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GC–Olfactometric Characterization of Aroma Volatiles from the Thermal Degradation of Thiamin in Model Orange Juice

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Model orange juice solutions containing 0.024 mM thiamin hydrochloride were stored for up to 8 weeks at 35 °C in amber glass containers. Volatiles were evaluated, primarily, using gas chromatography (GC) with olfactometry but also with flame ionization detector , pulsed-flame photometer detector (PFPD) (sulfur specific), and MS detection. Both 2-methyl-3-furanthiol (MFT) and its dimer, bis(2−methyl−3−furyl) disulfide (MFT−MFT) were identified thus confirming that thiamin could serve as the precursor to these potent off-flavors in thermally degraded citrus juices. Thirteen aroma active components were observed. MFT and MFT−MFT were observed after only a few days storage, and produced 33% of the total aroma activity after 7 d storage. Both compounds were observed olfactometrically earlier than they could be detected using PFPD. Other aroma-active compounds included 4,5-dimethylthiazole (skunky, earthy), 3-thiophenethiol (meaty, cooked), 2-methyl-4,5-dihydro-3(2H)-thiophenone (sour-fruity, musty, green), 2-acethylthiophene (burnt), 2-formyl-5-methylthiophene (meaty), and 2-methyl-3-(methyldithio) furan (meaty).

KEYWORDS: Thiamin; 2-methyl-3-furanthiol; bis(2−methyl−3−furyl) disulfide; aroma; gas chromatography-olfactometry; GC−O; Pulsed Flame Photometric Detection; PFPD.

INTRODUCTION

Thiamin (vitamin B1) can thermally decompose to produce potent aroma sulfur compounds which have roasted, meaty notes. Thiamin's thermal decomposition has been examined in both low and high water activity systems, usually at high temperatures (110−130 °C) for short times (1−6 h). Model studies have demonstrated that pH, temperature, and heating time determine which decomposition pathway will be favored and ultimately determine the final products observed (*1−4*). Recent studies have focused on the formation of meat flavors from the thermal degradation of thiamin (*5−7*), as well as through a Maillard reaction involving cysteine and various sugars (*8−10*). In meat systems, both reaction pathways are active.

One of the most significant thiamin thermal degradation products is 2-methyl-3-furanthiol (MFT). Both it and its dimer, bis(2−methyl−3−furyl) disulfide (MFT−MFT) produce intense savory, meaty aromas. MFT−MFT is the most potent food aroma reported to date and has an odor threshold as low as 8.9 $\times 10^{-11}$ mM water (*11*), while its monomer has a threshold of 6.14 $\times 10^{-8}$ mM water (*12*). They are well-documented components of meat flavors (*6, 13, 14*). MFT−MFT is also responsible for the characteristic aroma of thiamin in vitamin B1 tablets (*11*). Both MFT and bis(2−methyl−3−furyl)disulfide have been found in cooked brown rice (*15*) and recently reported in reconstituted grapefruit juice (*16*). MFT has recently been identified in coffee (*17*) and as an off-flavor in stored orange juice (*18*). Both compounds are extremely difficult to quantify, as they are potent aroma-active components that typically exist in food systems at concentrations well below the detection level of most instrumental detectors.

Thiamin is the second most abundant water-soluble vitamin in orange juice, and is a more concentrated source for vitamin B1 than many foods that are better known sources of this vitamin, such as whole wheat bread (*19, 20*). The thermal degradation of thiamin at high temperature for short times has been well studied as have room temperature photochemical degradations, but no prior work was found on the thermal degradation of thiamin at elevated room temperature. Because orange juice is a relatively rich source of thiamin, our goal was to determine if thiamin was the probable source of these observed off-flavors in nonrefrigerated juices. To achieve this goal, the aroma active volatiles formed in thiamin-containing model orange juice solutions stored at 35 °C in the absence of light will be identified and characterized.

MATERIALS AND METHODS

Materials. The following compounds were obtained commercially from Acros Chemical (New Jersey): glucose, sucrose, citric acid, 2-formyl-5-methylthiophene, 2-methyl-3-furanthiol, dimethyl sulfide, 2-acetylthiophene, and bis(2−methyl−3−furyl) disulfide. Fructose and

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tripotassium citrate were obtained from Fisher (New Jersey). Thiamin hydrochloride, 2-methyl-4,5-dihydro-3(2H)-thiophenone, and 2-Methyl-3-(methyldithio) furan were obtained from Sigma (Steinheim, Germany). 4,5-Dimethylthiazole was a gift from Florida Treatt Inc. Hydrogen sulfide was obtained from Matheson Gas Products (Montgomeryville, PA).

Preparation of Model Orange Juice Solutions. Model orange juice (MOJ) solutions, at an adjusted pH of 3.8, were prepared according to Peleg and co-workers (*21*), with modifications. A 100 g MOJ solution (% w/w) contained the following compounds: sucrose, 5.0; fructose, 2.5; glucose, 2.5; citric acid, 1.0; tripotassium citrate, 0.5, double distilled water, 88.5. Thiamin hydrochloride was added at 0.024 mM. Fifty mL aliquots were transferred to 120 mL amber vials, and a nitrogen atmosphere was added by gently flowing N_2 into the vials before sealing. Samples were then stored in the dark at 35 °C for up to 8 weeks to eliminate possible photochemical reactions. A control sample was also prepared under the same conditions, except without thiamine hydrochloride.

Sample Preparation. Thiamin-MOJ samples were pulled on the following days: 0, 1, 7, 14, 28, 42, and 56. Ten mL aliquots were then taken and placed into a 30 mL vial with a septum lid and given a nitrogen headspace. Samples were placed in a 40 °C water bath and equilibrated for 15 min. Samples were then exposed to SPME: 50/30μm DVB/Carboxen/PDMS StableFlex (Supelco, Bellefonte, PA) for 30 min.

Gas Chromatography−Pulse Flame Photometric Detector (GC−PFPD). Samples were separated by SPME using an HP-5890 series II GC (Palo Alto, CA) using an O-I-Analytical 5380 PFPD with a DB-5 column (30 m × 0.32 mm i.d. x 0.25 μm) from J&W Scientific (Folsom, CA). Initial oven temperature was 40 °C and increased to a final temperature of 290 °C at 7 °C/min. Injector (Gerstel, Baltimore, MD, model CIS-3) and detector temperatures were 200 and 250 °C, respectively. Helium was used as the carrier gas at a flow rate of 2 mL/min. Compounds were monitored on the PFPD for sulfur in two different manners: linear and exponential responses. Chromatograms were recorded using Chromperfect (Justice Innovations, Inc., Mountain View, CA). Samples were run in triplicate.

Quantitative Analysis. MFT and MFT−MFT were quantified by means of standard calibration curves containing 0.007, 0.01, 0.05, 0.1 ppm and 0.001, 0.01, 0.1 ppm of MFT and MFT−MFT, respectively. The standards were prepared in MOJ solutions that did not contain thiamin. The samples were extracted and analyzed in triplicate using the GC−PFPD under identical conditions as the storage samples that contained thiamin.

Gas Chromatography. An HP-5890A GC (Agilent Technologies, Palo Alto, CA) with a standard flame ionization detectorr was used to separate the model orange juice extracts using either a DB-5 (30 m × 0.32 mm i.d., 0.5 μm film thickness, J&W Scientific (Folsom, CA)) or DB−Wax (30 m × 0.25 mm i.d., 0.5 μm film thickness, J&W Scientific (Folsom, CA)). Initial oven temperature was 40 °C and increased to a final temperature of 265 °C at 7 °C/min with no hold. Injector and detector temperatures were 220 and 250 °C, respectively. Data were collected and recorded using Chromperfect Software.

GC−Olfactometry. GC−O equipment and conditions were identical to those described in earlier studies (*22*). The olfactometry panel consisted of two trained panelists, 1 male and 1 female, between 25 and 30 yrs old. Panelists were trained in a manner similar to Rouseff and co-workers (*23*), using a standard solution of 11 compounds typically found in citrus juice (ethyl butanoate, *cis*-3-hexenol, *trans*-2-hexenal, α−pinene, myrcene, linalool, citronellol, carvone, terpin-4-ol, geranial, and neral). The standard mixture helped train panelists in a time-intensity scale, optimum positioning, and breathing techniques. Panelists also were trained by evaluating at least 10 commercial orange juice flavor extracts in order to gain experience and consistency. Panelists were not used for this study until they demonstrated the ability to replicate aroma intensity responses in the practice juice extracts. Panelists ran each experimental sample in duplicate and summary reports were generated for each aromagram. Only peaks detected at least 50% of the time were included in this study. Results from each panelist's aromagram were normalized with their own maximum peak intensity (set to 100) before being averaged.

Gas Chromatography−Mass Spectrometry (GC−MS). Sample separation was performed on a Finnigan GCQ Plus system (Finnigan Corp., San Jose, CA), using a J&W Scientific DB-5 column (60m × 0.25 mm i.d. × 0.25 μm film thickness (Folsom, CA)). The MS was operated under positive ion electron impact conditions: ionization energy, 70 eV; mass range, 40−300 amu; scan rate, 2 scans/s; electron multiplier voltage, 1050 V. Transfer line temperature was 275 °C. Initial column oven temperature was 40 °C and increased at 7 °C/min to a final temperature of 275 °C. Injector temperature was 250 °C. Helium was used as the carrier gas at a linear velocity of 32 cm/s. When searchable spectra could not be obtained for compounds of interest because of low signal-to-noise ratio, chromatograms of selected masses were reconstructed from the MS data matrix. These selected ion chromatograms (SIC) employed at least three unique *m/z* values from the mass spectrum of standards were used as identification aides. Whenever possible, the molecular ion (M^+) was chosen as one of the three *m/z* values.

Injector Decomposition Study. A standard solution of MFT was injected onto the GC−PFPD under similar chromatographic conditions outlined above with changes to the injector temperature. Samples were injected at three temperatures: 160, 180, and 200 °C.

Microbiological Analysis. Thiamin MOJ samples from day 0 and day 56 were plated for microbial counts using standard microbial techniques (*24*). Samples were run in duplicate using orange serum agar (OSA), acidified potato dextrose agar (APDA), and plate count agar (PCA) plates. OSA and PCA plates were incubated at 30 and 35 °C, respectively, for 24 h, while APDA plates were incubated at 25 °C for 48 h. Dehydrated media was purchased from Difco (Becton, Dickindon and Company, Sparks, MD.). Each medium was prepared according to manufacturer's directions, and plates were poured using standard aseptic techniques.

RESULTS AND DISCUSSION

This study differs from previous thiamin thermal degradation studies (*4, 5, 25−28*) in terms of time−temperatures, sample matrix, detection devices, and thiamin levels employed. Whereas previous studies were conducted at high temperatures (110−130 °C) and short times (1−6 h), this study was conducted at relatively low temperature (35 °C) and long times (8 weeks). The former conditions are typical for cooking and roasting, whereas the time−temperature conditions chosen for this study represent the most extreme conditions a juice would likely encounter during storage. In this study, GC−O is employed to identify the number, quality, and the relative aroma intensity of the thiamin degradation products. Prior studies primarily employed GC-MS to determine total volatiles without directly determining their aroma activity. Finally, thiamin concentrations chosen for this study are more typical of those found in citrus juices (0.024 mM), whereas prior studies employed considerably higher concentrations, some as great as 296 mM or more than 12,000 × higher concentrations(*29*).

Day 7 and 42 Aromagrams. Normalized aromagrams from thiamin model orange juice solutions stored at 35 °C for 7 and 42 d are compared in **Figure 1**. These two dates were chosen to represent short and long-term storage conditions. Thirteen aroma volatiles were observed between the two storage times; 11 aroma active volatiles were found after 7 day storage, but only 8 aroma volatiles were observed after 42 day storage. Six of the eight aroma active volatiles found in the day 42 samples were also found in the day 7 samples. Thus almost half of the aroma volatiles observed after 7 day storage were no longer observed after 42 day storage. Although 5 aroma volatiles were lost between day 7 and day 42 samples (peaks 1, 2, 6, 8, and 11), two new aroma volatiles were generated (peaks 5 and 10). Total aroma intensity also decreased from day 7 to day 42.

Of the aroma components detected, MFT (peak 4), roasted meaty aroma, and its dimer, MFT−MFT (peak 14), roasted meat/savory aroma, were among the most intense. MFT is a

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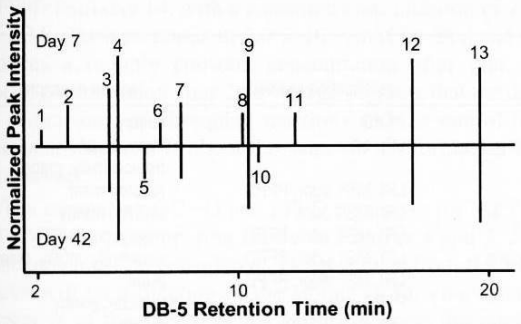
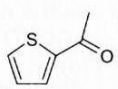


Figure 1. SPME headspace samples of GC–O aromagrams comparing day 7 and 42, where peak intensities are inverted for day 42 data. Peak number corresponds to compound numbers in Table 1.

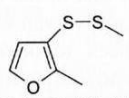
well-established thermal degradation product of thiamin (*7*) and has been reported in stored orange juice (*18*). The intensity of MFT−MFT peaks in the aromagrams in **Figure 1** is only slightly less than that of the monomer, MFT, strongly suggesting that it could be a potent storage off-flavor as well. Combined, these two compounds comprise 33% of the total aroma activity after 7 day storage and 48% of the aroma peak area after 42 d storage. Because the dimer (peak 13) has only slightly less aroma intensity than MFT (peak 4) at both sampling times and there are fewer aroma volatiles at day 42, the relative impact of dimer will increase with increased storage time.

Peaks 3, 9, and 12 are common to both sampling times and have been characterized but not identified (see **Table 1**). These peaks were characterized as having tropical fruity/grape, fertilizer/earthy, and savory/meaty/sulfury attributes, respectively. All three peaks diminish between 7 d storage and 42 d storage. Many of the peaks that are lost after extended storage also remain to be identified. However, peak 6, with meaty, cooked attributes; peak 8, with a burnt aroma; and peak 11, with a meaty aroma, have been identified as 3-thiophenethiol, 2-acetylthiophene, and 2-methyl-3-(methyldithio)furan. The two new compounds found after 42 day storage, peak 5 with skunky/earthy attributes and peak 10 with a meaty aroma, have been identified as 4,5 dimethylthiazole and 2-formyl-5-methylthiophene, respectively. Their structures are shown below:

3-Thiophenethiol (peak 6)

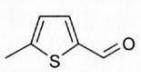


2-Acetylthiophene (peak 8)



2-methyl-3-(methyldithio) furan (peak 11)

4,5-Dimethylthiazole (peak 5)



2-Formyl-5-methylthiophene (peak 10)

Aroma Volatile Identifications. Table 1 lists the aroma active compounds observed, their linear retention index values (LRI) on DB-5 and DB−Wax columns, aroma descriptors, and identification procedures employed. Linear retention index values and aroma descriptors were used to make preliminary identifications; these aroma descriptors and retention values were confirmed using authentic standards. Final confirmation was achieved by comparing GC−MS data from the sample with that of standards. The PFPD is one of the most sensitive and selective detectors for studying sulfur containing volatiles. The responses from this detector were used as further confirmation for peaks thought to be due to sulfur volatiles. PFPD peaks in the sample that occurred at the same retention time as an authentic standard were considered additional proof of the peaks' identity.

Peaks 4 and 13 are the major flavor impact compounds from the thermal degradation of thiamin and have been identified as 2-methyl-3-furanthiol, MFT, and bis(2−methyl−3−furyl) disulfide, MFT−MFT, the dimer of MFT. Identification was based on the cumulative evidence of retention matching on both DB-5, carbowax columns, aroma characteristics, PFPD data, and MS evidence. MFT was confirmed using SIC chromatograms at *m/z* 114(M^+), 106, and 86. In the case of MFT, all three SIC's produced distinct peaks at the identical LRI value as the standard.

The first aroma active peak shown in **Figure 1** occurs in the region where hydrogen sulfide and dimethyl disulfide would be expected to elute. Both hydrogen sulfide (*1*, *5*) and dimethyl disulfide (*4*, *26*) have been reported as thiamin degradation products. Therefore, the first 6 min of the day 7 aromagram and corresponding PFPD response is shown in **Figure 2,** to better illustrate which sulfur compound corresponds best with the first aroma peak. Hydrogen sulfide elutes before dimethyl sulfide and an unidentified sulfur peak. It is readily apparent that the first aroma peak elutes at the same time as dimethyl sulfide.

As illustrated in **Figure 1,** aroma peaks 1, 2, 6, 8, and 11 were only detected during the first few days of storage at 35 °C storage. These were weak intensity aroma peaks that were completely absent after 42 d storage. Peak one has already been identified as dimethyl sulfide. The second GC−O peak has been tentatively identified as 1-pentanol, based on its aroma description of fruity/green and its LRI values. Aroma peak 6 had a meaty, cooked aroma. It has been tentatively identified as 3-thiophenethiol on the basis of its aroma characteristics and retention characteristics on DB-5. SIC−MS chromatograms using *m/z* 116(M^+) and 71 (the only major peaks in the Wiley library spectra for this compound) produced peaks at the same retention time as a PDPF peak and the GC−O peak in question. All of these peaks occur at the literature LRI for this compound. However, this identification must be considered tentative as no standard could be obtained for comparison purposes. Aroma peak 8 was identified as 2-acetylthiophene on the basis of the match between its retention characteristics on DB-5 and carbowax, MS−SIC's of *m/z* of 110, 125, and 83 peaks, PFPD response with identical LRI and odor match with a standard. Aroma peak 11 was identified as 2-methyl-3-(methyldithio) furan on the basis of the matching of its aroma characteristics, retention characteristics, and MS characteristics of SIC's of *m/z* 160, 113, and 85, compared to an authentic standard.

The identities of peaks 3, 9, and 12 could not be determined. As seen in **Figure 1**, all three peaks were observed in samples stored for both 7 and 42 d. Peak 3 displayed a topical fruit aroma and probably does not contain sulfur, for there was no associated PFPD peak (see **Figure 2**). Its fruity aroma and early retention value suggests it might be an ester (fruity) or a potent sulfur volatile whose concentration was above its threshold but below the detection limits of the sulfur detector. Peaks 9 and 12 were major aroma components in the 7 d sample, but were only about half as intense after 42 d storage. Peak 12 had a DB-5 LRI value of 1403, with an aroma that was described as savory, meaty, and sulfury. It may also be due to the same aroma volatile reported by Baek and co-workers (*30*) in a process flavor, because it had similar retention and aroma characteristics. It had a DB-5 LRI of 1393 and described its aroma as spicy, burnt, meaty, and roasty. They were also unable to identify this material.

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Table 1. Aroma Active Compounds Detected in a Model Orange Juice Solution

no.	LRI[a] (DB5)	LRI[a] (DB–Wax)	compound name	identification method	aroma descriptor
1	681		dimethyl sulfide[e]	PFPD	sulfury
2	766		1-pentanol[e]	LRI, odor	fruity, green
3	843		unknown		tropical fruity, grape
4	863	1305	2-methyl-3-furanthiol	LRI, MS[c], odor, PFPD	roasted meat
5	928	n.d.[b]	4,5-dimethylthiazole	LRI, MS[c], odor	skunky, earthy
6	967		3-thiophenethiol[e]	LRI, MS[d], odor, PFPD	meaty, cooked
7	998	1506	2-methyl-4,5-dihydro-3(2H)-thiophenone	LRI, MS[c], odor, PFPD	sour-fruity, musty, green
8	1085	1785	2-acetylthiophene	LRI, MS[c], odor, PFPD	burnt
9	1095		unknown	PFPD	fertilizer, earthy
10	1112	1785	2-formyl-5-methylthiophene	LRI, odor, PFPD	meaty
11	1178	n.d.	2-methyl-3-(methyldithio) furan	LRI, MS[c], odor, PFPD	meaty
12	1403		unknown	PFPD	savory, meaty, sulfury
13	1543	2150	bis(2-methyl-3-furyl) disulfide	LRI, MS[c], odor, PFPD	roasted meat, savory

[a] Linear Retention Indices. [b] Not detected. [c] Identified by comparison of the compound's mass spectrum compared with those of an authentic standard. [d] Identified by comparison with spectra from Wiley Spectral library. [e] Tentative identification. PFPD = PFPD peak observed at DB-5 LRI.

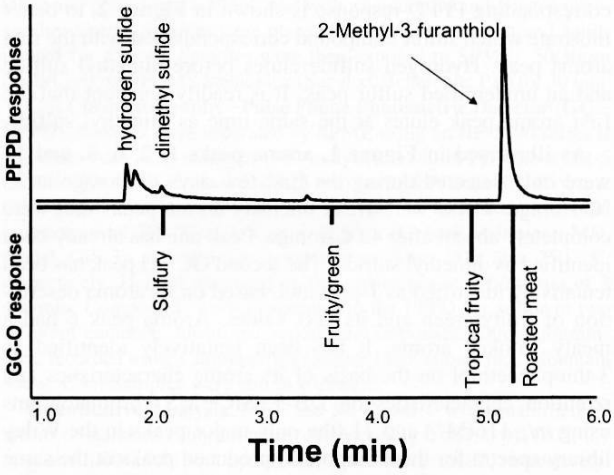

Figure 2. Comparison between PFPD chromatogram and corresponding aromagram from a model orange juice stored for 7 d at 35 °C. First 6 min shown, to clearly illustrate which of the early PFPD peaks were aroma active as well as to demonstrate that there was no sulfur activity associated with aroma peaks 2 and 3. SPME injection using a DB-5 column. See methods section for additional experimental details.

Of those aroma peaks that were only seen toward the end of the storage study, peak 5 was identified as 4,5-dimethylthiazole (**5**), and peak 10 was identified as 2-formyl-5-methythiophene. SIC's of *m/z* 114, 98, and 71 produced peaks at the identical retention values as authentic 4,5-dimethylthiazole. Aroma quality and retention values were also identical to an authentic standard. Earlier studies had found this compound in greatest concentration at pH 9.5 under high-temperature short-time conditions (*4*, *5*, *27*). However, at the low-temperature, acidic pH of the model orange juice in this study, it was only a minor aroma peak. Because citrus juices are highly unlikely to be stored at this temperature for this length of time, it is also unlikely that this compound would be found in many commercial juices. The identification of peak 10 was based on its meaty aroma and the fact that it also produced a PFPD peak at the exact retention time as 2-formyl-5-methythiophene. This peak also matched the FID−LRI values on DB-5 and carbowax and the MS fragmentation data of 5-formyl-5-methylthiophene.

Peak 7 has been identified as 2-methyl-4,5-dihydro-3(2H)-thiophenone, because its sensory, chromatographic, and mass spectral properties were identical to that of an authentic standard.

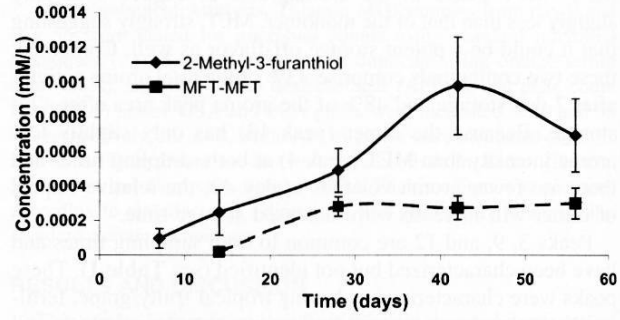

Figure 3. MFT and MFT–MFT concentrations in thiamin model orange juice solutions stored at 35 °C in the absence of light as determined by PFPD.

SIC's of *m/z* of 116, 88, and 60 produced peaks at the identical retention value as the standard.

Quantification of MFT and MFT−MFT. Both compounds possess a roasted meat or savory aroma, which is highly desirable in meat and savory flavors but are definite off flavors in citrus juices. MFT−MFT is one of the most potent food aromas ever measured. It produces an aroma peak at levels well below that of the PFPD detector (1 pgS/s) and is thus difficult to quantify even with the most sensitive detectors. MFT−MFT has been reported in a recent GC−O study of thermally concentrated grapefruit juice (*16*), but no quantitation was attempted.

Thiols are known to readily oxidize into disulfides (thiol dimers). This was demonstrated in a model study on the oxidative stability of odor-active thiols, which included MFT (*31*). MFT and its dimer were quantified during the course of this storage study using the PFPD. Results are shown in **Figure 3**. Even though the PFPD detector is one of the most sensitive sulfur detectors, appreciable aroma peaks for both MFT and MFT−MFT were perceived by GC−O before any PFPD peaks were observed. For example, MFT−MFT was first detected on d 14 using the PFPD, whereas it produced a significant aroma peak on day 7. Using a similar extraction procedure (SPME), panelists in another GC−O study could detect as little as 26 fM MFT in orange juice (*18*). As shown in **Figure 3**, MFT concentration begins to increase with increasing storage time up to 42 d of storage then decreases from 9.8×10^{-4} mM at d 42 to 7.0×10^{-4} mM at d 56. As expected, the dimer of MFT, MFT−MFT, cannot be formed until a certain amount of the monomer has formed. Thus, its concentration will always lag behind that of the monomer. The dimer is not detected with

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the PFPD until day 14, with a measured concentration of 2.0×10^{-5} mM, which increases to 3.0×10^{-4} mM by 28 d and then maintains a roughly constant concentration after that. The constant concentration after 28 d storage suggests that the dimer also participates in subsequent reactions and the rate of these subsequent reactions is about the same as the formation from the monomer.

When comparing GC−O and PFPD responses for MFT and MFT−MFT as in comparing results in **Figures 1** and **3**, a few distinctions must be considered. The response from the PFPD detector will be a function of the atomic sulfur concentration irrespective of the source of the sulfur, whereas the intensity indicated by human assessors for GC−O aromagrams will be a function of the human sigmoidal dose−response to aroma. The aroma intensities for both MFT and MFT−MFT in **Figure 1** do not change appreciably between 7 and 42 d, whereas changes in PFPD responses were observed. Human olfactory detection limits for some thiols are appreciably lower than that of the PFPD. For example, at day 14 the concentration of MFT−MFT was 2.3×10^5 times greater than its aroma threshold and increased to 3.39×10^6 times greater than threshold at day 42. At these levels, it should not be surprising that GC−O aroma responses did not vary as they were saturated, but the PFPD response (being less sensitive) was not saturated.

Thiamin as a Source of MFT and MFT−MFT in Citrus Juices. It is generally accepted that both MFT and its dimer are formed during the thermal decomposition of thiamin in acid media at high temperature (*25*, *32*). However, MFT can potentially be formed from two other pathways. It can be produced through a Maillard reaction involving cysteine and various simple sugars (*8*, *9*), as well as from the reaction of norfuraneol and cysteine (*33*). Bolton et al. (*34*) studied a thiamin/cysteine model system in order to determine the role of cysteine in the formation of MFT. Using labeled ^{34}S-cysteine, they determined that cysteine can contribute to MFT formation in the presence of thiamin, but that thiamin was required for the formation of MFT. Few studies have examined orange juice for the presence of cysteine. However, a recent report by Heems et al. (*35*) reported no measurable amounts of cysteine in orange juice (limits of detection = 152 μg/L). Because both alternate pathways for the formation of MFT require the presence of cysteine and cysteine is apparently absent from orange juice (and probably grapefruit juice), it is therefore unlikely that MFT can be formed in any way other than the direct decomposition of thiamin. MFT can also form from the reaction of 4-hydroxy-5-methyl-3(2H)-furanone, norfuraneol, and either cysteine or hydrogen sulfide (*33*, *36*). Norfuraneol's presence is considered a degradation product of pentoses; however, a reaction pathway from hexoses was proposed by Hofmann et al. (*33*). The presence of norfuraneol in the control model orange juice solution could point toward the formation of MFT through the mechanism with hydrogen sulfide. To test for the presence of norfuraneol, GC−O and GC-MS analyses were performed on the control model orange juice solution after 56 d storage. No norfuraneol was detected, thus eliminating the last alternate MFT formation pathway.

Possible GC Injector Thermal Artifacts. Because thiols are unstable and readily dimerize, and because there are literature reports (*37*) of sulfur artifact creation after exposure to the high temperature of the gas chromatograph injector, additional experiments were conducted to determine if MFT−MFT was formed from MFT in the GC injector. Three injector temperatures were chosen, 160, 180, and 200 °C. In each case, a standard containing 0.1 ppm MFT was injected onto the GC to determine if any dimer could be detected. In all cases, only MFT was detected by the PFPD, and its peak height did not increase with decreasing injector temperature. Therefore, it appears that MFT was not degraded in the injector, and that the MFT−MFT detected in this study was not an injector port artifact.

Possible Microbiological Artifacts. Microbial activity is a well-known means of producing of aroma compounds, providing they are present. However, extensive precautions were observed in this study to maintain microbial sterility in the storage samples. To confirm that none of the aroma-active compounds observed in this study were derived from microbial organisms, samples were evaluated for microbial content. Samples from day 0 and day 56 were plated using OSA for an aciduric count, APDA for a yeast/mold count, and PCA for a total plate count. Results from all plates indicated counts less than 10 cfu/mL with no visible growth. Therefore, the aroma compounds detected in this study were not the result of microbiological contamination.

CONCLUSIONS

Thiamin has been shown to be the precursor to the potent aroma compounds MFT and its dimer, MFT−MFT, in model orange juice solutions stored at 35 °C. Although the study lasted for eight weeks, both compounds produced major aroma peaks after 7 d storage. Both compounds have been shown to have a profound impact on the aroma of these stored solutions, responsible for 33 and 48% of the total aroma at day 7 and 42, respectively. The relative aroma contribution of these two compounds was shown to change with storage time. Both these meaty off flavors have been reported in prior stored and/or heated orange and grapefruit juices. Because citrus juices are rich sources of thiamin, and our model juice studies have demonstrated that, from an olfactory point of view, these two compounds are among the major aroma impact compounds formed, it appears that thiamin is the precursor for these off flavors in citrus juices. However, to definitively prove that thiamin is the source of these off flavors in citrus juices, it remains for isotopically labeled thiamin to be exposed under similar conditions to see if isotopically labeled MFT or its dimer could be detected.

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