

Identification of Metallic-Smelling 1-Octen-3-one and 1-Nonen-3-one from Solutions of Ferrous Sulfate

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Taste threshold tests of ferrous sulfate (FeSO₄) solutions have been confounded by the presence of putative odorants. To detect the presence of odorants released from these solutions solid-phase microextraction (SPME) was used to collect volatiles in the headspace above FeSO₄ solutions. Gas chromatography–olfactometry of samples collected over three time periods (1, 5, and 16 h) and at two temperatures (22 and 37 °C) revealed the presence of several metallic-smelling odorants in the headspace. Using authentic standards, two of the odorants were conclusively identified as 1-octen-3-one and 1-nonen-3-one. Trace levels of other odorants were also detected, but dilution experiments indicated that 1-nonen-3-one was at least 10 times more potent than anything else released from the solutions. 1-Octen-3-one and 1-nonen-3-one are excellent candidates for the metallic odor responses often observed in threshold testing of solutions of FeSO₄.

KEYWORDS: Metallic taste; ferrous sulfate; lipid oxidation

INTRODUCTION

The term metallic has been interchangeably used to describe taste, mouthfeel, and aroma, depending on the context. Research has shown that for some metal salts, taste is the primary cue for metallic perception, whereas for others tactile or chemesthetic cues are more important, and for even others retronasal and/or olfactory cues are important (1–4). For example, when solutions of ferrous sulfate (FeSO₄) in water are presented to subjects with and without the nose pinched, reports of metallic taste are reduced when the nose is occluded. These results imply that retronasal olfactory perception plays a role in the metallic percepts noted in the solutions (1). Using psychophysical scaling, this experiment was replicated and the same effects were noted for solutions of FeSO₄ (2). Panelists, however, could not distinguish between FeSO₄ solutions and water during a sniff analysis, again suggesting that the retronasal route of perception plays an important role. Additionally, these results suggest that the volatile substances responsible for the metallic taste of FeSO₄ may be formed in the mouth during mastication.

The sniff analysis experiment performed by Lawless et al. (2) used concentrations of ferrous sulfate in water of 0.001 M. Odor ratings over the headspace of the FeSO₄ solutions were not different from those for water using same–different tasks or intensity ratings. According to Civille and Lyon (5), however, the reference for metallic aroma is 0.01% (0.036 M) FeSO₄

diluted in distilled, filtered water. Preliminary benchtop analysis showed that concentrations of ferrous sulfate as low as 0.003 M seem to have a faint metallic odor.

Although the volatile substances seem to be formed in the mouth during mastication, another possibility is that they are formed in solution and become more volatile when in the mouth due to the increase in temperature. If so, when sniff analysis is performed at temperatures equivalent to those in the mouth, solutions of ferrous sulfate in water seem to elicit an olfactory cue. In fact, preliminary threshold work showed that subjects were able to detect FeSO₄ at concentrations >0.001 M when solutions were heated to 37 °C. Therefore, we conducted experiments to further understand the conditions and compounds responsible for metallic odor sensations from ferrous sulfate. These conditions included concentrations higher than those in the previous psychophysical studies as well as a comparison of body temperature and room temperature conditions. The purpose of these experiments was to identify the most potent metallic-smelling odorants released from solutions of FeSO₄.

MATERIALS AND METHODS

FeSO₄ Solutions. Four stimuli solutions of FeSO₄ (0, 0.003, 0.03, and 0.3 M) (Aldrich, Milwaukee, WI) were prepared in odor-free distilled water (Food Club, Skokie, IL) before each experiment. All glassware was washed with soap and water, rinsed with in-house distilled water, and washed with distilled Freon 113 prior to use.

Sample Preparation. Each sample consisted of 5 mL of FeSO₄ solution placed in a 20 mL amber glass headspace vial (22 × 75 mm MicroLiter Analytical Supplies, Inc., Suwanee, GA) containing a glass-encased magnet. The commercially cleaned vials were used as supplied

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and capped with Teflon-lined silicone crimp caps. A blank sample containing distilled water was used as the control. Solutions were prepared prior to each analysis and were allowed to stir for 1 h at 22 ± 2 °C (room temperature) or at 37 ± 2 °C (water bath) prior to odorant extraction. All solutions were then discarded (including headspace vials) after 4 h of use.

SPME. Volatiles were extracted by exposing 0.5 cm of a three-phase SPME fiber (Supelco, Bellefonte, PA; divinylbenzene/carboxen/PDMS) to the headspace in a sample vial for 1, 5, or 16 h. The fibers were immediately desorbed in a GC injector at 250 °C operating in the splitless mode. Samples were prepared in duplicate.

GC. All gas chromatography–olfactometry (GCO), GC–mass spectrometry–olfactometry (GCMSO), and GC–flame ionization detection (GCFID) samples were desorbed in an HP 6890 series GC (Agilent Inc., Avondale, PA) using He at 250 °C and absorbed at 35 °C on a DB-5 column (30 m \times 0.32 mm, film thickness = 0.25 μ m) (J&W Scientific, Folsom, CA) for 3 min, heated at 6 °C/min to 160 °C, then heated at 30 °C/min to 240 °C, and held for 2 min. Retention times were transformed to retention indices (RI) from the chromatographic data for C-7–C-18 *n*-paraffins in Freon 113 collected on each GC system (6). GCFID used H₂ at 0.6 mL/min. The GCMSO effluent was split at 3 to 1 using a splitter (Gerstel MD) with one-third going to the Agilent 5973 in EI mode at 70 eV and two-thirds going to an olfactometer (Datu Inc., Geneva, NY). GCO was conducted on an Agilent 6890 GC modified for GCO and using CharmAnalysis software (Datu Inc.).

GCO. Three subjects collected data during the GCO analysis. Subject 1, a female, age 23, participated in all of the experimental conditions. Subject 2, a female, age 19, sniffed the samples from the 5 h collection. Subject 3, a female, age 26, collected data from the 5 and 16 h sample collections. All assessors were trained to use GCO by smelling a set of standard odorants (7) including 1-octen-3-one, an odorant that has been characterized as mushroom and metallic smelling (8–10). The descriptors “mushroom, metallic, green, rust, and other” were used in the CharmAnalysis software (Datu Inc.) during data collection.

Reference Odorants. *trans*-4,5-Epoxy-*E*-hept-2-enal (CAS Registry No. 78307-41-2) was synthesized courtesy of Dr. Ippolliti (University of St. Thomas, St. Paul, MN). 1-Octen-3-one (CAS Registry No. 4312-99-6) was purchased from Bedoukian Research (Danbury, CT). 1-Nonen-3-one (CAS Registry No. 24415-26-7) was synthesized according to the method of Corey and Suggs (11) in which pyridinium chlorochromate (9.23 g) (Acros Chemicals, Geel, Belgium) was suspended in 60 mL of anhydrous methylene chloride (Sigma-Aldrich, Steinheim, Germany) in a 500 mL round-bottom flask fitted with a reflux condenser. 1-Nonen-3-ol (4 mL, ~20 mmol) in 20 mL of anhydrous methylene chloride was slowly added to the magnetically stirred solution over 2.5 h. Then 60 mL of anhydrous methylene chloride was added and the supernatant decanted from the dark brown/black granular slurry. The solution was passed through a short pad of Florisil [Aldrich, Steinheim, Germany; diluted in Freon 113 1:100 and injected into the GCMSO; 55 (100%), 70 (57.79%), 83 (41.72%), 97 (11.89%), and 111 (15.69%)]. Odorants were identified when their retention indices, mass spectra, and odor character under GCO matched those of authentic standards. Tentative identifications are not reported.

High-Volume Purge and Trap. One hundred milliliters of 0.3 M FeSO₄ was placed into a 200 mL three-neck flask with one arm fitted with an adapter to a 10 \times 0.25 cm Pyrex glass trap packed with 100 mg of a porous polymer resin based on 2,6-diphenylene oxide (Tenax TA 60/80, Supelco, Bellefonte, PA), a plug in the middle, and an adapter to a Teflon gas line delivering dry He for 3 h at 25 mL/min. Prior to trapping, the FeSO₄ solution was stirred for 30 min in the presence of oxygen. The trap was flushed for 1 h with helium (25 mL/min) prior to desorption with a Gerstel thermal desorption system (TDS) (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) set at –10 °C for 5 min, then heated at 60 °C/min to 260 °C, and held for 5 min. All traps were thermally desorbed once prior to use. The transfer was at 300 °C. The volatiles transferred from the trap were absorbed cryogenically (CIS from Gerstel) initially at –60 °C and then heated at 12 °C/min to 260 °C and held for 3 min. Blank traps flushed with He after being thermally desorbed once produced controls with no odor.

Table 1. Odorants from Different Concentrations of FeSO₄ Solutions As Detected by Subject 1 Using GCO

odorant	odor quality	retention index	concn of FeSO ₄ (M)	temp (°C)
	no odor		0	22
	no odor		0.003	22
	no odor		0.03	22
1-nonen-3-one	metallic	1080	0.3	22
unknown	metallic	1168		
	no odor		0	37
1-nonen-3-one	metallic	1080	0.003	37
1-nonen-3-one	metallic	1080	0.03	37
1-nonen-3-one	metallic	1080	0.3	37
unknown	metallic	1168		

Table 2. Odorants Detected in 0.3 M Ferrous Sulfate at 37 °C, Their Odor Qualities, Retention Indices, and Purge Times

odorant	odor quality	retention index	purge time (h)	subject(s)
1-nonen-3-one	metallic	1080	1	1
unknown	metallic	1168	1	1
1-octen-3-one	mushroom	980	5	2, 3
1-nonen-3-one	mushroom/metallic	1080	5	1
1-octen-3-one	mushroom/metallic	980	16	1, 3
1-nonen-3-one	mushroom/metallic	1080	16	1
unknown	metallic	1096	16	1
unknown	other	1104	16	1
unknown	other	1175	16	1

RESULTS AND DISCUSSION

Metallic Odorants in FeSO₄. Table 1 shows the odorants detected by subject 1 during the GCO of FeSO₄ solutions (0.003, 0.03, and 0.3 M) purged at 22 and 37 °C for 1 h. Subject 1 detected odors with a clear metallic smell only at retention index (RI) 1080 and at RI 1168 also reporting a tingling irritation as they eluted. Both odorants were detected by GCO at the highest concentration, however; only the odorant at RI 1080 was detected in the lower concentrations at 37 °C. This suggests that the temperature in the mouth seems to play a role in metallic perception of FeSO₄ solutions. Tests of several water sources all produced background odors of various qualities, however, no odorants were detected in any of the blanks or at the lower concentrations and purge temperatures using the commercial distilled water described above. This would indicate that for subject 1 the odorant detected at RI 1080 was the most potent odorant released from FeSO₄ solutions after a 1 h collection.

The odors detected by GCO analysis of FeSO₄ solutions at the highest concentration and temperature (0.3 M and 37 °C) purged for 5 and 16 h are listed in Table 2. In the 16 h purge of the solutions subjects 1 and 3 detected a total of five odors. Only one odor was detected by both of the subjects at a RI of 980. Three of the five odors were described as having a metallic odor component. Two of these odors (RI 980 and 1080) were also detected in the 5 h collections. Subject 1 was the only subject to detect the odor at RI 1080, whereas subjects 2 and 3 were the only subjects to detect the odor at RI 980 after the 5 h purge. The differences in the responses of the subjects (all duplicated) are consistent with the well-documented presence of odor-specific sensory deficits found extensively in the human population (12).

Using a high-volume purge and trap (4.5 L: 3 h at 25 mL/min) and TDS desorption into a GCMSO, the odorant at RI 980 was identified as 1-octen-3-one. The odor quality and RI of 1080 were consistent with *trans*-4,5-epoxy-*E*-hept-2-enal and

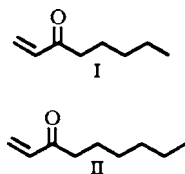


Figure 1. Metallic-smelling compounds detected in FeSO_4 solutions, 1-octene-3-one (I) and 1-nonen-3-one (II).

1-nonen-3-one. Comparison with authentic standards ruled out *trans*-4,5-epoxy-*E*-hept-2-enal. 1-Nonen-3-one, however, matched the RI, odor character, and mass spectral data of the metallic odorant at 1080. The remaining metallic odorants detected at 1096 and 1168 were less potent than the others and produced very little response even in the high-volume purge and trap. Therefore, 1-octene-3-one (I) and 1-nonen-3-one (II) are the best candidates for the metallic odors detected by the three subjects under the various conditions (Figure 1).

1-Nonen-3-one has been identified as a volatile component of Spanish aged red wines (13), raspberry aroma (14), and thermally oxidized polyethylene (9). In all of the reports of the detection of 1-nonen-3-one in foods, 1-octen-3-one was also found. The reported threshold for 1-nonen-3-one is 8×10^{-6} $\mu\text{g}/\text{kg}$ (15), which is markedly lower than the threshold of 1-octen-3-one: 5.5–110 $\mu\text{g}/\text{kg}$ in oil to 0.06–0.13 $\mu\text{g}/\text{kg}$ in water (10).

In conclusion, metallic-smelling odorants were detected in solutions containing FeSO_4 and water using SPME and purge and trap. These results suggest that the metallic-smelling odorants are formed in FeSO_4 solution, although they may be formed in the mouth as well and that temperature plays a role in the perception of their metallic odor. Just where and how these odorants originate remain to be determined, but the two most potent have been conclusively identified as 1-octen-3-one (I) and 1-nonen-3-one (II).

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