraction and a careful cleanup step (i.e., SAFE) to yield a representative aroma extract, which when evaluated using a flavor blotter possessed the characteristic aroma expected for the roasted chicory brew. To simplify subsequent analyses by GC−O and AEDA, the aroma extract was further separated into neutral, basic, and acidic fractions.

A combined total of 54 compounds were detected by GC−O and AEDA (Tables 4 and 5). A total of 24 compounds are reported for the first time as aroma-active components of roasted chicory. Among the compounds listed in Tables 4−6, those identified in previous studies on roasted chicory include dimethylsulfide (**1**), 2-methylbutanal (**3**), 3-methylbutanal (**4**), 2,3-butanedione (**5**), 2,3-pentanedione (**6**), hexanal (**7**), 1-octen-3-one (**11**), nonanal (**15**), 3-ethyl-2,5-dimethypyrazine (**16**), 2-ethyl-3,5-dimethylpyrazine (**17**), phenylacetaldehyde (**30**), cyclotene (**36**), vanillin (**56**), and phenylacetic acid (**57**).[8,9,11] Additional volatile compounds previously identified in the aerial parts of chicory (not the roasted root) are also reported in the present study, including 1,8-cineole (**8**), dimethyltrisulfide (**14**), decanal (**20**) (*E*,*Z*)-2,6-nonadienal (**27**), 3-methylbutanoic acid (**31**), (*E*,*E*)-2,4-nonadienal (**32**), (*E*,*E*)-2,4-decadienal (**34**), *β*-ionone (**40**), and 1-octen-3-ol (**60**).[10] Volatiles previously reported in roasted chicory brew included (*Z*)-4-heptenal (**9**), 2-acetyl-1-pyrroline (**12**), (*Z*)-1,5-octadien-3-one (**13**), 2-methoxyphenol (**38**), (*E*,*Z*)-2,6-nonadienal (**27**), *β*-damascenone (**35**), HDMF (**44**), and 4-vinylguaiacol (**48**).[11] These researchers reported eight additional compounds not identified in the present study, including methanethiol, 2-methyl-3-furanthiol, 2-furfurylthiol, 3-mercapto-3-methylbutyl formate, 2-thenythiol, 3-(*sec*-butyl)-2-methoxypyrazine, 2-methyl-[3-methyldithio]furan, and 3-isobutyl-2-methoxypyrazine.[11]

AEDA. The results of AEDA (Tables 4 and 5) indicated that, on the basis of its overall highest FD factor of 729, sotolon (**49**) was potentially the most potent odorant in roasted chicory. Dihydromaltol (**37**; FD_{wax} = 243), HDMF (**44**; FD_{wax} = 243), guaiacol (**38**; FD_{wax} = 243), and rotundone (**50**; FD_{wax} = 243) were indicated as additional potent odorants and compounds that likely contribute greatly to roasted chicory aroma. Collectively, compounds with caramel/

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Table 6. Concentrations, Odor Detection Thresholds, and Odor-Activity Values (OAVs) for Selected Potent Odorants in Roasted Chicory Coffee Brews

number[a]	compound	concentration (μg/L)[b]	odor threshold[c]	OAV[d]
50	rotundone	4.22 (±0.15)	0.008[31]	528
49	3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (sotolon)	89.6 (±7.0)	0.3[32]	298
4	3-methylbutanal	26.2 (±0.6)	0.2[33]	131
2	2-methylpropanal	93.2 (±1.3)	1[34]	93
35	(*E*)-β-damascenone	0.157 (±0.002)	0.002[34]	79
37	dihydromaltol	14900 (±220)	250[35]	60
11	1-octen-3-one	2.87 (±0.05)	0.05[33]	57
18	2-ethyl-3,5-dimethylpyrazine	1.39 (±0.09)	0.04[33]	35
3	2-methylbutanal	91.1 (±2.0)	3[33]	30
6	2,3-pentanedione	380 (±13)	20[33]	19
26	2-methylpropanoic acid	777 (±18)	50[34]	16
38	2-methoxyphenol (guaiacol)	46.0 (±0.3)	3[33]	15
10	octanal	10.3 (±21)	0.7[33]	15
19	2,3-diethyl-5-methylpyrazine	1.30 (±0.05)	0.09[34]	14
29	butanoic acid	240 (±1)	17[36]	14
15	nonanal	13.9 (±0.1)	1[33]	14
44	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF)	288 (±11)	21[34]	14
30	phenylacetaldehyde	48.1 (±0.7)	4[33]	12
1	dimethyl sulfide	3.47 (±0.07)	0.3[34]	12
34	(*E*,*E*)-2,4-decadienal	0.782 (±0.006)	0.07[33]	11
42	eugenol	57.7 (±1.3)	6[34]	9.6
40	β-ionone	0.0434 (±0.0017)	0.007[34]	6.2
31	3-methylbutanoic acid	1350 (±10)	250[33]	5.4
47	ethyl (*E*)-cinnamate	0.299 (±0.004)	0.06[34e]	5.0
20	decanal	6.63 (±0.21)	2[34]	3.3
5	2,3-butanedione	9.15 (±0.05)	3[36]	3.0
22	propanoic acid	4680 (±82)	2000[33]	2.3
60	1-octen-3-ol	2.26 (±0.002)	1[33]	2.3
56	vanillin	130 (±0.36)	58[33]	2.2
59	(*E*)-2-octenal	6.68 (±0.18)	3[33]	2.2
36	cyclotene	605 (±59)	300[37]	2.0
7	hexanal	5.13 (±0.09)	4.5[33]	1.1
16	3-ethyl-2,5-dimethylpyrazine	6.63 (±0.09)	8.6[33]	0.77
45	*p*-cresol	15.5 (±0.03)	55[36]	0.28
17	acetic acid	12800 (±15)	50000[34]	0.26
58	trimethylpyrazine	3.94 (±0.16)	23[32]	0.17
41	maltol	5480 (±140)	35000[33]	0.16
55	3-methylindole (skatole)	0.451 (±0.003)	3[33]	0.15
57	phenylacetic acid	1260 (±26)	10000[33]	0.13
65	nonanoic acid	286 (±4)	3000[36]	0.095
46	*m*-cresol	10.7 (±5)	680[37]	0.016
63	heptanoic acid	14.8 (± 0.16)	3000[37]	0.0049
62	hexanoic acid	19.4 (±0.9)	3000[33]	0.0065
54	indole	0.836 (±0.021)	90[34]	0.0093
64	octanoic acid	3.78 (±0.03)	3000[36]	0.0013
66	decanoic acid	0.102 (±0.0003)	10000[36]	0.000010
53	(*E*)-isoeugenol	45.1 (±0.4)	0.1−0.2[24f]	
61	(*Z*)-isoeugenol	15.4 (±0.6)	0.4−0.6[24f]	

[a]Numbers corresponded to those in Tables 2−5. [b]Average concentration [±standard deviation (SD); n = 3]. [c]Orthonasal odor detection thresholds in water (μg/L), with the literature source. [d]Odor-activity value (OAV) determined by dividing the concentration of an odorant by its respective odor detection threshold. [e]Retronasal odor detection threshold. [f]Odor detection threshold in air.

sweet aroma notes (**36**, **37**, **41**, **44**, and **49**) and rotundone (**50**) with its characteristic incense and spicy/peppery note may be of particular importance in the aroma of roasted chicory coffee brew.

Quantitation of Selected Compounds. Compounds listed in Table 6 were quantitated using SIDA. In SIDA, a deuterium or ^{13}C-labeled isotopologue of the target analyte serves as the perfect internal standard, because the isotopologue and unlabeled (target) compound are nearly identical (i.e., chemical and physical properties), with the exception of their mass spectra. As a result of the similarity between a labeled isotopologue and target analyte, quantitative analyses performed by SIDA are highly accurate and precise. However, for some of the compounds in Table 6, a deuterium

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or ^{13}C-labeled isotopologue was not available. In such cases, an internal standard compound with a similar structure was used. For example, cyclotene (**36**) and dihydromaltol (**37**) were quantitated using [$^{2}H_3$]-maltol (**I-37**) as the internal standard, and 2,3-butanedione (**5**) was quantitated against [$^{2}H_2$]-3-methybutanal (**I-4**) as the internal standard.

OAVs. OAVs were calculated for 49 odorants based on the quantitation results and published odor detection thresholds in water (Table 6). Overall, rotundone (**50**) had the highest OAV. Other potent aroma compounds with relatively high OAVs (>50) in roasted chicory were sotolon (**49**), 3-methylbutanal (**4**), 2-methylpropanal (**2**), (*E*)-*β*-damascenone (**35**), dihydromaltol (**37**), and 1-octen-3-one (**11**).

Aroma Chemistry of Roasted Chicory Coffee. *Rotundone.* On the basis of the results of this study, rotundone [5-isopropenyl-3,8-dimethyl-3,4,5,6,7,8-hexahydro-1(2*H*)-azulenone] with its special aromatic, woody and peppery aroma note is believed to be responsible for much of the unique and distinctive flavor of chicory coffee. With its low odor detection threshold (8 ng/L in water), only a small amount of rotundone may be required to make chicory coffee distinguishable from coffee. To our knowledge, rotundone has never been reported in coffee. The incense- or sandalwood-smelling compound was first reported in (agarwood) essential oil of *Cyperus rotundus*.[38] It is not usually identified as a food odorant and only occasionally as a fragrance component. As mentioned earlier, it is found in agarwood oil, a rare and expensive oil extracted from the heartwood of a mold-infected tree, and used in perfume formulations. Most recently, it was noted as a potent odorant in grapes and has also been found in other products, including white pepper, black pepper, wine, marjoram, nut grass, geranium, rosemary, saltbush, basil, thyme, and oregano.[40] Rotundone concentrations can range from 0.15 to 2025 μg/kg, and the highest concentration was found in white pepper (2025 μg/kg).[39]

The precursor of rotundone may be *α*-guaiene, which is a sesquiterpene hydrocarbon found in various plants. Rotundone is generally believed to be formed by aerial oxidation of *α*-guaiene.[40] The formation of rotundone in processed plant products, such as dried herbs and spices, may be the result of the air interaction rather than rotundone being a product of enzymatically controlled processes or biosynthesis. Furthermore, this autoxidation sequence is enhanced at higher temperatures and may have significance when considering the amount of rotundone that may be formed during the cooking, heating, or drying of foodstuffs.[40]

Threshold of Rotundone in a Coffee Brew Matrix and Potential Sensory Impact. Wood et al. reported that the odor detection threshold of rotundone is 8 ng/L [parts per trillion (pptr)] in water and 16 ng/L (pptr) in red wine.[31] In the present study, the threshold of rotundone was determined in a coffee brew matrix. In a complex coffee brew matrix, the overall group threshold was 2.27 μg/L (ppm) for 26 panelists. Using this threshold value, the new calculated OAV based on the quantitative results in Table 6 would be 1.86. For the group of panelists (n = 6) sensitive to rotundone, the threshold was 0.62 ppm (OAV = 6.81). For moderately sensitive panelists (n = 11), the threshold was 2.67 ppm (OAV = 1.58). For insensitive panelists (n = 9), the threshold of rotundone was 16.95 ppm (OAV = 0.25). Among the initial 30 panelists recruited for threshold determination, 4 were unresponsive or anosmic to the smell of rotundone. Consequently, results for the 4 anosmic panelists were omitted from the threshold calculations. In a previous study, approximately 20% of "experienced" sensory panelists could not detect rotundone.[39]

The average threshold result of 2.27 ppm in the coffee brew matrix is about 1000-fold higher than the threshold in water (8 pptr).[35] There are at least two possible reasons why rotundone is harder to detect in a roasted coffee brew matrix. First, matrix components and/or other volatile constituents may have interacted or reacted with rotundone (e.g., flavor binding). Second, the strong aroma of the coffee brew matrix may mask the odor of rotundone.

An additional sensory study was conducted to determine if spiking of a regular (instant) coffee matrix with a suprathreshold level of rotundone (34 ppm, equal to approximately 2-fold higher than the highest group threshold determined above) would cause panelists to rate the perceived aroma of the rotundone-spiked coffee as being more chicory-like compared to an unspiked regular coffee. The panel was able to distinguish among all three samples ($p \leq 0.05$), which consisted of chicory coffee, rotundone-spiked coffee, and regular coffee. With respect to their similarity to the control (chicory coffee), the chicory coffee (control sample, same as the reference) received a similarity score (average ± SD) of 8.65 ± 1.2, while the rotundone coffee and regular coffee received scores of 5.28 ± 1.49 and 2.77 ± 1.24. These results demonstrate that rotundone is an important component of chicory "coffee" aroma.

Caramel and Sweet Aromatic Compounds. Caramel- and sweet-smelling odorants contributed greatly to the characteristic aroma of roasted chicory. Within this group, the compounds cyclotene, maltol, dihydromaltol, HDMF, and sotolon are known to be formed via the Maillard reaction. Maltol and dihydromaltol are reported for the first time in roasted chicory and were successfully quantitated. Dihydromaltol has caramel and sweet odor and has a much lower odor detection threshold than maltol; therefore, it readily contributes to a caramel and sweet aromatic odor when present compared to maltol. Dihydromaltol was first reported as a food flavor in barley malt.[41] Dihydromaltol is formed during thermal treatment (e.g., roasting) by degradation of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-3-one, which is a relatively stable degradation product of hexoses.[42]

Lipid-Derived Compounds. Many volatiles derived from lipid oxidation/degradation were detected in roasted chicory. The compounds 1-octen-3-ol, 1-octen-3-one, and (*Z*)-1,5-octadien-3-one have metallic and mushroom-like notes and might be undesirable in chicory. Other lipid oxidation volatiles identified in this study include hexanal, octanal, nonanal, decanal, (*E*)-2-nonenal and (*Z*)-2-nonenal, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4-nonadienal, and (*E*,*E*)-2,4-decadienal. For example, (*E*)-2-nonenal and 1-octen-3-one can be formed from linoleic acid; (*E*)-2-decenal can be formed from oleic acid; and octanal can be formed from either oleic or linoleic acid.

The compound *trans*-4,5-epoxy-(*E*)-2-decenal can be formed by thermal degradation of fatty acids.[43] Certain aldehydes can result in formation of new aroma compounds, such as (*Z*)-4-heptenal by retro-aldol degradation of (*E*,*Z*)-2,6-nonadienal and (*E*)-2-octenal by retro-aldol degradation of (*E*,*E*)-2,4-decadienal.[44]

Malty, Nutty, and Floral Compounds. The nutty- and earthy-smelling odorant, 2-ethyl-3,5-dimethylpyrazine, and other pyrazines identified in this study can be formed via the Maillard reaction.[45] The Strecker degradation (part of the Maillard reaction) is responsible for the formation of a number

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of malty- and floral-smelling odorants, such as 2-methypropanal (from valine), 2- and 3-methylbutanal (from isoleucine and leucine, respectively), and phenylacetaldehyde (from phenylalanine). The Strecker degradation of some amino acids during roasting of chicory may be important in the formation of the typical aroma of roasted chicory.

Carotenoids are precusors of norisoprenoids. Norisoprenoids could be formed by direct degradation of carotenoids, such as β-carotene and lutein, which are precursors to (*E*)-β-damascenone and β-ionone.[46]

Phenols and Guaiacols. Some important aroma compounds can be derived from pyrolysis of lignin during heating.[47] These compounds share many of the same functional groups and an aromatic structure, including guaiacol, eugenol, isoeugenols, 4-vinylguaiacol, and vanillin, which may come from coniferyl alcohol. The decomposition of sinapyl alcohol can lead to the formation of *p*- and *m*-cresol. Vanillin may be formed from eugenol by biosynthesis, e.g., by biodegradation of ferulic acid via the β-oxidative pathway, or thermal degradation.

In conclusion, this study is the first to report a comprehensive and quantitative listing of the predominant odorants in roasted chicory brew and is the first to indicate the existence and potential importance of rotundone, an odorant possessing a distinctive "aromatic woody and chicory-like" note. In addition, many other odorants were identified for the first time in chicory, including the caramel and sweet aromatic compounds sotolon and dihydromaltol as well as malty-smelling Strecker aldehydes. It is suggested that recombination and omission sensory studies be conducted to fully determine the importance of rotundone and other potent odorants identified in this study to the characteristic aroma of chicory "coffee" brew.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.9b00776.

Synthesis, analytical methods, and working solutions and spike volumes used in SIDA−SPME−GC−MS of roasted chicory brews (Table S1) (PDF)

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Notes

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