

Character Impact Odorants of Fennel Fruits and Fennel Tea

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The flavor of fennel fruits and fennel tea was examined by aroma extract dilution analysis of the respective dichloromethane extracts. In both fennel fruits and tea, *trans*-anethole, anisaldehyde, and *trans*-4,5-epoxy-2(*E*)-decenal showed high flavor dilution (FD) factors followed by fenchone, 1,8-cineole, (*R*)- α -pinene, estragole, and β -myrcene. On the basis of these results, the odorants showing higher FD factors were quantified in tea as well as in fruits, and odor activity values (OAV) in tea were calculated by dividing the concentration of the compound by its recognition threshold in water. The highest OAV was found for *trans*-anethole, followed by estragole, fenchone, 1,8-cineole, (*R*)- α -pinene, β -myrcene, and anisaldehyde. From a comparison of the concentrations of odorants in fruits and tea, *trans*-anethole and estragole showed similar extraction rates of approximately 10–15%, whereas the extraction rates for (*R*)- α -pinene, β -myrcene, and limonene were below 2%. In contrast to this, fenchone, camphor, linalool, and carvone showed higher extraction rates (26–50%), whereas the high apparent extraction rates of anisalcohol (393%) and vanilline (480%) were attributed to the formation from precursors. Sensory studies of aqueous models containing odorants in the amounts quantified in fennel teas revealed high similarity of the models with the tea and proved that all impact odorants had been identified in their correct concentrations. Further sensory experiments showed that estragole had no odor impact on the overall flavor of fennel tea, and, therefore, a reduction of estragole in fennel products would have no negative impact on their sensoric quality. In contrast to this, *trans*-anethole and fenchone were found to be character impact compounds of fennel.

KEYWORDS: Aroma extract dilution analysis; estragole; fennel; fennel tea; odor activity value

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.) is an aromatic plant belonging to the family Apiaceae. Bulbs of some fennel varieties are consumed as a vegetable, whereas the fruits are used to flavor bread and liquors. Moreover, the fruits serve as a remedy against catarrh of the respiratory tract and gastrointestinal disorders. Therefore, fennel infusions are the classical tea for nursing babies to prevent flatulence and spasms. The pharmacological effects of fennel fruits generally are attributed to its essential oil, which is composed mainly of *trans*-anethole, estragole, and fenchone together with minor compounds such as α - or β -pinene, limonene, and *p*-cymene (1). Of the main compounds, in particular estragol has become a cause for concern, as the structurally similar methyleugenol has been recently found to be a potent carcinogen (2). Although until recently it seemed to be widely accepted that carcinogens possess no minimum effective dose, there are now indications that there might be a general threshold for carcinogenic compounds of about 10^{20} molecules/kg of body weight (bw)/day, which is equivalent to 0.17 mmol or 25 mg/kg of bw/day (3). Far from being accepted, this new hypothesis led in the

European Union (EU) to discussions of a new legal limit for estragole of 10 mg/kg in nonalcoholic beverages (4). Moreover, the variety of estragole contents in fennel teas ranging from 0.11 to 4.6 mg/L (5) has also to be considered in the assessment of whether fennel is a potent hazard to consumers' health. As the essential oil of fennel resembles in its sensoric properties and composition anise, *trans*-anethole is often considered to be the main contributor to fennel flavor (1, 6). However, the impact of estragole and of the other essential oil constituents on the aroma is widely unknown. Likewise, there is only limited information on the flavor impact compounds of fennel tea. Although Bilia et al. (6) examined several commercial fennel teas by gas chromatography–mass spectrometry (GC-MS), Fehr (7) studied the transfer of odorants from the fruits into infusions, and Diaz-Maroto et al. (8) compared different extraction methods by GC–olfactometry, neither unequivocal sensory studies nor exact quantitations have been performed.

A straightforward approach to identify the main contributors to the odor of foods is the following, well-documented procedure. First, screening for potent odorants by aroma extract dilution analysis (AEDA), second, calculating odor activity values, and third, sensory studies of flavor models are performed. Numerous foods such as coffee (9, 10) and cheese (11) and also spices such as dill (12, 13) or pepper (14, 15) have

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already been successfully analyzed by this procedure, demonstrating its superiority over other methods.

The aim of the present study was, therefore, to apply this methodology also to fennel fruits and fennel infusions and to clarify the impact of potent odorants on fennel flavor.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially from the sources given in parentheses: (*R*)- α -pinene, (*S*)- α -pinene, β -myrcene, (*R*)-limonene, (*S*)-limonene, 1,8-cineole, fenchone, 2-isopropyl-3-methoxy-pyrazine, (*R/S*)-linalool (*R*)-campher, estragole, carvone, *trans*-anethole, β -ionone, methylisoeugenol, sotolone, myristicin, anisalcohol, vanillin, 1,2,4-trimethoxybenzene, and menthone (Aldrich, Steinheim, Germany); anisaldehyde, eugenol, and (*S*)-campher (Merck, Darmstadt, Germany); anisketone, 4-methoxyphenol (Acros Organics, Geel, Belgium); guaiacol (Serva, Heidelberg, Germany); menthene, (*R*)-linalool, and 1,4-cineole (Fluka, Neu Ulm, Germany); 4-propyl-methoxybenzene (Lancaster, Mülheim am Main, Germany). *trans*-4,5-Epoxy-2(*E*)-decenal was synthesized according to the literature (16).

Isolation of Volatiles in Fennel Fruits and Fennel Tea. Ripe fruits of bitter fennel from Hungary (*Foeniculum vulgare* ssp. *vulgare* var. *vulgare*, Martin Bauer, Vestenbergsgreuth, Germany) were broken by a squeezer (Korn Quetsche, Eschenfelder, Hauenstein, Germany). Immediately afterward, broken fruits (2.5 g) were extracted with 100 mL of freshly distilled dichloromethane by stirring for 1 h. After filtration, the extract was dried over anhydrous sodium sulfate, and nonvolatile compounds were removed by solvent-assisted flavor evaporation (SAFE) (17) at 40 °C. The distillates were concentrated at 47 °C to 400 μ L using a Vigreux column (50 \times 1 cm).

Fennel tea was prepared by extracting 2.5 g of freshly broken fennel fruits with 150 mL of boiling water for 10 min and subsequently filtering the extract. The hot aqueous infusion was cooled to room temperature and extracted with 2 \times 50 mL of dichloromethane in a separation funnel. The organic phase was dried over anhydrous sodium sulfate and concentrated at 47 °C to 400 μ L using a Vigreux column (50 \times 1 cm).

All extracts were analyzed by high-resolution gas chromatography–olfactometry (HRGC-O) and aroma extract dilution analysis (AEDA) directly after isolation and stored at –20 °C overnight.

HRGC-O and High-Resolution Gas Chromatography–Mass Spectrometry (HRGC-MS). HRGC-O was performed by means of a gas chromatograph (Trace GC, 2000 series, Thermo Finnigan, Bremen, Germany) using the following capillaries: DB-FFAP (30 m \times 0.32 mm i.d., film thickness = 0.25 μ m, J&W Scientific, Fisons, Germany), DB-5 (25 m \times 0.32 mm i.d., film thickness = 0.25 μ m, J&W Scientific), and DB-1701 (30 m \times 0.25 mm i.d., film thickness = 0.25 μ m, J&W Scientific). The samples were injected on-column at 40 °C. After 1 min, the oven temperature was raised by 6 °C/min to the final temperature of 230 °C. Helium was used as the carrier gas, and the flow rate was set to 2 mL/min. The gas flow was split 1+1 (v/v) at the end of the capillary using a Y-shaped glass splitter (Chrompack/Varian, Darmstadt, Germany) to a flame ionization detector (FID) and a sniffing port made of alumina, both held at 250 °C.

HRGC-MS for identification of flavor compounds was performed by means of a gas chromatograph 5890 series II (Hewlett-Packard, Waldbronn, Germany) connected to a sector field mass spectrometer type MAT 95 S (Finnigan, Bremen, Germany). Electron impact ionization (EI) was performed at 70 eV and chemical ionization (CI) at 115 eV using isobutane as reactant gas. The capillaries and temperature program were the same as used for HRGC-O.

AEDA. Flavor dilution (FD) factors were determined by AEDA (11). The extracts (400 μ L) were diluted stepwise with dichloromethane (1:1 v/v) and 1 μ L of the concentrate (FD 1), and each diluted sample was analyzed independently by three persons by HRGC-O using capillary DB-FFAP (30 m \times 0.32 mm i.d., film thickness = 0.25 μ m, J&W Scientific).

Identification of Odorants. Odorants in fennel fruits and fennel tea were identified by retention indices (RI), mass spectra obtained from HRGC-MS, odor quality, and threshold perceived by HRGC-O

compared to reference substances. RI were calculated from the retention times of *n*-alkanes by linear interpolation.

Quantification of Phenylpropanes and Terpenoids. 4-Propyl-methoxybenzene, 4-methoxyphenol, and 1,2,4-trimethoxybenzene were used as internal standards (IS) for quantification of phenylpropanes. Terpenoids were quantified by using menthene [1-methyl-4-(1-methylethyl)cyclohexen], menthone [5-methyl-2-(1-methylethyl)cyclohexanone], and 1,4-cineole [1-methyl-4-(1-methylethyl)-7-oxabicyclo-[2.2.1]heptane] as IS. In the first experiments, the samples were analyzed for the IS to prove their absence in fennel. For quantification of fennel tea, solutions of the IS in dichloromethane were added to the cooled aqueous extract and stirred for 1 h before extraction with dichloromethane. Fennel fruits were quantified by adding the internal standards to the dichloromethane extract and stirring for 1 h followed by SAFE distillation. The extraction procedure was performed as detailed before, and two or more replicate analyses were carried out. The samples were analyzed by a different HRGC-MS system, using a gas chromatograph (CP 3800, Varian, Darmstadt, Germany) coupled with an ion trap detector (Saturn 2000, Varian, Darmstadt, Germany) running in the CI mode with methanol as the reactant gas. A DB-FFAP (30 m \times 0.32 mm i.d., film thickness = 0.25 μ m, J&W Scientific) capillary was used for analysis. The samples were injected on-column at 40 °C. After 1 min, the oven temperature was raised by 6 °C/min to the final temperature of 230 °C. Helium was used as the carrier gas, and the flow rate was set to 2 mL/min. Flavor compounds were quantified by relative area counts of analyte and IS using a response factor detailed in Table 1, which was determined by analyzing definite mixtures of analyte and IS as described previously (18).

Quantification of Minor Compounds by Stable Isotope Dilution Assays (SIDA) Using Two-Dimensional HRGC-MS. Minor flavor compounds were quantified by SIDA (19). The labeled compounds were synthesized according to the literature given in parentheses: [²H₃]-guaiacol (20), [²H₃]-vanillin (21), [¹³C₂]-3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (22), [²H₄]-*trans*-4,5-epoxy-2(*E*)-decenal (16), [²H₃]- β -ionone (23), [²H₃]-2-isopropyl-3-methoxy-pyrazine (24), [²H₂]-myristicin (25), [²H₂₋₄]-4-propyl-2-methoxyphenol (26), and [²H₂]-linalool (18). The preparation of fennel fruit extracts and fennel tea extracts for quantification was performed as detailed before. The extracts were analyzed by two-dimensional HRGC-MS. In the first dimension, flavor compounds were separated by capillary DB-FFAP (30 m \times 0.32 mm i.d., film thickness = 0.25 μ m, J&W Scientific) after on-column injection in a gas chromatograph (Trace GC, 2000 series, Thermo Finnigan). The samples were injected on-column at 40 °C. After 1 min, the oven temperature was raised by 6 °C/min to the final temperature of 230 °C. Helium was used as the carrier gas, and the flow rate was set to 2 mL/min. The effluent was quantitatively transferred to the second dimension by a moving column stream switching system (MCSS, Thermo Finnigan). As the second dimension a DB-1701 column (30 m \times 0.32 mm i.d., film thickness = 0.25 μ m, J&W Scientific) or DB-5 column (30 m \times 0.32 mm i.d., film thickness = 0.25 μ m, J&W Scientific) was used in a gas chromatograph (CP 3800, Varian, Darmstadt, Germany) coupled with an ion trap detector (Saturn 2000, Varian) running in the CI mode with methanol as reactant gas. The following temperature program in the second dimension was used: 40 °C was held for 1 min, then the oven temperature was raised by 8 °C/min to the final temperature of 230 °C. Flavor compounds were quantified by relative area counts of analyte and IS in their respective mass traces using the response factors detailed in Table 1.

Determination of the Enantiomeric Distribution of Odorants in Fennel Tea Using Two-Dimensional HRGC-MS. For chiral odorants such as limonene, α -pinene, linalool, and camphor the enantiomeric distribution was determined by chiral analysis. The preparation of fennel tea extracts and the analysis by two-dimensional HRGC-MS was performed as detailed before. As the second dimension a chiral capillary BGB 176 (30 m \times 0.25 mm i.d., film thickness = 0.25 μ m, BGB Analytik, Rothenfluh, Switzerland) was used coupled with an ion trap detector (Saturn 2000, Varian) running in the CI mode with methanol as reactant gas. The temperature program in the second dimension started at 40 °C held for 1 min, and then the oven temperature was raised by 6 °C/min to 80 °C, followed by a heating rate of 3 °C/min until 150 °C. Then, the temperature was raised by 20 °C/min to 230

Table 1. Fragment Ions and Response Factors Used for Quantification of Odorants in Fennel Fruits and Fennel Tea

analyte	internal standard	m/z used for quantification in CI mode		response factor
		analyte	internal standard	
(R)- α -pinene	menthene	137	139	0.30
β -myrcene	menthene	137	139	0.56
(R/S)-limonene	menthene	137	139	0.29
1,8-cineole	1,4-cineole	137	137	1.05
fenchone	menthone	153	155	0.87
2-isopropyl-3-methoxypyrazine	[² H ₃]-2-isopropyl-3-methoxypyrazine	153	156	0.83
(S)-camphor	menthone	153	155	0.96
(S)-linalool	[² H ₂]-linalool	137	139	0.90
estragole	4-propylmethoxybenzene	149	151	0.72
carvone	menthone	151	155	1.40
cis-anethole ^a	4-propylmethoxybenzene	149	151	1.16
trans-anethole	4-propylmethoxybenzene	149	151	1.16
guaiacol	[² H ₃]-guaiacol	125	128	0.89
β -ionone	[² H ₃]- β -ionone	193	196	0.77
trans-4,5-epoxy-2(E)-decenal	[² H ₄]-trans-4,5-epoxy-2(E)-decenal	139	143	1.01
anisaldehyde	1,2,4-trimethoxybenzene	137	169	0.70
aniseton	1,2,4-trimethoxybenzene	165	169	0.49
eugenol	[² H ₂ -4]-4-propyl-2-methoxyphenol	165	169–171	1.01
methylisoeugenol	4-propylmethoxybenzene	179	151	0.93
sotolone	[¹³ C ₂]-3-hydroxy-4,5-dimethyl-2(5H)-furanone	129	131	1.11
myristicin	[² H ₂]-myristicin	193	195	0.91
anisalcohol	4-methoxyphenol	121	125	1.14
vanillin	[² H ₃]-vanillin	153	156	1.08

^a The response factor determined for *trans*-anethole was used.

°C. The enantiomeric distribution was determined by comparison of reference compounds with the fennel tea extract and calculation of the enantiomers' peak areas in the mass traces given in **Table 1**.

Odor Recognition Threshold Determination and Triangle Tests.

The odor recognition thresholds of odorants in water were determined by triangle tests (27). A stock solution of the flavor compound dissolved in demineralized water was diluted stepwise (1:3 v/v), and each dilution was compared with demineralized water in a triangle test. This sensory testing is performed by presenting three samples, two of which are identical and have to be correctly differentiated from the different, third sample.

The odor activity of estragole and fenchone, respectively, in solutions containing *trans*-anethole was determined by triangle tests. A *trans*-anethole solution (10 mg/L water) was combined with various amounts of estragole (0.4, 4, 5, 8 mg/L) and fenchone (1, 2, 3, 4, 5, 8 mg/L), respectively, to give a total concentration of flavor compounds of 10 mg/L water. These mixtures were compared with a *trans*-anethole solution (10 mg/L water) by triangle tests. In addition, an estragole solution (10 mg/L of water) or fenchone solution (10 mg/L of water) was compared with a *trans*-anethole solution (10 mg/L of water). Significance levels of difference were determined according to the German official collection of test methods (27).

Sensory Quantitative Descriptive Analysis (QDA) of Aqueous Sensory Models of Fennel Tea. Fennel tea was evaluated by sensory QDA in comparison to an aqueous sensory model. The model contained the odorants with OAVs exceeding 1 in the amounts quantified in fennel tea. Ten odor attributes were assessed by a panel consisting of 18 trained persons on a scale from 0 (not detectable) to 3 (high odor intensity). For the 10 odor attributes reference solutions of the compounds given in parentheses were presented: anise-like (*trans*-anethole), eucalyptus-like (1,8-cineole), almond-like (anisaldehyde), fir tree-like [(R)- α -pinene], moldy (fenchone), geranium-like (β -myrcene), citrus-like [(S)-linalool], vanilla-like (vanillin), seasoning-like (sotolone), and metallic [*trans*-4,5-epoxy-2(E)-decenal].

RESULTS AND DISCUSSION

AEDA of Fennel Fruits and Fennel Tea. Fennel fruits and fennel tea both possess a characteristic odor. To obtain representative material for our investigations, several commercial fennel types were evaluated sensorially, and seeds of a bitter

fennel variety (*F. vulgare* ssp. *vulgare* var. *vulgare*) were chosen as a typical sample. For the analysis of the impact odorants, solvent extraction proved to be suitable as the first step. The odor of dichloromethane extracts resembled that of fennel fruits and tea and, therefore, these were used for further examination of odorants. As fruit extracts contained nonvolatile compounds, the volatiles were isolated by SAFE, which is known to yield high recoveries of volatiles along with a low thermal impact on the sample (17).

Tea was prepared from the broken fruits of bitter fennel by water extraction at 100 °C for 10 min according to a general procedure reported by Bilia et al. (6). After cooling, the tea was extracted with dichloromethane, and the volatiles could be analyzed directly by GC without preceding distillation. The principle odor-active compounds in both extracts were evaluated by AEDA, which gave the results shown in **Table 2**. Identification of the compounds was achieved on the basis of odor quality, odor intensity, retention index, and mass spectra in comparison with reference substances. For chiral odorants, the assignment of FD factors to the respective enantiomer was based on the results of a chiral analysis given in **Table 3**. In both extracts the anise-like smelling *trans*-anethole was the most odor-active compound with FD factors of 8192 and 1024 in fennel fruits and tea, respectively. Further odorants showing high FD factors in both extracts were the anise and almond-like smelling anisaldehyde followed by the metallic smelling *trans*-4,5-epoxy-2(E)-decenal, whereas the fir tree-like smelling (R)- α -pinene revealed a very high FD factor only in fennel fruits. Similarly, further nonoxygenated terpenoids such as the geranium-like smelling β -myrcene and the citrus-like smelling limonene appear to have a low odor impact in fennel tea, as they were not detectable by HRGC-O in the tea extract. The anise-like and herbaceous smelling estragole was identified in both extracts showing average FD factors, so a high flavor activity cannot be confirmed doubtlessly by AEDA. In fennel tea two herbaceous and sweet smelling flavor compounds (unknowns 3 and 7) with high FD factors could not be identified, although mass

Table 2. Odor-Active Compounds in Fennel Tea and Fennel Fruits

odorant ^a	systematic name or mass spectral information	odor quality ^b	earlier identified in fennel ^c	RI ^d on			FD factor ^e	
				DB-FFAP	DB-1701	DB-5	tea	fruit
(R)- α -pinene	2,6,6-trimethylbicyclo[3.1.1]hept-2-ene	fir tree-like	29	1016	946	935	128	2048
β -myrcene	7-methyl-3-methylene 1-6-octadiene	geranium-like	30	1161	1021	990	nd ^f	128
(R/S)-limonene	1-methyl-4-isopropenyl-1-cyclohexene	citrus-like	29	1191	1062	1026	nd	1
1,8-cineole	1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane	eucalyptus-like	31	1195	1079	1030	128	512
fenchone	1,3,3-trimethylbicyclo[2.2.1]heptan-2-one	eucalyptus-like, moldy	29	1389	1184	1086	128	512
2-isopropyl-3-methoxypyrazine		green bell pepper-like		1435	1147	1104	32	64
(S)-camphor	1,7,7-trimethylbicyclo[2.2.1]-2-heptanone	campher-like	32	1509	1257	1142	nd	1
(S)-linalool	3,7-dimethyl-1,6-octadien-3-ol	citrus-like	32	1526	1200	1100	16	64
estragole	4-allylmethoxybenzene	anise-like, herbaceous	29	1671	1289	1196	32	128
carvone	2-methyl-5-isopropenyl-2-cyclohexenone	caraway-like	6	1735	1372	1244	nd	1
cis-anethole	1-methoxy-4-cis-propenylbenzene	anise-like, sweet	30	1759	1352	1258	16	128
trans-anethole	1-methoxy-4-trans-propenylbenzene	anise-like	30	1830	1395	1283	1024	8192
guaiaicol	2-methoxyphenol	smoky		1875	— ^g	—	32	nd
β -ionone	4-(2,6,6-trimethylcyclohex-1-enyl)-3-buten-2-one	flowery		1947	—	1500	1	64
trans-4,5-epoxy-2(E)-decal		metallic		2009	1537	1378	512	512
anisaldehyde	4-methoxybenzaldehyde	anise-like, almond-like	29	2038	1433	1252	512	4096
unknown 1		smoky, seasoning-like		2158	—	—	nd	64
anisketone	1-(4-methoxyphenyl)-2-propanone	herbaceous, sweet	31	2170	1572	1384	4	16
eugenol	4-allyl-2-methoxyphenol	clove-like	1	2179	1510	1356	128	nd
methylisoeugenol	1,2-dimethoxy-4-trans-propenylbenzene	smoky		2189	1647	1495	2	8
sotolone	3-hydroxy-4,5-dimethyl-2(5H)-furanone	seasoning-like		2192	—	1116	32	32
unknown 2	Cl: ^h m/z 150 (M ⁺); El: ⁱ m/z 135, 150, 91, 136, 107, 115	honey-like, flowery		2236	1629	1315	16	16
unknown 3	Cl: ^h m/z 164 (M ⁺); El: ⁱ m/z 135, 164	herbaceous, sweet		2248	1517	—	512	128
myristicin	3-methoxy-4,5-methylenedioxyallylbenzene	herbaceous, carrot-like	33	2272	1665	1520	64	128
anisalcohol	4-methoxybenzyl alcohol	herbaceous, sweet		2290	1495	—	64	4
unknown 4	Cl: ^h m/z 178 (M ⁺); El: ⁱ m/z 135, 77, 92, 107	herbaceous, flowery		2320	1665	—	64	128
unknown 5		herbaceous, sweet		2371	—	—	nd	16
unknown 6	Cl: ^h m/z 208 (M ⁺); El: ⁱ m/z 121, 148, 147, 208, 117, 133, 91, 77, 105, 135	seasoning-like		2380	1644	1448	128	512
vanillin	4-hydroxy-3-methoxybenzaldehyde	vanilla-like		2584	1653	1400	64	16
unknown 7	Cl: ^h m/z 180 (M ⁺); El: ⁱ m/z 135, 77, 137, 136, 92, 107	herbaceous, sweet		2593	1781	1554	512	32

^a The compound was identified by comparison of retention index (RI) on the stationary phases given in the table, mass spectra obtained by MS (EI) and odor quality and intensity perceived at the sniffing port with reference substances. *cis*-Anethole was tentatively identified on the basis of mass spectra and RI in comparison with the literature. ^b Odor quality perceived at the sniffing port. ^c Reported earlier as volatile compound in fennel in the literature cited. ^d Retention index. ^e Flavor dilution factor obtained by aroma extract dilution analysis (AEDA) on capillary DB-FFAP. ^f Not detectable. ^g Not determined. ^h Molecular ion. ⁱ Fragment ions measured in EI modus in decreasing order of relative abundance.

Table 3. Enantiomeric Distribution of Odorants in Fennel Tea Determined by Chiral Analysis

odorant	R-isomer (%)	S-isomer (%)
α -pinene	100	0
linalool	0	100
limonene	64	36
camphor	0	100

spectra in CI and EI were obtained. However, the spectra did not allow an unequivocal structure assignment, and the amounts present in the extracts were too low for NMR experiments. In the extract of fennel fruits both unknowns had a lower aroma impact. Moreover, the smoky smelling guaiacol and the clove-like smelling eugenol were detected by HRGC-O only in the extract of fennel tea. In contrast to this, some aroma compounds showed detectable FD factors only in fennel fruits such as β -myrcene, limonene, camphor, carvone, and two unknown compounds (no. 1 and 5). These compounds were also detected by HRGC-MS in fennel tea but not by HRGC-O.

The literature survey indicates that some flavor compounds were identified for the first time in fennel fruits and fennel tea. The green bell pepper-like smelling 2-isopropyl-3-methoxypyrazine, the smoky smelling guaiacol, the flowery smelling β -ionone, the metallic smelling *trans*-4,5-epoxy-2(E)-decal, the smoky smelling methylisoeugenol, the seasoning-like smelling sotolone, the herbaceous, sweet smelling anisalcohol, and the vanilla-like smelling vanillin have not been described as

flavor compounds in fennel before. However, guaiacol, β -ionone, and vanillin have been identified as flavor compounds in anise (28), which also belongs to the family Apiaceae. The latter authors also described the unknown substance 7 as an unidentified flavor compound in anise by presenting the same mass spectra. The other substances given in **Table 2** have been found in fennel before, but their flavor contribution has not been examined yet.

Odor Activity Values (OAVs) in Fennel Tea. The determination of FD factors gives a first impression of potent odor-active compounds. Nevertheless, the flavor contribution of these odorants can be better estimated by calculating their OAVs. These values are defined by dividing the concentrations of the compound by its recognition threshold in a suitable matrix. For OAVs > 1, an impact of the respective compound on the overall flavor can be assumed. The recognition thresholds in water were determined by triangle tests and are presented in **Table 4** along with some thresholds already reported in the literature. The quantification of flavor compounds in fennel tea was performed by the internal standard method. Considering their structural similarity, 4-propylmethoxybenzene, 4-methoxyphenol, and 1,2,4-trimethoxybenzene were chosen as internal standards (IS) for phenylpropanes, whereas the terpenoids were quantified by menthene, menthone, and 1,4-cineole as IS. In contrast to this, the minor compounds were quantified by SIDA using stable isotopomers of the analytes and separation by two-dimensional HRGC. The results of the quantifications in fennel tea and the

Table 4. Concentrations and Odor-Activity Values (OAV) of Odorants in Fennel Tea

odorant	concn ^a ($\mu\text{g/L}$)	<i>n</i> ^b	RSD ^c (%)	threshold ^d ($\mu\text{g/L}$)	OAV ^e
(<i>R</i>)- α -pinene	140	7	19	4.6	30
β -myrcene	140	7	8	4.9	29
(<i>R/S</i>)-limonene	190	7	16	34 ^f	6
1,8-cineole	160	6	9	4.6	35
fenchone	28000	6	8	440	64
(<i>S</i>)-camphor	760	7	6	1470	0.52
(<i>S</i>)-linalool	20	2	4	5 ^g	4
estragole	2200	4	2	16	138
carvone	160	10	10	20 ^g	8
<i>cis</i> -anethole	80	7	14	nd ^h	nd
<i>trans</i> -anethole	60000	10	11	73	822
guaiaicol	0.25	3	4	3 ^g	0.08
<i>trans</i> -4,5-epoxy-2(<i>E</i>)-decenal	0.20	2	4	0.12 ^g	2
anisaldehyde	630	4	5	47	13
anisetonone	60	10	11	1800	0.03
eugenol	0.18	7	11	2.5	0.07
methylisoeugenol	110	11	10	1600	0.07
sotolone	2.3	2	2	1.1	2
myristicin	110	8	6	88	1
anisalcohol	190	10	10	38000	0.005
vanillin	40	6	6	25 ^g	2

^a Concentration determined by internal standards and stable isotope dilution analysis (SIDA). ^b Number of replicate analyses. ^c Relative standard deviation. ^d Recognition threshold in water determined by triangle tests. ^e Odor-activity values were calculated by dividing the concentrations of the compound by its recognition threshold. ^f According to ref 34. ^g According to ref 35, the threshold of racemic linalool was used. ^h Not determined.

resulting OAVs are presented in **Table 4**. Regarding their concentrations, the most abundant odorants in fennel tea were *trans*-anethole, followed by fenchone and estragole. These results were in good accordance with the findings of Fehr (7), who found a likewise high concentration of 60 mg of *trans*-anethole in 1 L of fennel tea. Further odorants such as guaiaicol, *trans*-4,5-epoxy-2(*E*)-decenal, eugenol, and sotolone were found in only minute amounts, but were successfully quantified by SIDA. The resulting OAVs revealed *trans*-anethole as the most odor-active compound in fennel tea. Although fenchone was also present in high amounts in fennel tea, its OAV was much lower than that of *trans*-anethole due to the lower threshold of the latter. Moreover, the likewise highly abundant estragole showed a lower threshold than fenchone and therefore displayed the second highest OAV in fennel tea. For the terpenoids fenchone, β -myrcene, (*R*)- α -pinene, and 1,8-cineole, OAVs ranging between 29 and 64 were calculated, whereas most of the remaining odorants showed OAVs only marginally exceeding 1. The compounds with OAVs of <1 obviously do not contribute to the overall flavor of fennel tea.

Quantitation of Odorants in Fennel Fruits and Extraction Yield in Fennel Tea. The quantification of flavor compounds in fennel fruits was accomplished analogously to fennel tea. The contents are presented in **Table 5** together with the respective amounts extracted into fennel tea from 1 g of fruits. Calculated from these data, the rates of extraction are also given. In fennel fruits the major compound was also *trans*-anethole followed by fenchone. In contrast to fennel tea the nonoxygenated terpenoids α -pinene, β -myrcene, and limonene were also found in high amounts in the fruits. Obviously, their lower concentration in the tea was caused by a lower rate of extraction ranging from 0.5 to 2%. Estragole was also present in high concentrations in fennel fruits. Regarding its content in fennel tea, estragole was extracted into fennel tea to a similar extent as *trans*-anethole. Some oxygenated terpenoids such as fen-

Table 5. Concentrations of Odorants in Fennel Fruits and Rate of Extraction in Fennel Tea

odorant	concn in fruits ^a ($\mu\text{g/g}$)	<i>n</i> ^b	RSD ^c (%)	amount in tea extracted from 1 g of fruits ^d (μg)	rate of extraction ^e (%)
(<i>R</i>)- α -pinene	1800	2	8	8.4	0.5
β -myrcene	1150	4	2	8.4	1
(<i>R/S</i>)-limonene	690	4	3	11	2
1,8-cineole	170	4	2	9.6	6
fenchone	6500	7	5	1700	26
2-isopropyl-3-methoxy- pyrazine	0.007	2	10	nd ^f	nd
(<i>S</i>)-camphor	180	6	1	46	26
(<i>S</i>)-linalool	2.8	2	7	1.2	43
estragole	1080	3	12	132	12
carvone	19	6	7	9.6	50
<i>cis</i> -anethole	68	6	9	4.8	7
<i>trans</i> -anethole	23000	4	5	3600	16
guaiaicol	0.027	2	5	0.02	74
β -ionone	0.058	2	13	nd	nd
<i>trans</i> -4,5-epoxy- 2(<i>E</i>)-decenal	nd	nd	nd	0.012	nd
anisaldehyde	130	8	13	38	29
anisetonone	45	4	3	3.6	8
eugenol	0.067	2	14	0.01	16
methylisoeugenol	10	6	11	6.6	66
sotolone	0.15	3	4	0.14	94
myristicin	14	6	9	6.6	47
anisalcohol	2.8	2	7	11	393
vanillin	0.50	2	1	2.4	480

^a Concentration determined by internal standards and stable isotope dilution analysis (SIDA). ^b Number of replicate analyses. ^c Relative standard deviation. ^d 16.6 g of fruits used for preparation of 1 L of fennel tea. ^e Rates of extraction were calculated by dividing the concentrations of the compound in fennel tea by its concentration in fruits. ^f Not determined.

chone, camphor, linalool, and carvone showed even better extraction rates (26–50%). Interestingly, the extraction rates of anisalcohol and vanillin were around or exceeded 400%, which might be explained by their formation from precursors in the fruits. However, as the latter compounds showed only low OAVs (**Table 4**) in the tea, further studies to verify this assumption were not performed. The solubility of *trans*-anethole and fenchone in fennel tea has already been examined by Fehr (7). The latter author observed a better extraction rate of fenchone compared to that of *trans*-anethole, which is well in line with our results.

Sensory Studies of Models. To verify that all impact odorants have been identified, aqueous sensory models containing the odorants in the amounts quantified in fennel tea were evaluated in comparison to fennel tea. The sensory testing showed very similar odor profiles of the model and the tea (**Figure 1**). Although the model appeared to be slightly more citrus-like and slightly less anise-like than the tea, the differences were statistically not significant due to the relative standard deviation (RSD) of sensory ratings of approximately 10%.

The quantitations have revealed *trans*-anethole (OAV 822), estragole (OAV 138), and fenchone (OAV 64) to show the highest OAVs in tea, all three belonging also to the major compounds in fennel fruits. The anise-like odor quality of *trans*-anethole and estragole is quite similar, whereby estragole smells much more herbaceous and less sweet than *trans*-anethole (**Table 2**). In contrast, fenchone exhibits a significantly different, eucalyptus-like and moldy note. Because there is increasing emphasis on removing estragole from fennel tea, we first studied in triangle tests whether the absence of estragole in tea could be recognized sensorially. Therefore, an aqueous *trans*-anethole

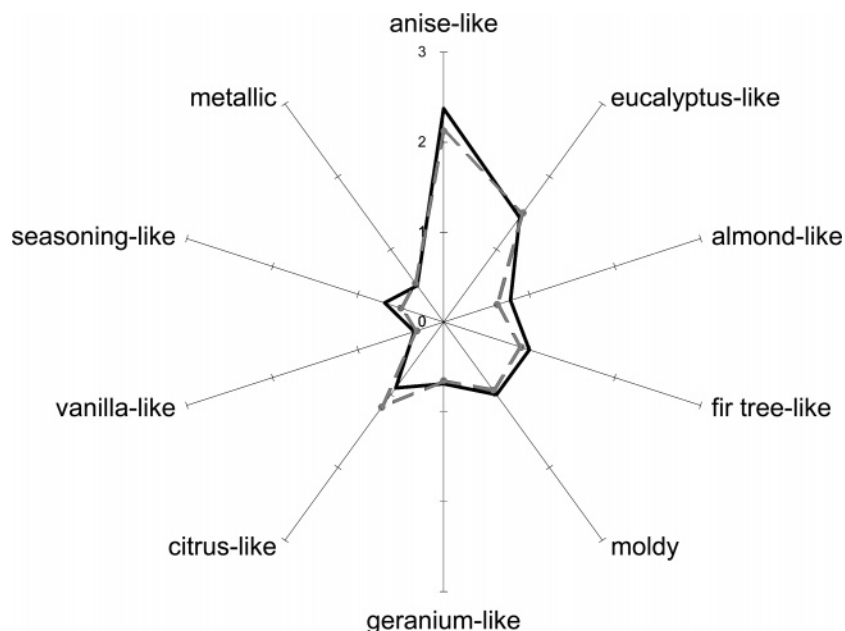


Figure 1. Sensory profiles of fennel tea (—) and its aqueous sensory model (---).

Table 6. Odor Activity of Estragole Compared to *trans*-Anethole Analyzed by Triangle Tests

% of estragole in <i>trans</i> -anethole solution ^a	positive results ^b	significance level ^c (%)
5	9/18	>5
40	9/18	>5
5	9/18	>5
80	10/18	5
100	14/18	0.1

^a A *trans*-anethole solution (sum of *trans*-anethole and estragole content = 10 mg/L) with percent of estragole was analyzed by triangle tests in comparison with *trans*-anethole solution (10 mg/L). ^b Number of panelists with positive results/total number of panelists. ^c Significance level according to ref 27.

solution with a similar concentration (10 mg/L) to that of fennel tea was used as the basis, and various amounts of estragole were added to give a total concentration of 10 mg/L for the sum of both odorants. These mixtures were analyzed by triangle tests, and the results are given in Table 6. The mixtures containing 5, 40, and 50% estragole could not be distinguished from pure *trans*-anethole at a significance level below 5%. Only the mixture of 80% estragole along with *trans*-anethole could be differentiated from *trans*-anethole solution by a significance level of 5%, which still meant only a slight difference between the samples. A significant differentiation was observed only when a pure estragole solution was compared to that of *trans*-anethole. Because the estragole concentration in fennel tea is only 3.4% of the *trans*-anethole content, it can be assumed that removal of estragole in fennel tea would not be recognized olfactorily. Analogously, it can be suggested that estragole does not have an impact on the flavor of fennel fruits as it is present only at a percentage of 4.7 compared to *trans*-anethole. To clarify the role of fenchone in combination with *trans*-anethole, we performed sensory experiments of binary anethole/fenchone mixtures analogously to anethole/estragole mixtures. The results of these studies given in Table 7 show that even 10% of fenchone can be detected sensorially at a significance level of 5%. As the fenchone content in fennel tea is about 50% of the anethole content, it contributes obviously to the flavor of fennel.

Conclusions. On the basis of AEDA, calculations of OAVs, and sensory studies, it can be concluded that estragole does not

Table 7. Odor Activity of Fenchone Compared to *trans*-Anethole Analyzed by Triangle Tests

% of fenchone in <i>trans</i> -anethole solution ^a	positive results ^b	significance level ^c (%)
10	10/18	5
20	12/18	1
30	14/18	0.1
40	14/18	0.1
50	17/18	0.1
80	16/18	0.1
100	18/18	0.1

^a A *trans*-anethole solution (sum of *trans*-anethole and fenchone content = 10 mg/L) with percent of fenchone was analyzed by triangle tests in comparison with *trans*-anethole solution (10 mg/L). ^b Number of panelists with positive results/total number of panelists. ^c Significance level according to ref 27.

have an impact on the flavor of fennel tea. This result for a number of reasons is also valid for other teas prepared from commercially available fennel. First, the estragole contents in fennel teas were reported to amount only to a maximum of 4.6 mg/L (5) compared to 2 mg/L in the tea under study. Second, fennel oil for pharmaceutical purposes has to contain not less than 60% *trans*-anethole, according to the European Pharmacopoeia (36), and, third, the essential oils of commercial fennel teas have been reported to contain at least 50% *trans*-anethole (6).

Therefore, even for a tea containing 4.6 mg/L estragole, the latter does not exceed the content of anethole and, by considering our sensory studies, does not play a role in the flavor of fennel teas.

In view of the public concern about possible hazards caused by estragole, a reduction of estragole in fennel tea, therefore, would have no negative effects on its flavor. Regarding the reduction of estragole, biotechnological methods, on the one hand, are not very promising as biosyntheses of anethole and estragole are closely linked and reduction of estragole would cause also a decrease of the impact compound anethole. On the other hand, physical processes such as rectification have been reported in the literature (37) and may open in-roads into the development of new tea products, if effective separation of estragole from anethole is achieved.

In contrast to that, fenchone has been demonstrated to play an important role in fennel tea flavor besides *trans*-anethole.

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