

# Comparison of the Most Odor-Active Compounds in Fresh and Dried Hop Cones (*Humulus lupulus* L. Variety Spalter Select) Based on GC–Olfactometry and Odor Dilution Techniques

Martin Steinhaus and Peter Schieberle\*

Deutsche Forschungsanstalt für Lebensmittelchemie and Institut für Lebensmittelchemie der Technischen Universität München, Lichtenbergstrasse 4, D-85748 Garching, Germany

Application of aroma extract dilution analysis on the volatiles obtained from dried cones of Spalter Select hops grown in the German hop-growing area of Hallertau revealed 23 odorants in the flavor dilution (FD) factor range of 16–4096, 20 of which could be identified. On the basis of high FD factors, *trans*-4,5-epoxy-(*E*)-2-decenal, linalool, and myrcene were identified as the most potent odorants, followed by ethyl 2-methylpropanoate, methyl 2-methylbutanoate, (*Z*)-1,5-octadien-3-one, nonanal, (*E,Z*)-1,3,5-undecatriene, 1,3(*E*),5(*Z*),9-undecatetraene, propyl 2-methylbutanoate, 4-ethenyl-2-methoxyphenol, and 1-octen-3-one. Ten of the high-impact hop aroma compounds had previously not been identified as hop constituents and, in particular, 1,3(*E*),5(*Z*),9-undecatetraene has not yet been reported as a food odorant. In an extract obtained from fresh hops, in addition to the odorants found in dry hops, (*Z*)-3-hexenal was characterized as a further key odorant rendering an additional green aroma note to the fresh material.

**Keywords:** *Aroma extract dilution analysis; hops; trans-4,5-epoxy-(E)-2-decenal; linalool; myrcene; (E,Z)-1,3,5-undecatriene; 1,3(E),5(Z),9-undecatetraene; (Z)-3-hexenal*

## INTRODUCTION

The dried cones of the female plant of the species *Humulus lupulus*, known as hop, are used by the brewing industry throughout the world to render especially a bitter taste and a more or less pronounced “hoppy” aroma to beer. Both the bitter-tasting acids and the aroma compounds are known to be present in the “lupulin” glands of the hop cone.

Compounds evoking the typical aroma of dried hop cones are constituents of the essential oil fraction, amounting to ~0.5–3.0% of the dry weight. Since the very early studies of Chapman (1895a,b, 1928) and Semmler and Mayer (1911), who identified humulene, myrcene, linalool, and 3-methylbutanoic acid as predominant constituents in hop oil, numerous investigations have been performed aimed at identifying volatile components [cf. reviews by Bullis and Likens (1962), Stevens (1967), and Sharpe and Laws (1981)]. A very comprehensive study on the volatile constituents of Spalter hops has been published by Tressl and Friese (1978), who identified and quantified >140 volatiles. On the basis of these studies, to date, >400 hop volatiles have been reported and summarized [cf. review by Nijssen et al. (1996)].

It is well accepted for a number of foods or their raw materials, respectively, that only a limited number of volatile compounds are responsible for the overall aroma (Acree, 1993; Grosch, 1993; Schieberle, 1995). Such compounds can be differentiated from the bulk of the rather odorless compounds by applying odor dilution techniques, such as CHARM analysis and the aroma

extract dilution analysis (AEDA) or the odor activity value concept [cf. review by Schieberle (1995)]. By applying the last mentioned approach to Brewers Gold hops, Guadagni et al. (1966) found that the major fraction of the essential oil, the hydrocarbons, accounted for ~69% of the total odor activity, whereas the minor oxygenated fraction accounted for ~34%. When calculating odor activity values (OAVs; ratio of concentration to odor threshold) of single volatiles, the authors found myrcene to represent 58% of the total hop aroma followed by methyl thiohexanoate (4.8%), methyl 4-decenoate (3%), caryophyllene (1.6%), and humulene (1.5%). Linalool, however, showed a low contribution (0.3%).

By applying gas chromatography–effluent sniffing (GCH-O), Seaton et al. (1981a,b) detected several odor-active regions in the chromatogram of extracts of Wye Target and Wye Saxon hops (Seaton and Moir, 1987). Although numerous odor impressions such as burnt, sweet, spicy, geraniums, garlic, rubbery, fruity, estery, green, or cucumber were described, no attempts were made to systematically correlate the identified compounds with the odor-active regions perceived during sniffing runs.

Oxygenated fractions of Hallertauer Mittelfrüh and two hop varieties developed in the United States were recently evaluated by Sanchez et al. (1992) using a descriptive sensory panel and a gas chromatography–olfactometry (GC-O) technique called “Osme” (McDaniel et al., 1992). Nine odor-active substances were detected in all three varieties, three of which could be identified as linalool, neral, and humulene monoepoxide III. A significant correlation was reported for the description of the overall aroma of the oxygenated fractions and the intensities of several odor-active regions in the chromatograms. On the basis of their data, the authors

\* Author to whom correspondence should be addressed (telephone +49-89-289-13265; fax +49-289-14183; e-mail peter.schieberle@Lrz.tum.de).

(Sanchez et al., 1992) proposed linalool and oxidation products of caryophyllene and humulene to contribute significantly to the overall odor of all three hop varieties.

After harvest, hop cones are usually dried by hot air. Howard and Slater (1958) found that drying of hops generally reduced the total amount of essential oil without noticeable influence on its composition. Narziss and Forster (1971) reported losses of hop essential oil of up to 50% during kilning when higher temperatures were applied. Most of the volatiles investigated, for example, monoterpenes, sesquiterpenes, linalool, and esters, were decreased; an increase in oxidation products of sesquiterpenes and of compounds proposed as degradation products of hop bitter substances was observed.

Studies correlating the different sensory properties of dried and undried hop cones with odor activities of single compounds are, however, lacking in the literature. The purpose of the following investigation was, therefore, to characterize the most odor-active compounds in fresh and dried cones of the same hop variety by means of odor dilution techniques and to compare their odor contributions based on flavor dilution (FD) factors.

## EXPERIMENTAL PROCEDURES

**Hop Samples.** Whole cones of Spalter Select hops were purchased from a hop farm in the German hop-growing area of Hallertau. Fresh hops were deep-frozen at the day of picking. Samples of dried hops were taken from the same hop garden. All samples were stored at  $-30\text{ }^{\circ}\text{C}$  prior to use.

**Chemicals.** Pure samples of the following compounds were obtained from commercial sources: acetaldehyde, dimethyl trisulfide, ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, hexanal, linalool, methional, 2-methylbutanal, 3-methylbutanal, methyl 2-methylbutanoate, methyl phenylacetate, myrcene, (*E,E*)-2,4-nonadienal, (*E,Z*)-2,6-nonadienal, octanal, and phenylacetaldehyde (Aldrich, Steinheim, Germany); 4-ethenyl-2-methoxyphenol, 1-octen-3-one (Lancaster, Mülheim/Main, Germany);  $\alpha$ -humulene (Fluka, Neu-Ulm, Germany); 2,3-butanedione (Merck, Darmstadt, Germany); (*Z*)-4-heptenal and a mixture of undecatetraenes (Haarmann & Reimer, Holzminden, Germany).

**Syntheses.** The following compounds were synthesized following procedures described in the literature given in parentheses: *trans*-4,5-epoxy-(*E*)-2-decenal (Schieberle and Grosch, 1991); (*Z*)-3-hexenal, and (*Z*)-1,5-octadien-3-one (Ullrich and Grosch, 1988); propyl 2-methylbutanoate (Fuhrmann, 1998).

**(*E,Z*)-1,3,5-Undecatriene.** In a first step (*E*)-1-bromo-2,4-pentadiene was synthesized by treatment of 1,4-pentadien-3-ol with HBr (Prévost et al., 1964). The preparation of a mixture of (*E,Z*)- and (*E,E*)-1,3,5-undecatriene by reacting (*E*)-1-bromo-2,4-pentadiene and hexanal was then performed according to the method of Näf et al. (1975) with some modifications: A stirred mixture of triphenylphosphine (35 mmol) and (*E*)-1-bromo-2,4-pentadiene (32 mmol) in toluene (40 mL) was kept for 3 days at room temperature in the dark. The crystals of the (*E*)-2,4-pentadienyltriphenylphosphonium bromide formed were filtered off, washed with toluene, and dried in vacuo at room temperature. Traces of the solvent were finally removed in a stream of nitrogen. (*E*)-2,4-Pentadienyltriphenylphosphonium bromide (5 mmol), suspended in dry dimethylformamide (DMF; 10 mL), was added to a solution of potassium *tert*-butoxide (6 mmol) in dry DMF (10 mL) under argon and stirred for 10 min at room temperature. Freshly distilled hexanal (5 mmol) in dry DMF (10 mL) was added dropwise at  $0\text{ }^{\circ}\text{C}$  to the intensely orange-red solution within 10 min. After stirring for 1 h at  $40\text{ }^{\circ}\text{C}$ , the mixture was cooled to room temperature, diluted with 10 mL of pentane, and filtered through Celite. The *n*-pentane phase was separated, and the DMF layer was extracted with another portion of *n*-pentane (100 mL). The

combined *n*-pentane phases were washed with a total of 400 mL of an aqueous saturated ammonium chloride solution and dried over anhydrous sodium sulfate. To remove nonvolatile impurities, the light yellow solution was purified by high-vacuum sublimation using the apparatus previously described (Guth and Grosch, 1989). As determined by HRGC, the (*E,Z*)- and (*E,E*)-1,3,5-undecatriene had been formed in a ratio of nearly 1:1. The following retention indices were determined for the 1,3(*E*),5(*E*)-isomer:  $\text{RI}_{\text{FFAP}}$ , 1397;  $\text{RI}_{\text{DB-1701}}$ , 1231; and  $\text{RI}_{\text{CPSi8CB}}$ , 1182. Retention indices for the 1,3(*E*),5(*Z*)-isomer were  $\text{RI}_{\text{FFAP}}$ , 1387;  $\text{RI}_{\text{DB-1701}}$ , 1215, and  $\text{RI}_{\text{CPSi8CB}}$ , 1174.

(*E,Z*)-1,3,5-Undecatriene was isolated by chromatography on silica gel containing 25% silver nitrate (25 g of silica in a water-cooled glass column; 30 cm  $\times$  1.6 cm i.d.;  $12\text{ }^{\circ}\text{C}$ ). Using 50 mL of pentane followed by a mixture of 1.5% diethyl ether in pentane (350 mL), the (*E,Z*)-isomer was eluted between 150 and 400 mL. After further purification by preparative GLC, which was performed as described previously (Schieberle, 1991), its structure was confirmed by mass spectrometry and proton magnetic resonance measurement. Characterization by  $^1\text{H}$  NMR afforded the following data:  $\delta$  [multiplicity, coupling constant (in hertz), and relevant H at carbon (numbering refers to Figure 5)] 0.88 (t, 3H,  $J_{11-10} = 7$ , C-11); 1.20–1.45 (m, 6H, C-8 to C-10); 2.18 (q, 2H,  $J_{7-6}$  and  $J_{7-8} = 7$ , C-7); 5.06 (d, 1H,  $J_{1-2} = 11$ , C-1); 5.20 (d, 1H,  $J_{1-2} = 16.8$ , C-1); 5.48 (dt, 1H,  $J_{6-5} = 11$ ,  $J_{6-7} = 7$ , C-6); 6.01 (t, 1H,  $J_{5-4}$  and  $J_{5-6} = 11$ , C-5); 6.19 (dd, 1H,  $J_{3-2} = 11$ ,  $J_{3-4} = 14.8$ , C-3); 6.40 [dt, 1H,  $J_{2-1} = 16.8$  (tr),  $J_{2-1}$  and  $J_{2-3} = 11$ , C-2]; 6.50 (dd, 1H,  $J_{4-3} = 14.8$ ,  $J_{4-5} = 11$ ; C-4). The  $^1\text{H}$  NMR data were in good agreement with those reported in the literature (Alexakis et al., 1992; Hyunh et al., 1994).

**Preparation of Undecatetraene Isomers.** A commercially available mixture of undecatetraenes was fractionated on a water-cooled ( $12\text{ }^{\circ}\text{C}$ ) glass column (30 cm  $\times$  1.6 cm) using silica dotted with silver nitrate (25 g; 3 + 1 by weight) as the stationary phase. The material was suspended in *n*-pentane, and elution was performed using *n*-pentane (200 mL), followed by *n*-pentane/diethyl ether (200 mL; 99 + 1 by volume) and finally *n*-pentane/diethyl ether (200 mL; 97 + 3 by volume) as the eluents. The effluents from 420 to 470 mL (fraction SIC I) and from 540 to 620 mL (fraction SIC II) were pooled and, after the solvent had been distilled off, analyzed by HRGC-MS and  $^1\text{H}$  NMR. For fractions SIC I and SIC II the mass spectra shown in Figures 1 and 2 were obtained. SIC II gave the following  $^1\text{H}$  NMR signals (numbering refers to Figure 2):  $\delta$  1.63 (d, 3H,  $J_{11-10} = 5$ , C-11); 2.05 (q, 2H,  $J_{8-7}$  and  $J_{8-9} = 7$ , C-8); 2.24 (q, 2H,  $J_{7-6}$  and  $J_{7-8} = 7$ , C-7); 5.06 (d, 1H,  $J_{1-2} = 10$ , C-1); 5.19 (d, 1H,  $J_{1-2} = 16$ , C-1); 5.39–5.50 (m, 3H, C-6, C-9, and C-10); 6.00 (t, 1H,  $J_{5-4}$  and  $J_{5-6} = 11$ , C-5); 6.18 (dd, 1H,  $J_{3-2} = 11$ ,  $J_{3-4} = 15$ , C-3); 6.39 [dt, 1H,  $J_{2-1} = 16$  (tr),  $J_{2-1}$  (cis) and  $J_{2-3} = 11$ , C-2]; 6.48 (dd, 1H,  $J_{4-3} = 15$ ,  $J_{4-5} = 11$ , C-4).

**Isolation of Hop Volatiles; Separation of the Acidic from Neutral and Basic Compounds.** Dried (5 g) or fresh hop cones (22.5 g) were frozen in liquid nitrogen and ground by means of mortar and pestle. To the sample of fresh hops was added anhydrous sodium sulfate (22.5 g). The powder was then extracted repeatedly with portions of freshly distilled diethyl ether (total volume = 100 mL) by stirring for 1 h at room temperature. After filtration, the residue was washed with diethyl ether (50 mL). To remove the nonvolatile material, the combined ethereal extracts were distilled in vacuo at room temperature as described earlier (Guth and Grosch, 1989). Acidic volatiles were separated from the neutral/basic volatile fraction (NBV) by treatment of the distillate with aqueous sodium bicarbonate (0.5 mol/L; total volume = 200 mL). The combined aqueous solutions were adjusted to pH 3 by the addition of hydrochloric acid and then extracted with diethyl ether (3 times; total volume = 150 mL). The solutions containing the NBV and the acidic volatiles (AV) were dried over anhydrous sodium sulfate and concentrated to 1 mL by distilling off the solvent by means of a Vigreux column (60  $\times$  1 cm) followed by microdistillation (Bemelmans, 1979). The odor-active compounds in the two fractions were evaluated by GC-O and AEDA.

**Column Chromatography.** To obtain enough material for the identification experiments, the NBV fraction isolated from a larger quantity of dried hop cones (500 g) was fractionated on a water-cooled (12 °C) glass column (50 × 3 cm) packed with a slurry of silica gel in pentane. After elution with pentane (500 mL) yielding the fraction of hydrocarbons (HCF), the oxygenated compounds were eluted with diethyl ether (500 mL; OF). The concentrated fraction of the oxygenated volatiles was further fractionated by flash chromatography using a glass column (40 × 2 cm) filled with silica gel (silica gel for flash chromatography; T. J. Baker, Deventer, The Netherlands). Stepwise elution was performed with pentane (150 mL), pentane/diethyl ether (95:5 v/v; 150 mL), pentane/diethyl ether (90:10 v/v; 150 mL), pentane/diethyl ether (85:15 v/v; 150 mL), pentane/diethyl ether (80:20 v/v; 150 mL), and finally diethyl ether (150 mL). The eluates were collected in 12 fractions: fraction 1 (150 mL), fractions 2–10 (50 mL each), and fractions 11 and 12 (150 mL each).

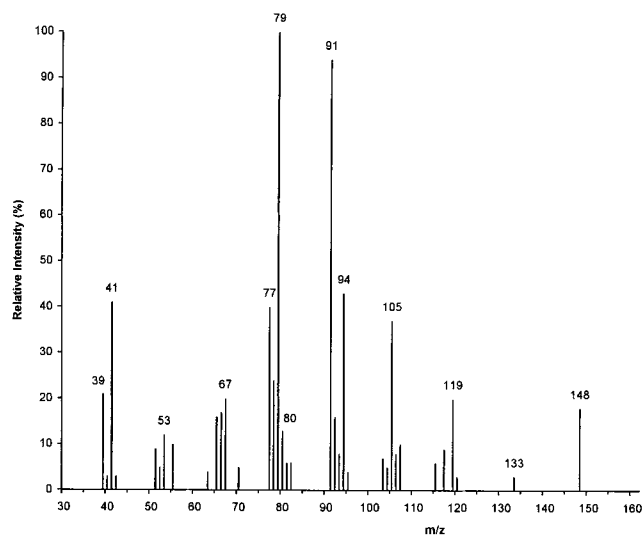
**Capillary Gas Chromatography (HRGC); HRGC–Olfactometry (GC-O).** HRGC was performed by means of a gas chromatograph type 8160 (Fisons Instruments, Mainz, Germany) using the following fused silica capillaries: CP Sil 8CB (equivalent to silicone SE-54; 30 m × 0.32 mm; Chrompack, Frankfurt/Main, Germany); DB-1701 (30 m × 0.32 mm fused silica column coated with OV-1701; J&W Scientific, Fisons); and FFAP-CB (25 m × 0.32 mm; free fatty acid phase; Chrompack). The samples were applied by cold on-column injection at 35 °C. After 2 min, the oven temperature was raised by 40 °C/min to 50 °C (60 °C for FFAP), held for 2 min isothermally, and then raised by 6 °C/min to 180 °C and finally by 10 °C/min to 240 °C. For HRGC-O, at the end of the capillary the effluent was split 1:1 into a flame ionization detector (FID) and a heated (220 °C) sniffing device made from alumina using a Y-shaped quick-seal glass splitter (Chrompack) and deactivated fused silica capillaries (50 cm × 0.10 mm i.d.). Helium at a flow rate of ~2.2 mL/min was used as the carrier gas. Retention indices (RI) were calculated from the retention times of *n*-alkanes by linear interpolation.

**AEDA.** FD factors of odorants in fractions AV and NBV were determined by AEDA (Schieberle, 1995) using capillary FFAP for fraction AV and capillary CP Sil 8CB for fraction NBV. Extracts were stepwise diluted with diethyl ether (1+1 by volume) and 0.5 μL of the concentrate (FD = 1), and each diluted sample (FD = 2, 4, 8, etc.) was separated and in parallel evaluated by HRGC-O.

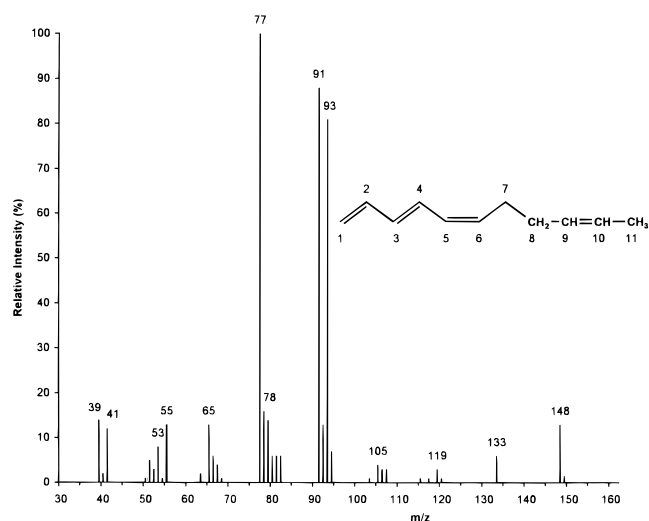
**Static headspace olfactometry (SHO)** was performed using the equipment described previously by Guth and Grosch (1993). Hop cones were frozen using liquid nitrogen and then ground. Hop powder (0.1 g of dried hops; 0.45 g of fresh hops) was put into a septum-sealed vessel (volume = 144 mL) and kept for 30 min at 20 °C for equilibration. Decreasing volumes (20, 10, 5, 2.5, 1.25, 0.63, 0.31, 0.16, and 0.08 mL, respectively) were withdrawn from freshly equilibrated aliquots by means of a gastight syringe and injected into the purge system operating in the desorption mode (10 min). The headspace volatiles were collected at -100 °C in a precooled trap and were flushed with the carrier gas onto the GC column, by heating the trap very rapidly to 200 °C. The fused silica capillary used was an RTX-5 (equal to an SE-54; 30 m × 0.53 mm, film thickness = 1.5 μm). The oven was held at 0 °C for 2 min and then raised by 6 °C/min to 200 °C. The effluent of the column was split 1:1 into an FID and a sniffing device as described above.

**HRGC–Mass Spectrometry (HRGC-MS).** Using the capillaries described above, mass spectra were generated by means of an MAT 95 S (Finnigan, Bremen, Germany) at 70 eV in the electron impact mode (MS/EI) and at 110 eV in the chemical ionization mode (MS/CI; reagent gas was isobutane). For the identification of very low boiling substances, the gas chromatograph used for SHO was coupled to an MS-System IncoS XL (Finnigan).

**Proton magnetic resonance spectra** were recorded in CDCl<sub>3</sub> solution using a Bruker AM 360 spectrometer (360.13 MHz) and tetramethylsilane as the internal standard.



**Figure 1.** Mass spectrum (MS/EI) obtained for fraction SIC I isolated from a commercial mixture of undecateetraenes.



**Figure 2.** Mass spectrum (MS/EI) obtained for fraction SIC II isolated from a commercial mixture of undecateetraenes.

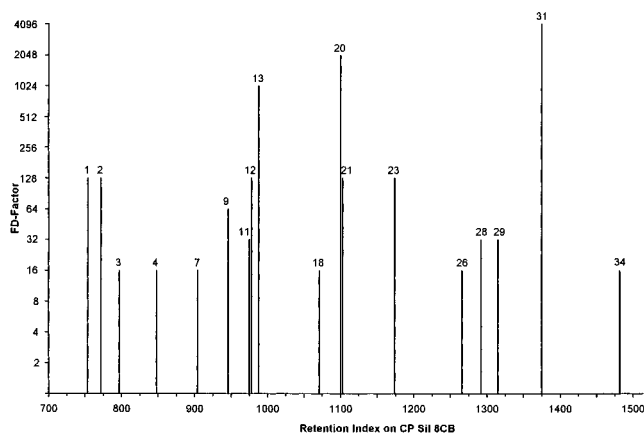
**UV spectra** were recorded in *n*-pentane using a Hitachi spectrometer (U 2000).

## RESULTS AND DISCUSSION

**Odorants in Dried Hop Cones.** The volatile fraction isolated from a small sample of Hallertauer Spalter Select hop cones (5 g), eliciting a very intense typical hop aroma, was carefully isolated by solvent extraction followed by sublimation in vacuo at 25 °C. Sniffing of an aliquot of the distillate on a strip of filter paper fully represented the characteristic aroma impression evoked by the hop cones.

After separation of the volatiles into the NBV and AV, the most odor-active compounds in both extracts were evaluated by means of AEDA. In the NBV fraction, 18 odor-active regions were detectable in the FD factor range of 16–4096 (cf. Figure 3). Among them, the metallic smelling compound **31**, followed by the sweet, flowery smelling compound **20** and odorant **13**, having a geranium-like odor, showed the highest FD factors of 1024 or 4096, respectively.

In the hydrocarbon fraction, the predominant compound **13** could readily be identified as myrcene, which



**Figure 3.** FD chromatogram obtained by application of AEDA on a hop extract containing the NBV (odorants with FD  $\geq 16$  are displayed).

most probably rendered its typical geranium-like odor to the overall aroma of the HCF. Contrarily, in the oxygenated fraction many odorants were found to belong to the trace constituents. To obtain enough material for the identification experiments, the NBV fraction was isolated from 500 g of dried hop cones and separated into the hydrocarbon subfraction (HCF) and the oxygenated fraction (OF). The latter was further separated on silica into 10 subfractions (OF1–OF10). Each fraction was then submitted to GC-O to localize the odorants. Compound **10** was enriched in fraction OF10 and was identified as *trans*-4,5-epoxy-(*E*)-2-decenal by comparing the analytical data (MS/EI and MS/CI; retention indices on two stationary phases) as well as its odor quality and odor intensity with those of the reference compound (Table 1). The epoxyaldehyde was previously unknown in hops. Compound **20**, which was next in rank according to the FD factors, having a flowery note, was also enriched in OF10 and was identified as linalool (Table 1). This result confirmed the very early suggestions of Chapman (1895) on the flavor contribution of this terpene alcohol.

Besides the three high-impact odorants described above, high FD factors (FD 128) were found for five other odorants (**1**, **2**, **12**, **21**, and **23**). Four of them could be identified by means of reference odorants based on the criteria given in Table 1: ethyl 2-methylpropanoate (**1**, Table 1; fruity), methyl 2-methylbutanoate (**2**; fruity), (*Z*)-1,5-octadien-3-one (**12**; geranium-like), and nonanal (**21**; soapy).

**Identification of Compound 23.** The mass spectrum obtained at RI 1174 on the CPSil8 C8 column for compound **23** was not very conclusive. Enrichment of **23** in the HCF followed by preparative gas chromatography and subsequent GC-O analysis on an FFAP column revealed two odor-active compounds at RI 1387 (compound **23a**) and RI 1448 (compound **23b**), each of them eliciting a fresh, balsamic odor and having identical FD factors. Analysis by MS/EI gave the mass spectra shown in Figures 4 and 5. Comparison of the spectrum obtained for compound **23a** (Figure 4) with that of the synthesized reference odorant unambiguously suggested its structure as 1,3(*E*),5(*Z*)-undecatriene. Identical retention indices on HRGC columns of three different polarities as well as odor intensity and odor quality in the sniffing port strongly corroborated this result.

The molecular mass of compound **23b** (Figure 5) was confirmed by mass spectrometry in the chemical ioniza-

tion mode to be 148, indicating that this odorant might be an undecatetraene. Berger et al. (1985a,b) had previously reported that (*E,Z*)-1,3,5-undecatriene and 1,3(*E*),5(*Z*),8(*Z*)-undecatetraene nearly coelute on an SE-54 column, and they have reported identical, low odor thresholds in air for both compounds (1 pg for the triene and 2–4 pg for the tetraene).

To obtain enough material for the identification experiments, a commercial mixture containing several undecatetraenes was separated by HRGC (Figure 6). Sniffing of the eluate revealed the same fresh, balsamic odor at RI 1174 as found for compound **23** in the hop extract. Separation of the crude mixture by silver ion chromatography yielded two odor-active fractions (SIC I and SIC II) showing the mass spectra displayed in Figures 1 and 2.

The spectrum for SIC I (Figure 1) was in good agreement with data reported by Marner et al. (1982) for 1,3,5,8-undecatetraenes in general. Because this compound was not found in hops, no attempts were made to clarify the conformation of its double bonds. However, the retention indices suggested the 1,3(*E*),5(*Z*),8(*Z*)-isomer.

The data obtained for SIC II (Figure 2) were identical with those obtained for compound **23b** in the hop extract (Figure 5). The fragmentation pattern of **23b** in MS/EI also suggested an undecatetraene structure (cf. Figures 5 and 1) but excludes the 1,3,5,8-structure because of higher intensities of the fragments  $m/z$  77 and 93.

For further structure elucidation the following analyses were performed using fraction SIC II: First, by UV measurement (Figure 7) a conjugated triene system was established in SIC II. To clarify the position and the conformation of the double bonds, SIC II was analyzed by  $^1\text{H}$  NMR. The signals obtained for carbons 1–6 nearly agreed with those determined for the 1,3(*E*),5(*Z*)-undecatriene (cf. Experimental Procedures). In particular, the downfield shifted signals of two methylene groups at  $\delta$  2.24 and 2.05 and a strongly deshielded methyl group at  $\delta$  1.63 suggested the presence of an isolated double bond, which would be possible only between carbons 9 and 10. The conformation of this double bond could, however, not unambiguously be confirmed.

In conclusion, compound **23b** was identified as 1,3(*E*),5(*Z*),9-undecatetraene. To our knowledge, this odorant is reported here for the first time as a flavor constituent of a food.

In the fraction of the acidic volatiles, five additional odorants showing comparatively low FD factors were detected, four of which were identified as 2- and 3-methylbutanoic acid, butanoic acid, and pentanoic acid (Table 2).

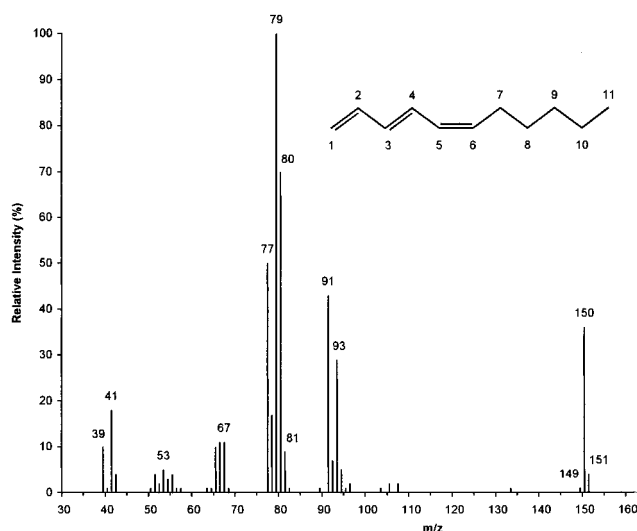
For AEDA experiments, solvent extracts have to be prepared by distillation and concentration steps. This approach might cause, for example, losses of very volatile odorants and, consequently, an underestimation of their flavor contribution when AEDA is applied. By applying GC-O on static headspace samples of decreasing gas volumes, a technique assigned as SHO (Guth and Grosch, 1993), this gap in the evaluation of the flavor contribution of volatiles can be overcome.

In a static headspace volume of 20 mL, taken from a sample of 0.1 g of hop powder in a 144 mL vessel, 24 odor-active compounds were detectable (Table 3). Due to the very low amounts of each odorant present, the

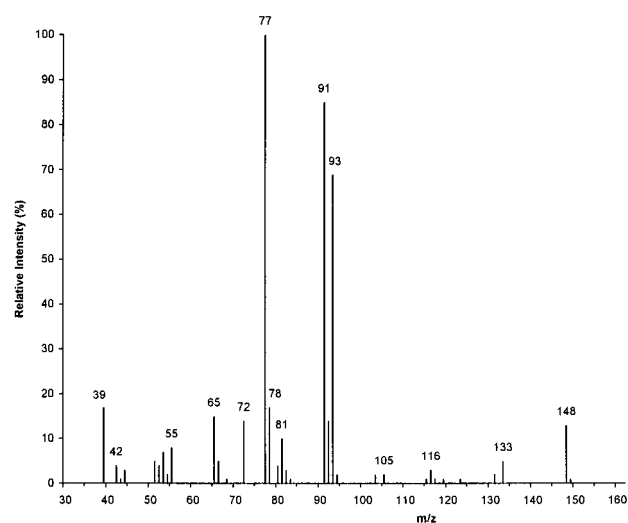
**Table 1. Most Odor-Active NBV (FD Factor  $\geq 4$ ) in Dried Hop Cones**

peak no. <sup>a</sup>	odorant <sup>b</sup>	odor quality <sup>c</sup>	fraction	RI <sup>d</sup> on		FD factor	reported earlier <sup>e</sup>
				CPSi8	FFAP		
1	ethyl 2-methylpropanoate	sweet, fruity	OF4	754	964	128	
2	methyl 2-methylbutanoate	sweet, fruity	OF4	772	1002	128	
3a	(Z)-3-hexenal	green	OF5	797	1132	16	
3b	hexanal	green	OF5	797	1073	16	(1)
4	ethyl 2-methylbutanoate	sweet, fruity	OF4	848	1040	16	
7	3-(methylthio)propanal (methional) <sup>f</sup>	cooked-potato-like		904	1452	16	(2)
8	unknown	sweet, fruity		936		8	
9	propyl 2-methylbutanoate	sweet, fruity	OF4	946	1132	64	
10	unknown	sweet, fruity		969		8	
11	1-octene-3-one	mushroom-like	OF5	975	1296	32	
12	(Z)-1,5-octadien-3-one	geranium-like	OF6	978	1367	128	
13	myrcene	geranium-like	HCF	988	1154	1024	(3)
14	octanal	citrus-like, soapy	OF5	1002	1282	8	(4)
16	unknown	fruity, banana-like		1022		4	
18	unknown <sup>g</sup>	mushroom-like		1071	1452	16	
19	unknown	citrus-like		1075		8	
20	linalool	sweet, flowery, citrus-like	OF9,10	1100	1537	2048	(5)
21	nonanal	citrus-like, soapy	OF4	1103	1387	128	(4)
22	(E,Z)-2,6-nonadienal	cucumber-like	OF6	1153	1579	4	
23a	1,3(E),5(Z)-undecatriene	fresh, balsamic	HCF	1174	1387	128	
23b	1,3(E),5(Z),9-undecatetraene	fresh, balsamic	HCF	1174	1448	128	
26	unknown	sweet, musty, coconut-like		1266	1865	16	
28	unknown	citrus-like		1292		32	
29	4-ethenyl-2-methoxyphenol	spicy		1315	2200	32	
30	unknown	citrus-like		1356		8	
31	trans-4,5-epoxy-(E)-2-decenal <sup>f</sup>	metallic	OF10	1375	2007	$\geq 4096$	
32	unknown	suffocating		1378	2251	8	
33	$\alpha$ -humulene	balsamic	HCF	1457	1656	8	(6)
34	unknown	mushroom-like	HCF	1482		16	
35	unknown	sweet, balsamic	HCF	1528		8	
36	unknown <sup>h</sup>	mushroom-like, balsamic	HCF	1561	1820	8	

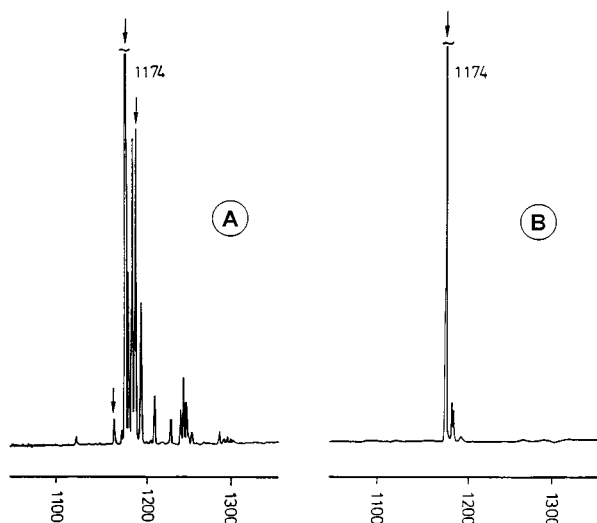
<sup>a</sup> Numbers refer to Figure 3. <sup>b</sup> The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention indices on the capillaries detailed in the table; mass spectra obtained by MS/EI and MS/CI; odor quality and odor threshold at the sniffing port. <sup>c</sup> Odor quality perceived at the sniffing port. <sup>d</sup> Retention index. <sup>e</sup> Reported earlier as volatile compound in hops: (1) Tressl and Friese, 1978; (2) Anderson et al., 1974; (3) Semmler and Mayer, 1911; (4) Naya and Kotake, 1971; (5) Chapman, 1903; (6) Chapman, 1895a,b. <sup>f</sup> The MS signal was too weak for unequivocal interpretation. Identification of the compound was based on the resting criteria given in footnote b. <sup>g</sup> Mass spectral data are as follows: MS/EI 55 (100), 41 (50), 95 (37), 70 (31), 69 (26), 123 (23), 83 (20), 67 (19), 39 (13), 82 (11), 43 (10), 97 (10), 68 (9), 56 (8), 96 (8), 109 (8), 53 (7), 81 (6), 105 (6), 138 (5), 77 (5); MS/CI 139 (100), 157 (75), 140 (9), 158 (7). <sup>h</sup> Because the reference substances were not available, the compound was proposed as germacrene B (Hartley and Fawcett, 1969) on the basis of MS data and by comparing the RI with published data (Le Quere and Latrasse, 1990).

**Figure 4.** Mass spectrum (MS/EI) obtained for compound **23a** after separation on a DB-OV-1701 GC stationary phase.

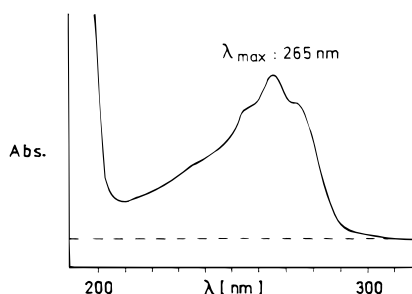
identification experiments for most of the odorants could be performed on the basis of odor quality, odor intensity, and retention index. A comparison with the results given in Tables 1 and 2 revealed that acetaldehyde, 2-methylpropanal, and 2,3-butandione as well as 2- and 3-methylbutanal and dimethyl trisulfide were detected as additional odorants by application of the SHO

**Figure 5.** Mass spectrum (MS/EI) obtained for compound **23b** after separation on a DB-OV-1701 GC stationary phase.

technique. This is well in line with the high volatility of most of these compounds, which were obviously lost during the concentration and enrichment steps. There is, however, no satisfactory explanation for the absence of dimethyl trisulfide in the solvent extract. However, it has to be pointed out that the FD factors of these volatiles were quite low.



**Figure 6.** HRGC separation (capillary FFAP) of a commercial mixture of undecatenes (A) and fraction SIC II (B). Arrows indicate an odor-active region.



**Figure 7.** UV spectrum obtained for fraction SIC II isolated from a commercial mixture of undecatenes.

**Table 2. Most Odor-Active AV (FD Factor  $\geq 4$ ) in Dried Hop Cones**

no.	odorant <sup>a</sup>	odor quality <sup>b</sup>	RI <sup>c</sup> on FFAP	FD factor	reported earlier <sup>d</sup>
37	butanoic acid	buttery, cheesy	1626	32	(1)
38a	2-methylbutanoic acid				(2)
38b	3-methylbutanoic acid	cheesy	1669	64	(3, 4)
39	pentanoic acid	cheesy	1736	4	(1)
32	unknown	suffocating	2251	32	

<sup>a</sup> The compound was identified by comparing it with the reference substance using the following criteria: retention indices on capillary FFAP; MS obtained by MS/EI and MS/CI; odor quality and odor threshold at the sniffing port. <sup>b</sup> Odor quality perceived at the sniffing port. <sup>c</sup> Retention index. <sup>d</sup> Reported earlier as volatile compound in hops: (1) Tressl et al., 1978; (2) Tressl and Friese, 1978; (3) Chapman, 1928; (4) Hartley and Fawcett, 1969.

On the other hand, compounds showing high FD factors in the solvent extracts, particularly *trans*-4,5-epoxy-(*E*)-2-decenal, were not detected by this technique. This might be explained by its relatively low volatility.

By sniffing decreasing headspace volumes to only 80  $\mu$ L, the important contributors to the "top-note" flavor of the hops were shown to be myrcene and linalool. Also, (*E,Z*)-1,3,5-undecatriene, 1,3(*E*),5(*Z*),9-undecatetraene and ethyl 2-methylpropanoate were detectable when low headspace volumes of 630  $\mu$ L were used, suggesting their significant contribution to the hop aroma.

**Important Odorants in Fresh Hop Cones.** In the overall aroma of the fresh sample from the same batch of Hallertauer Spalter hops, besides the typical hop aroma, an intense green, grassy odor was perceiv-

**Table 3. Odorants in Dried Hop Cones Detected by SHO**

odorant <sup>a</sup>	odor quality <sup>b</sup>	vol <sup>c</sup> (mL)	relative FD <sup>d</sup>
acetaldehyde <sup>e</sup>	solvent-like	20	1
2-methylpropanal	sweet, malty	10	2
2,3-butanedione	sweet, buttery	5	4
3-methylbutanal	sweet, malty	10	2
2-methylbutanal	sweet, malty	20	1
ethyl 2-methylpropanoate	sweet, fruity	0.63	32
methyl 2-methylbutanoate	sweet, fruity	1.25	16
( <i>Z</i> )-3-hexenal	green		
hexenal	green	10	2
hexenal <sup>e</sup>	sweet, unpleasant	20	1
ethyl 2-methylbutanoate	sweet, fruity	1.25	16
( <i>Z</i> )-4-heptenal	sweet, unpleasant	20	1
propyl 2-methylbutanoate	sweet, fruity	2.5	8
unknown	sweet, fruity	1.25	16
dimethyl trisulfide	sulfurous, putrid	1.25	16
1-octen-3-one	mushroom-like	20	1
( <i>Z</i> )-1,5-octadien-3-one	geranium-like	10	2
myrcene	geranium-like	0.08	256
octanal	citrus-like, soapy	20	1
unknown	sweet, fruity	20	1
linalool	sweet, flowery	0.08	256
( <i>E,Z</i> )-2,6-nonadienal	cucumber-like	20	1
1,3( <i>E</i> ),5( <i>Z</i> )-undecatriene	fresh, balsamic		
1,3( <i>E</i> ),5( <i>Z</i> ),9-undecatetraene	fresh, balsamic	0.63	32

<sup>a,b</sup> Refer to Table 1. <sup>c</sup> Lowest headspace volume in which the odorant was detected by HRGC-O. <sup>d</sup> Calculated by dividing the largest volume analyzed (20 mL) by the lowest volume in which the odorant was detected by HRGC-O. <sup>e</sup> Tentatively identified on the basis of MS data.

able. Application of AEDA on the NBV fraction revealed 36 odor-active regions in the FD factor range of 4–2048 (Table 4). The highest factor of 2048 was approximated for linalool (flowery) and (*Z*)-3-hexenal (green, grassy). With somewhat lower FD factors myrcene, (*E,Z*)-1,3,5-undecatriene, 1,3(*E*),5(*Z*),9-undecatetraene, and *trans*-4,5-epoxy-(*E*)-2-decenal were identified in the extract from the fresh hops. In the AV fraction, the same odor-active short-chain fatty acids as found in the dry hop cones were identified (data not shown). Furthermore, also their FD factors did not differ significantly in both samples.

**Changes in the Odor Activities Caused by the Drying Process.** On the basis of dry weight, extracts from the same amount of fresh and dry hop cones were isolated, allowing a comparison of changes in the odor activities caused by the drying procedure. The most significant differences were evaluated for the green, grassy-smelling (*Z*)-3-hexenal, which was much higher in the fresh sample, and *trans*-4,5-epoxy-(*E*)-2-decenal (metallic) which, on the other hand, was much higher in the dried sample (cf. Tables 1 and 4). (*Z*)-3-Hexenal is known to be formed in plants from linoleic acid by an enzymic reaction cascade (Hatanaka, 1993). During food processing, such as drying or cooking, the aldehyde is relatively unstable and may be converted either enzymatically to the less flavor-active volatiles (*E*)-2-hexenal and/or (*Z*)-3-hexenol or by chemical degradation into, for example, 3-hydroxyhexenal, particularly when weakly acidic conditions are applied (Fischer and Grosch, 1988). It can, therefore, be concluded that (*Z*)-3-hexenal is significantly degraded during drying of the hop cones. Similar results on the instability of the aldehyde have also been obtained for tomatoes (Buttery et al., 1990), olives (Blekak et al., 1994), and strawberries (Schieberle and Hofmann, 1997).

*trans*-4,5-Epoxy-(*E*)-2-decenal was shown to be formed by thermal cleavage of 12,13-epoxy-9-hydroperoxy-11-

**Table 4. Most Odor-Active NBV (FD Factor  $\geq$  4) in Undried Hop Cones**

peak no. <sup>a</sup>	odorant <sup>b</sup>	odor quality <sup>c</sup>	fraction	RI <sup>d</sup> on		FD factor
				CPSil8CB	FFAP	
1	ethyl 2-methylpropanoate	sweet, fruity	OF 4	754	964	128
2	methyl 2-methylbutanoate	sweet, fruity	OF 4	772	1002	256
3a	(Z)-3-hexenal	green	OF 5	797	1132	2048
3b	hexanal	green	OF 5		1073	
4	ethyl 2-methylbutanoate	sweet, fruity	OF 4	848	1040	32
5	unknown	sweet, fruity		890		16
6	(Z)-4-heptenal	sweet, unpleasant	OF 4	896	1236	4
7	3-(methylthio)propanal (methional) <sup>e</sup>	cooked-potato-like		904	1452	128
8	unknown	sweet, fruity		936		4
9	propyl 2-methylbutanoate	sweet, fruity	OF 4	946	1132	16
10	unknown	sweet, fruity		969		4
11	1-octene-3-one	mushroom-like	OF 5	975	1296	32
12	(Z)-1,5-octadien-3-one	geranium-like	OF 6	978	1367	32
13	myrcene	geranium-like	HCF	988	1154	512
14	octanal	citrus-like, soapy	OF 5	1002	1282	8
15	unknown	sweet, fruity		1005		16
16	unknown	fruity, banana-like		1022		16
17	phenylacetaldehyde <sup>e</sup>	honey-like, floral		1040	1640	16
18	unknown <sup>f</sup>	mushroom-like		1071	1452	8
19	unknown	citrus-like		1075		16
20	linalool	sweet, flowery	OF 9,10	1100	1537	2048
21	nonanal	citrus-like, soapy	OF 4	1103	1387	64
22	(E,Z)-2,6-nonadienal	cucumber-like	OF 6	1153	1579	16
23a	1,3(E),5(Z)-undecatriene	fresh, balsamic	HCF	1174	1387	512
23b	1,3(E),5(Z),9-undecatetraene	fresh, citrus-like	HCF	1174	1448	
24	methyl phenylacetate	honey-like		1176	1758	4
25	(E,E)-2,4-nonadienal <sup>e</sup>	fatty, waxy	OF 6	1218	1698	4
26	unknown	sweet, musty, coconut-like		1266	1865	32
27	unknown	sweet, musty		1284		16
28	unknown	citrus-like		1292		32
29	4-ethenyl-2-methoxyphenol	spicy		1315	2200	32
30	unknown	citrus-like		1356		16
31	trans-4,5-epoxy-(E)-2-decenal	metallic	OF 10	1375	2007	512
32	unknown	suffocating		1378	2251	4
33	$\alpha$ -humulene	balsamic	HCF	1457	1656	16
34	unknown	mushroom-like	HCF	1482		32
35	unknown	sweet, balsamic	HCF	1528		32
36	unknown <sup>g</sup>	mushroom-like, balsamic	HCF	1561	1820	32

<sup>a</sup> Numbers refer to Figure 3. <sup>b</sup> The compound was identified by comparing it with the reference substance using the following criteria: retention indices on the capillaries detailed in the table; MS obtained by MS/EI and MS/CI; odor quality and odor threshold at the sniffing port. <sup>c</sup> Odor quality perceived at the sniffing port. <sup>d</sup> Retention index. <sup>e</sup> The MS signal was too weak for unequivocal interpretation. Identification of the compound was based on the resting criteria given in footnote b. <sup>f</sup> MS data are as follows: MS/EI 55 (100), 41 (50), 95 (37), 70 (31), 69 (26), 123 (23), 83 (20), 67 (19), 39 (13), 82 (11), 43 (10), 97 (10), 68 (9), 56 (8), 96 (8), 109 (8), 53 (7), 81 (6), 105 (6), 138 (5), 77 (5); MS/CI 139 (100), 157 (75), 140 (9), 158 (7). <sup>g</sup> Because the reference substances were not available, the compound was tentatively proposed as germacrene B (Hartley and Fawcett, 1969) on the basis of MS data and by comparing the RI with published data (Le Quere and Latrasse, 1990).

octadecenoates as precursors (Gassenmeier and Schieberle, 1994). It can, therefore, be proposed that such a precursor might also be present in the fresh hops, leading to the formation of the epoxydecenal during drying.

Less pronounced differences were evaluated for 3-(methylthio)propanal (potato-like) and phenylacetaldehyde (honey-like), which both were higher in the fresh sample, whereas (Z)-1,5-octadien-3-one (geranium-like) increased during drying (cf. Tables 1 and 4).

Oxidation of hop hydrocarbons has been proposed in the literature to occur during drying of hop cones. The results obtained in this study, however, propose that this type of reaction neither reduces the flavor potency of hydrocarbons already present in the fresh hop nor leads to the formation of new odor-active compounds in the dried hop.

**Conclusions.** Application of AEDA on Spalter Select hops has confirmed the well-known hop constituents myrcene and linalool as key contributors to the overall aroma. Furthermore, the previously unknown hop constituents trans-4,5-epoxy-(E)-2-decenal, (E,Z)-1,3,5-undecatriene, 1,3(E),5(Z),9-undecatetraene, (Z)-1,5-octadien-

3-one, and the two esters ethyl 2-methylpropanoate and methyl 2-methylbutanoate were identified as important hop odorants. Application of the SHO technique on hop powder confirmed the results obtained for myrcene, linalool, undecatriene, undecatetraene, and the esters. However, the data did not corroborate the key role of a few odorants identified by means of the AEDA, in particular trans-4,5-epoxy-(E)-2-decenal. Quantitative studies and flavor recombination experiments are, therefore, necessary further steps to confirm the aroma impact of the odor-active compounds identified.

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