

Identification of Prenyl Ethyl Ether as a Source of Metallic, Solvent-like Off-Flavor in Hazelnut

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In a large batch of ground hazelnuts, a metallic, solvent-like off-note was detected. In this investigation, the volatiles from the batch showing off-notes were compared to a batch without off-notes. On the basis of gas chromatography (GC) sniffing and instrumental analysis, a terpenoid compound, prenyl ethyl ether, was identified as a key contributor to the off-note. The compound was quantified, and its contribution to the metallic, solvent-like off-flavor was confirmed by spiking experiments and sensory evaluation. Analytical and sensory experiments found that the off-note was still present in hazelnut cakes. Fat oxidation did not contribute to the off-flavor. Analysis of market products demonstrated the correlation between the identified terpenoid and the off-flavor. It is assumed that fungi are involved in off-flavor formation.

KEYWORDS: Hazelnut; off-flavor; prenyl ethyl ether; terpenoids

INTRODUCTION

Hazelnut (*Corylus avellana* L.) is an important crop with a global production exceeding 800 000 tons, accounting for an economical value of more than \$800 million. Turkey is the number one producing country, providing about two-thirds of global production, followed by Italy, the United States, and Azerbaijan (1). Hazelnuts present an attractive snack as well as an important ingredient for the food industry, e.g., the chocolate industry. Their nutritional composition has been described by several studies (2–4). Hazelnuts are consumed or further processed as raw and roasted nuts. The attractive and typical aroma of roasted hazelnuts has been intensively investigated. 5-Methyl-(*E*)-2-hepten-4-one (filbertone) has been reported as a key compound for the hazelnut aroma (5, 6). It is found in larger amounts after roasting (6). In addition to filbertone, a very large number of chemicals has been found in raw and roasted hazelnuts, with pyrazines being an important group of compounds of the flavor for roasted nuts (7–10). Very recently, 5-methyl-4-heptanone, 2-methoxypyrazines, and other compounds were shown to be important odorants in raw Italian hazelnuts (11). In the context of off-flavors in hazelnuts, rancidity because of volatile aliphatic aldehydes and related compounds is often mentioned (12). However, other off-notes can be found in hazelnuts, which can render them unacceptable for consumption. Here, we report the detection of a new metallic, solvent-like off-flavor compound found in ground hazelnuts, being responsible for the loss of a major part of the production for a season. Analytical data are reported, including spiking experiments with ground hazelnuts and sensory evaluation with hazelnuts and cakes thereof, as well as data on market samples.

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MATERIALS AND METHODS

Ground hazelnuts of different origins were purchased at local supermarkets in Switzerland, and hazelnuts with off-note (HaS) and hazelnuts without off-note (HaG) were obtained from a Swiss nut trading company.

Reference substances (limonene, valencene, hexanal, octanal, nonanal, and methyl pelargonate) were obtained from Fluka (Buchs, Switzerland). Prenyl ethyl ether (PEE, ethyl 3-methyl-2-butenyl ether, CAS 22094-00-4) was provided by Givaudan (Dübendorf, Switzerland). Mass spectrum (EI+) of PEE *m/z*: 71 (100), 41(78), 99 (68), 29 (40), 69 (40), 27 (28), 39 (25), 43 (20), 114 (20), 68(16), 53 (16), 67 (15), 55 (12), 31 (10), and 57 (10). The retention index of PEE was 1004 on a DB WAX column (linear retention index based on alkane references).

The hazelnut cake was prepared according to the following standard recipe: butter (150 g), sugar (150 g), salt, and three eggs were mixed. After the addition of baking powder (8 g), ground hazelnuts (100 g) and milk (100 mL) were mixed with the other components. The dough was poured into a cake tin and baked in a household oven at 180 °C for 55 min.

Qualitative Solid-Phase Microextraction (SPME) Experiments with Gas Chromatography–Mass Spectrometry (GC–MS) and Gas Chromatography–Olfactometry (GC–O) Analysis. About 50 g of ground hazelnuts were placed in a 200 mL glass jar, which was then covered with a cling film and closed with a punctured lid. The SPME fiber was cleaned in the injection port of GC, before introducing it through the film into the headspace. The fiber (50/30 μm, DVB/Carboxen/PDMS StableFlex, Supelco, Bellefonte, PA) was loaded at room temperature for 30 min and then manually injected to the GC–MS system for thermal desorption (5 min). A dedicated SPME injection sleeve (0.7 mm inner diameter, Supelco) was used in the injection port. A gas chromatograph (6890, Agilent, Basel, Switzerland) coupled to a mass spectrometer (5973, Agilent) equipped with an autosampler (MPS2, Gerstel, Sursee, Switzerland) was used. An Optima δ-3 column (Macherey-Nagel, Dürren, Germany) with dimensions of 30 m × 0.32 mm (film thickness of 0.25 μm) was used. For confirmation, a column with different polarity was used: Restek 624Sil MS, 60 m × 0.32 mm, film thickness of 0.18 μm (BGB Analytik, Böckten, Switzerland). The injector was held at 250 °C

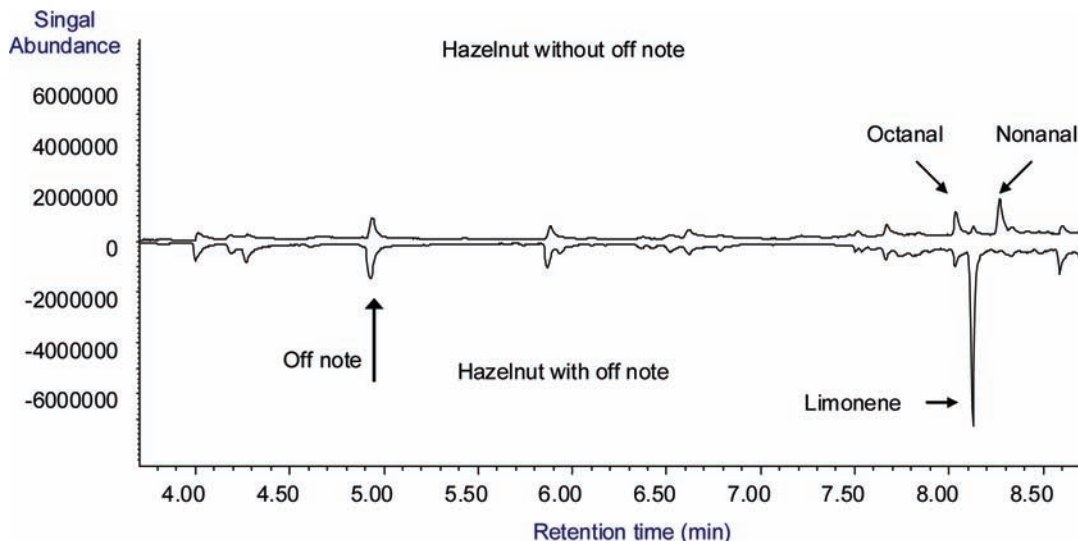


Figure 1. GC–MS chromatograms of SPME-sampled hazelnuts. (Upper trace) Hazelnuts without off-note (HaG). (Lower trace) Hazelnuts with off-note (HaS).

and operated in split mode (1:1). A column flow of 1 mL of helium/min was used. Temperature program: 40 °C for 1 min, increased to 100 °C at 10 °C/min, and then at 40 °C/min to 300 °C for 5 min. For sniffing, a 1:1 split at the end of the column was used to feed an olfactory detection port (ODP, Gerstel), which was flushed with humidified air. Ionization with electron impact was performed at 70 eV, and ions were scanned from 45 to 550 atomic mass units (amu). The quadrupole was held at 150 °C, and the source was held at 230 °C.

Semi-quantitative Analysis by GC–MS. For semi-quantitative analysis, the volatiles were isolated using a simultaneous distillation extraction (SDE) apparatus as described in ref 13. Hazelnuts (100 g) were mixed with water (600 mL), and an internal standard (methyl pelargonate, 1 mg) was added. Extraction was conducted for 1 h using methyl *tert*-butyl ether (100 mL) as the extraction solvent. The organic phase was concentrated on a Vigreux column to 5 mL and analyzed by GC–MS using the following equipment and conditions. A gas chromatograph (6890, Agilent, Basel, Switzerland) coupled to a mass spectrometer (5973, Agilent) equipped with an auto sampler (6890 series, Agilent) was used. A DB-WAX column (Agilent) with dimensions of 60 m × 0.25 mm (film thickness of 0.25 μm) was used. The injector was held at 250 °C and operated in split mode (1:1). The column flow was 1 mL of helium/min. Temperature program: 35 °C for 2 min, increased to 250 °C at 4 °C/min, and then held at 250 °C for 10 min. Ionization with electron impact was performed at 70 eV, and ions were scanned from 29 to 550 amu. The quadrupole was held at 150 °C, and the source was held at 230 °C. Compounds were quantified using the internal standard. Quantification was based on peak area [total ion current (TIC) signal]. Analyses were conducted in triplicate. Neither response factors nor recovery factors were determined.

In addition, PEE was quantified by standard additions using liquid extraction without a heating process: 5 g of ground hazelnuts ($n = 4$) were weighed into a Teflon tube (Oak Ridge, Nalgene, Thermo Fischer Scientific, Rochester, NY), and 4 different amounts of PEE (10 mg/L in ethanol, Fluka) were added to the tubes. A total of 10 mL of ethyl acetate (Scharlau, Barcelona, Spain) was added, followed by intensive shaking for 2 min at room temperature. After centrifugation, 1 μL of the raw extract was injected to the GC–MS system as described for the SPME experiments. m/z 99 was used as the quantifier ion, and m/z 71 and 114 served as qualifier ions. Precisions of the semi-quantitative methods are given as relative standard deviation (RSD).

Sensory Experiments. A blind triangle test was performed on hazelnut cake prepared from normal hazelnuts (HaG) as well as from hazelnuts having the distinct off-flavor (HaS). Three samples of freshly prepared cake (about 30 g each) were coded, placed on a cardboard plate, and presented to the panelist as a triangle test. The panelists ($n = 19$) were asked to identify the odd sample by tasting the samples. The triangle test was carried out according to the instructions of the Swiss Food Manual

(14), and calculations were made using the software difftest (StatBasics, Birmingham, U.K.).

A total of 50 g of ground hazelnuts without off-flavor was put in a 200 mL glass jar, spiked with a mixture of limonene, valencene, and PEE, as well as with PEE alone (all dissolved in ethanol), and immediately closed with a screw lid. The jar was shaken by hand and left for 15 min. The spiked concentrations referred to the results from SDE analysis of hazelnuts with off-flavor (HaS). In a blind sniffing test, these spiked samples were compared by six panelists to normal hazelnuts (with and without the addition of pure ethanol) as well as to hazelnuts with off-flavor (with and without the addition of pure ethanol). The panelists were asked in which samples they perceived the distinct off-flavor.

RESULTS AND DISCUSSION

Verification of the Off-Flavor. In 200 g packs of ground hazelnuts, a distinct metallic, solvent-like off-flavor was perceived by a large number of individuals (including consumer complaints). Because ground hazelnuts are hardly consumed as such, a hazelnut cake was selected as a model system. A cake was prepared from the hazelnuts with solvent-like off-flavor (HaS) and was compared to a cake prepared with a good quality batch of hazelnuts without off-flavor (HaG). In the blind triangle test with hazelnut cakes made from HaS and HaG nuts, 14 of 19 panelists correctly identified the odd sample. This demonstrated a highly significant difference between the two cakes ($p < 0.001$). After the test, panelists stated that the cake made from HaS had unpleasant, solvent-like off-notes. Obviously, the off-flavor is clearly perceived in the cake, and it is related to the hazelnuts used for preparation. This result initiated the present investigations.

Analytical Experiments. Comparing and analyzing the packaging material as such showed that the odor was not coming from the foil used for packaging (no results shown). Thus, various analytical approaches with the ground nuts were undertaken to identify the source of the solvent-like off-flavor. In a first attempt, the volatiles extracted by SPME from HaS were compared to those of HaG. Both chromatograms are displayed in **Figure 1**.

The comparison of the GC–MS chromatograms showed only a few major differences between the two samples in the first 9 min, when the off-note was suspected to elute. The obvious differences could be assigned to limonene in HaS (retention time = 8.1 min) and nonanal in HaG (retention time = 8.25 min). Another minor difference was the presence of valencene in the HaS sample (retention time = 14.4 min; not shown in **Figure 1**). Limonene

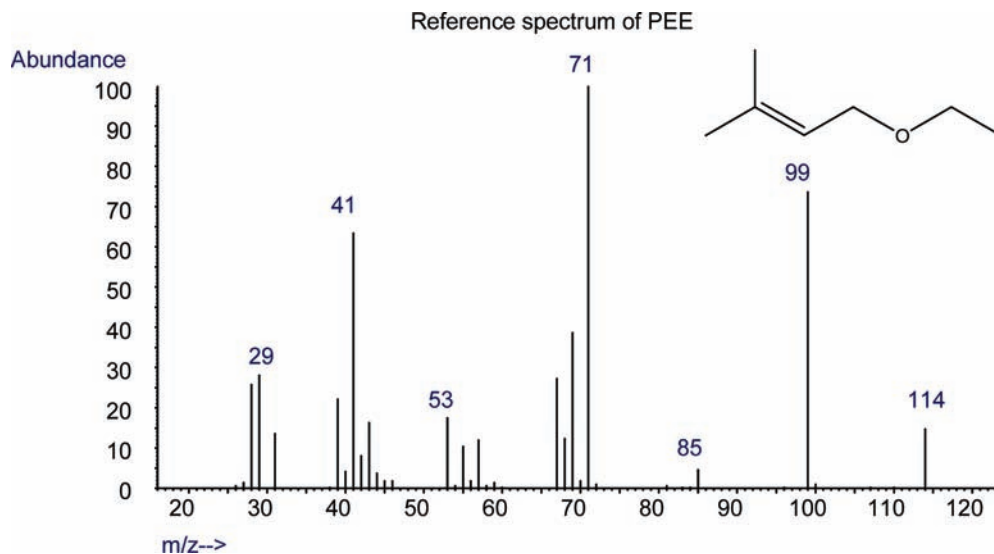


Figure 2. Mass spectrum and structure of PEE.

and other terpenes were already reported in raw earth almonds (15), roasted hazelnuts (9, 10), as well as hazelnut oil (16).

However, limonene, nonanal, and valencene have a different aroma profile than the perceived off-flavor. To obtain better insight into the aroma profiles of the volatile compounds, SPME was combined with GC–O. Three trained panelists conducted SPME–GC–MS sniffing experiments. All of them identified one specific area in the chromatogram with a clear metallic, solvent-like, ethery aroma note, which strongly resembled the off-flavor perceived in the hazelnuts. This smell was detected in HaS but not in HaG, and it was perceived at 4.8 min after injection. At that retention time, a peak was detected in both samples. Looking closer into the chromatogram, the main compound at 4.8 min in HaG was hexanal, which has a green aroma note. This compound did not match the off-note profile. However, at virtually the same retention time, a mass spectrum appeared in HaS that did not match hexanal. By screening through internal MS reference libraries, the spectrum was found to be similar to that of PEE (see **Figure 2** for the structure). It was only in the tailing of the PEE peak where some minor signals were observed that could be assigned to small amounts of hexanal eluting right after PEE. The identification of PEE was confirmed by co-injection of an authentic sample with a SDE extract, with a spiked liquid extract, and with a spiked SPME sample of HaS. The identification of PEE in ground hazelnuts was verified by SPME–GC–MS and sniffing using a different stationary phase (no results shown). The aroma profile of a diluted solution of PEE was strongly reminiscent of the solvent-like off-note in HaS. In analogy to ground hazelnuts, PEE was also detected in hazelnut cake made from HaS using SPME–GC–MS (no results shown).

Thus far, only qualitative data were obtained, and it was not clear if PEE was actually the cause of the described metallic, solvent-like off-flavor. Only sensory evaluation could reveal the actual contribution, which required quantification experiments. To obtain quantitative data, two semi-quantitative analyses were performed. For the analysis via SDE, no recovery was determined, and thus, the values are indicative. Nevertheless, on the basis of our experience, SDE allows for a good estimation of the concentration. The results of HaS and HaG are displayed in **Table 1**. The concentration of PEE in HaS was 0.8 mg/kg. It was not detectable in HaG (LOD = 0.02 mg/kg). The concentration of limonene was 8 times higher in the HaS sample. Myrcene and valencene were found in HaS only. Hexanal and nonanal were found in both samples in

Table 1. Concentration of Selected Volatiles in Ground Hazelnuts^a

	HaS	HaG
origin	Turkey	Turkey
compound		
PEE	0.84	nd
hexanal	7.00	8.80
myrcene	0.05	nd
limonene	3.30	0.38
octanal	2.00	2.42
nonanal	0.65	0.90
valencene	0.73	nd

^a HaS, hazelnuts with solvent-like off-flavor, HaG, hazelnuts without off-flavor. Data are in mg/kg. nd = not detected. Precision: RSD, 20%; *n* = 3.

similar amounts. The results from SDE were confirmed by the standard addition experiment with ethyl acetate extracts. Using this approach, a concentration of PEE of about 0.53 mg/kg (precision: RSD, 5.8%; *n*, 5) was found in HaS. This result excluded that PEE in the hazelnut was an artifact formed during SDE.

Sensory Experiments. To obtain more information on the potential role of PEE in hazelnut off-flavor, a simple sensory experiment was conducted using the semi-quantitative data. The good-quality hazelnuts were spiked with PEE alone (1 mg/kg), as well as with a mix of PEE (1 mg/kg), limonene (4 mg/kg), and valencene (1 mg/kg). These spiked samples were compared to HaS and HaG. The panelists were asked to name the samples having the distinct off-flavor. All panelists identified the spiked samples for clearly having the solvent-like off-flavor note. The off-note was more strongly perceived in the sample containing all three compounds. The HaS samples (with and without ethanol) were always identified as having the off-note. In the HaG samples, none of the panelists found the distinct off-note, which makes a confusing effect from the ethanol unlikely. Because of the setup of this experiment, a *p* value cannot be calculated as performed in the triangle test. Nevertheless, we think that this preliminary experiment indicates that PEE is an important contributor to the off-flavor in HaS. This is consistent with the information obtained by the SPME–GC–MS and SPME–GC sniff measurements. To the best of our knowledge, the identification of PEE as an off-note in hazelnut is new, and we wondered if this is a widespread phenomenon or a single incident. Therefore, we extended the investigation to commercially available samples of ground hazelnuts.

Table 2. Concentration of Selected Volatiles in Five Market Samples of Ground Hazelnuts^a

	A	B	C	D	E
origin	Turkey	Italy	Italy	Turkey	not defined
compound					
PEE	0.38	nd	nd	nd	nd
hexanal	5.30	1.90	1.60	2.30	2.00
myrcene	0.03	nd	nd	nd	nd
limonene	1.30	0.09	0.21	0.33	0.21
valencene	0.32	nd	nd	nd	nd
solvent off-note detected by expert panel	yes	no	no	no	no

^a Data are in mg/kg. nd = not detected. Precision: RSD, 20%; *n*, 3.

Investigation of Market Samples. Five samples of ground hazelnuts were bought from local markets. They were evaluated by the sensory expert panel and the concentration of selected compounds was measured. The data are displayed in **Table 2**. Only sample A showed a solvent-like off-note comparable to HaS. It was also the only sample in which PEE, myrcene, and valencene were found. Limonene was found in all samples, but its concentration was highest in sample A, which showed the off-note.

When off-notes in hazelnuts are discussed, compounds from fat oxidation, such as hexanal, are often described. It is a well-known and important compound for rancidity in many foods, including hazelnuts (12). However, the off-flavor we detected in this investigation was different from known rancidity. The amount of aliphatic aldehydes was even slightly higher in the HaG sample as compared to the HaS sample (see **Table 1** and **Figure 1**). The work described above clearly indicates that PEE and limonene and other terpenes are the compounds responsible. Obviously, these compounds are formed via a different pathway than the known pathway for aldehyde formation during fat oxidation. It is surprising that terpenoid compounds are the cause of the off-flavor. PEE in hazelnuts has not previously been described. It has been reported in tequila flavor (17), in citrus oil (18), and as a top note enhancer in flavorings (19).

It is not clear where the terpenoid compounds originate. One might suspect the off-flavor could be a contamination during transport or storage. Nuts readily absorb terpenes, such as limonene, emitted from, e.g., citrus fruits, which was confirmed by preliminary experiments (results not shown). However, the composition of terpenes that we found is not typical for citrus fruits. Therefore, we consider contamination by another raw material as unlikely. However, a concurrent contamination cannot be fully excluded because the signal for limonene in the SPME-GC-MS chromatogram of HaS was remarkably strong (see **Figure 1**).

Hazelnut quality is affected by variety, geographical origin, growing practices, harvesting time, and post-harvest treatment. This is reviewed in the literature (20). Mold contamination on hazelnuts is widespread. Microbiological changes can occur during growth in the field, harvesting, drying, storage, and shipment. Many microorganisms are described in hazelnuts, e.g., molds, such as *Penicillium*, *Aspergillus*, and *Eurotium*, or bacteria, such as *Staphylococcus* and *Salmonella* (20). Some microorganisms, especially bacteria, are inactivated over time (21) and may no longer be viable in the product. The source of PEE, valencene, and limonene in the hazelnut is not clear. However, some molds (e.g., *Penicillium*) that are known to occur on hazelnuts are capable of forming terpenes, such as limonene, myrcene, and valencene (22). Selected mold strains are also capable of forming prenol alcohols, such as farnesol and geranylgeraniol (23). Microbiological analysis of HaS samples showed that molds were present but a strong contamination was not detected (data not shown). In addition, in some samples of the HaS lot, an elevated water activity was detected (data not shown). The presence of molds and conditions allowing

for their growth indicate a possible impact from molds. Altogether, we assume that, during harvest, production, or storage, metabolic activity of molds has led to the formation of these compounds.

Analysis of five market samples showed that one of five samples had the described off-note that correlated with the presence of PEE. The two samples with off-note, i.e., HaS and sample A (**Table 2**), originated from Turkey. Turkish nuts are known to be more susceptible to kernel molds than the other major hazelnut varieties cultivated in the world (20). Thus, this problem might be more related to Turkish nuts, but the number of samples was too small to draw a final conclusion on the aspect of origin.

In this investigation, PEE was identified as a key compound for a metallic, solvent-like off-note in hazelnuts. Further research is needed to identify the production steps by which the off-flavor compound is formed. Once this has been performed, measures can be taken to avoid off-note formation in hazelnuts and improve product quality. This could not be addressed in the current work.

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