2-Methyl-3-furanthiol and Methional Are Possible Off-Flavors in Stored Orange Juice: Aroma-Similarity, NIF/SNIF GC−O, and GC Analyses

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The occurrence of methional in fresh orange juice, and possible occurrence of β-damascenone in heated orange juice, has been previously suggested. Here we report on the occurrence of 2-methyl-3-furanthiol in the headspace, collected by solid-phase micro-extraction, of fresh, pasteurized, and stored orange juice. The contents of 2-methyl-3-furanthiol and methional were quantified, and the relative level of β-damascenone was estimated, in the headspace of fresh, pasteurized, and stored orange juices using the nasal impact frequency (NIF) and surface of NIF (SNIF) GC−Olfactometry procedure. 2-Methyl-3-furanthiol concentrations were 2 ng/L in fresh and pasteurized Shamuti orange juice, and 270 ng/L in stored juice of the same variety. Methional concentrations were 550, 830, and 11550 ng/L in fresh, pasteurized, and stored pasteurized juices, respectively. β-Damascenone content appeared to have increased during pasteurization and storage. Aroma-similarity experiments strongly suggest that 2-methyl-3-furanthiol and methional, at the levels found in stored orange juice (21 days at 35 °C), contribute to stored orange juice off-flavor.

Keywords: *2-Methyl-3-furanthiol; methional; β-damascenone; orange juice; NIF; SNIF; GC−O; off-flavor; Citrus sinensis*

INTRODUCTION

The instability of fresh orange juice aroma during processing (e.g., heat treatment) and subsequent storage has been extensively studied (*1*−*3*). Decreased levels of characteristic fresh orange juice aroma compounds on one hand, and off-flavor formation on the other, lead to the distinct aroma differences between fresh and processed juice (*4*). 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol), 4-vinylguaiacol, and α-terpineol have been previously reported as storage-related off-flavors that may contribute to the typical overall aroma of stored commercial orange juice (*1*, *3*, *5*, *6*). Marin et al. (*4*) used a gas chromatography−olfactometry (GC−O) aroma dilution technique and Charm analysis for studying aroma activity in citrus juices. They noticed that processing altered the aroma profile of the juices, as the four most odor-intense peaks in the unprocessed juice were greatly diminished during processing. Rouseff and co-workers (*7*) used the same GC−O technique to identify volatiles in thermally abused orange juice. The aromagram of juice stored at 40 °C for 4 months revealed five intense aroma peaks characterized as bread, sulfur, rotten, and floral-like (two peaks), none of which were observed in similar aromagrams of juice stored at 4 °C for 2 weeks. Recently, Hinterholzer and Schieberle (*8*) analyzed fresh hand-squeezed Valencia orange juice using the GC−O aroma extraction dilution analysis (AEDA) procedure, and identified 42 volatiles with dilution values greater than 4.

Preliminary GC−O time−intensity experiments and previous observations (*7*) suggested the presence of unreported aroma active compounds that were formed during thermal processing and/or subsequent storage. Because these compounds might contribute to the objectionable odor of stored orange juice, it would be useful to characterize, identify, and evaluate their flavor contribution. Advanced GC−MS, sulfur detectors, and various GC−O techniques (Charm, ADEA, and Osme) can be used to identify aroma impact compounds in various food systems (*9*−*11*). However, due to the extremely low concentrations of potent odorants, direct identification in the headspace by means of physical methods such as GC−MS is sometimes difficult (*8*). The newly introduced nasal impact frequency (NIF) and surface of NIF (SNIF) GC−O procedures (*12*, *13*) enable, under certain circumstances, use of the high sensitivity of the human nose for the detection and even quantification of ppt levels of certain volatiles. NIF results are expressed as percentage detection frequency of an odor by panelists, and SNIF expresses the integrated time course of the NIF response. In the current study, we used the NIF/SNIF procedure to monitor the presence of three potent odor compounds in the headspace of fresh hand-squeezed, pasteurized, and stored Shamuti and Valencia orange juices.

MATERIALS AND METHODS

Materials. Fresh oranges (*Citrus sinensis* (L.) Osbeck), cultivars Valencia and Shamuti, from trees grown on the campus of The Hebrew University, in Rehovot, Israel, were used. Fruits were picked in March 2000 (Valencia) and in January 2001 (Shamuti). Fruits were then immediately processed as described below.

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Chemicals. 2-Methyl-3-furanthiol (95%) and 3-(methylthio)propionaldehyde (methional; 96%) were obtained from Aldrich (Milwaukee, WI), and β-damascenone (98%) was a gift of Dr. Gary Takeoka of the USDA (WRRC, Albany, CA). Also purchased were HPLC-grade methanol (J. T. Baker, Deventer, Holland), butylated hydroxytoluene (BHT) and gallic acid (Sigma, St. Louis, MO), and citric acid and potassium citrate (Frutarom Co., Haifa, Israel).

External Standard Solutions. Stock solutions (10000 ppm) were prepared as follows: 20 μL of methional or β-damascenone was dissolved in 1980 μL of methanol (0.05% w/w BHT); and 20 μL of 2-methyl-3-furanthiol was dissolved in 1980 μL of acetone (0.05% w/w BHT). These solutions were then stored at −20 °C until further use. Standard mixtures for calibration were obtained by serially diluting the stocks to the final concentrations. Calibration mixtures and dilutions were performed with citrate buffer (0.062 M citric acid, 0.018 M potassium citrate, and 3.0 mM gallic acid as antioxidant, pH 3.5). The final calibration solutions (50 mL each) were divided into aliquots of 1.5 mL and placed in 2-mL vials (Kimble Glass, Inc., Vineland, NJ), then stored immediately at −80 °C until analysis. Concentrations (ng/L) of volatiles in each calibration mixture were 0.5, 5, 50, and 250 (2-methyl-3-furanthiol); 100, 2000, 8000, and 10000 (methional); 10, 100, 2000, and 15000 (β-damascenone) for mixtures 1, 2, 3, and 4, respectively.

Juice Treatments for GC−O Analysis. Chilled oranges (4 °C) were washed and halved. Juice was then hand-squeezed with a kitchen juicer. A portion of the combined juice was divided into 1.5-mL aliquots, sealed in 2-mL vials, and immediately stored at −80 °C (designated fresh juice). The remaining juice was subjected to pasteurization with a microwave (500 W) for 4 min. Preliminary trials indicated that a temperature of 100 °C (from an initial 20 °C) was reached after heating 200 mL of juice for 3 min. After immediate cooling on ice, juice was supplemented with 500 ppm sodium benzoate. This laboratory procedure was aimed at simulating industrial conditions common in Israel (98 °C for 1 min). A portion of that juice (designated pasteurized) was divided into aliquots as described for fresh juice and stored at −80 °C. The remaining pasteurized juice was sealed in a 120-mL amber glass bottle and stored for 21 days at 35 °C. This juice was then divided into aliquots as described for fresh and pasteurized juices and stored at −80 °C.

Solid-Phase Micro-Extraction (SPME) of Juice Volatiles. Following sample thawing, fresh, pasteurized or stored-pasteurized orange juice (1 mL) was mixed with 1 mL of saturated $CaCl_2$ solution in a 4-mL amber vial (15 × 45 mm) and sealed with a screw-cap Teflon septum (National Scientific Company). The saturated $CaCl_2$ solution was used to deactivate enzymes (*14*) in fresh juice and also as a means of salting out volatiles. Each vial was then placed in a water bath set to 36 ± 0.3 °C and gently mixed. Samples were allowed to equilibrate for 5 min prior to SPME, and they were maintained at 36 °C throughout the 20 min assay. SPME of juice volatiles was conducted by inserting a 2-cm stable flex fiber coated with 50/30 μm of DVB/Carboxen/PDMS (Supelco, Bellefonte, PA). Preliminary trials at different SPME time periods (not shown) indicated that a 15-min SPME at 36 °C gave optimal extraction conditions for obtaining NIF/SNIF results of the orange juice headspace. Shorter time periods resulted in lower SNIF values, whereas times longer than 15 min showed lower SNIF values for 2-methyl-3-furanthiol and methional and no change in β-damascenone. The full length of the coated fiber was therefore exposed to the orange juice headspace for 15 min. The fiber was then removed from the headspace and immediately inserted into the GC injector.

High-Resolution GC−O. GC−O analyses were performed with an HP-4890 (Hewlett-Packard) gas chromatograph equipped with FID and with the following fused silica columns: a 5% phenyl-coated DB-5MS capillary column (30 m × 0.32 mm i.d., 0.5 μm) and a DB-WAX capillary column (60 m × 0.32 mm i.d., 0.25 μm) (J&W Scientific, Folsom, CA). The properties of these columns are very different and, therefore, when a marker is available, they can serve as an important indicator for identification of an unidentified volatile by matching the Kovats retention indices (RI). Following SPME, volatiles were desorbed in the injector (230 °C) for 3 min, in the splitless mode. Operating conditions were as follows: column held at 33 °C for 5 min (DB-5MS) or 30 °C for 4 min (DB-WAX) and then increased at 8 °C/min to 230 °C or 6 °C/min to 220 °C, respectively, then held for 8 or 10 min, respectively. Helium was used as the carrier gas with a linear velocity of 31 cm/s. A 0.75-mm i.d. SPME injector liner was used. At the end of the capillary column, the effluent was split (1:10 by volume) between the FID and a sniffing port (model ODP-2, Gerstel GmbH, Germany). The FID and the sniffing port were held at 250 and 270 °C, respectively. Linear Kovats RI of the compounds were calculated using a series of *n*-alkanes.

High-Resolution Gas Chromatography−Mass Spectrometry. 2-Methyl-3-furanthiol was identified in a Saturn 2000 mass spectrometer (Varian, Walnut Creek, CA) connected to a Varian 3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm; J&W Scientific). Following the aforementioned SPME procedure, samples were inserted into the injector (held at 250 °C) for 3 min, in splitless mode. The oven program was 30 °C for 5 min, increasing to 160 °C at 5 °C/min and holding for 10 min, then increasing to 280 °C at 40 °C/min and holding for 5 min. The transfer line was held at 300 °C and the source was held at 170 °C. Mass spectra in the electron impact (EI) mode were generated at 70 eV. Chromatograms and spectra were recorded with Saturn GC/MS Workstation software, version 5.41 (Varian).

For comparison with the SPME results, volatile compounds were analyzed with a purge and trap (Tekmar 3000, Tekmar-Dohrmann Co., Cincinnati, OH) coupled with the above GC and mass spectrometer. Twenty mL of orange juice (Shamuti, stored for 21 days at 35 °C) was placed in a 20-mL tube. Volatile compounds were purged for 15 min (ambient temperature) and trapped on Tenax Trap A, following by 2 min of desorption at 200 °C, then 1 min of cryofocusing at −145 °C (CRYOFocusing Module, Tekmar). Volatiles were then desorbed at 200 °C for 1 min and introduced into a GC column, SPB-624, 1.4-mm film thickness, 60 m × 0.25 mm (Supelco, Supelco Park, Bellefonte, PA). The column was programmed at 40 °C (1 min), heated at 4 °C/min to 150 °C, then 10 °C/min to 200 °C (held for 10 min), and then 20 °C/min to 240 °C (held for 10 min). Mass spectra were conducted as described above with the transfer line held at 200 °C and the source held at 170 °C.

Sniffing Procedure. On the basis of the approach of Pollien et al. (*12*), 10 panelists (7 females and 3 males) for the Valencia experiment and 12 panelists (10 females and 2 males) for the Shamuti experiment, all students 20−25 years of age, were randomly selected. Panelists were experienced with odor recognition of various volatiles and with the sniffing of the GC−O apparatus. Sniffing of samples derived from fresh, pasteurized, or stored juices was recorded at ranges of Kovats RI values from 755 to 969 and from 1211 to 1599. Preliminary GC−O screenings verified that methional and β-damascenone are eluted during these time ranges. Elution of each aroma compound through the sniffing port was recorded (HP-3398A GC software) by pressing a button during the entire sensation of an odorant, resulting in a square signal. Panelists were requested to assign odor properties to each volatile detected. The 10/12 individual chromatograms for each juice treatment were summed, yielding the final aromagram (detection frequency versus RI). Peak heights were related to their percentage detection frequencies (NIF) and peak areas (expressing the detection frequencies and the detection time duration) were the SNIF.

In addition, GC−O time−intensity experiments were performed with three trained panelists in the indicated RI ranges for the aforementioned orange juice treatments and authentic markers.

Quantification of 2-Methyl-3-furanthiol, Methional and β-Damascenone in Shamuti Orange Juice Head-

Table 1. Kovats RI Values Resulting from FID Chromatograms and GC−O Aromagrams of Authentic Markers of 2-Methyl-3-furanthiol, Methional, and β-Damascenone, and of Valencia Orange Juice, Using DB-WAX and DB-5 Capillary Columns

	DB-WAX			DB-5			
	markers		juice	markers		juice	odor
compound	FID	GC−O[a]	GC−O[a]	FID	GC−O[a]	GC−O[b]	description
2-methyl-3-furanthiol	1358	1357	1357	875	875	877	meaty/vitamin B
methional	1431	1431	1430	917	917	918	cooked potato
β-damascenone	1887	1886	1886	1365	1368	1366	rose/tobacco

[a] GC−O responses by three trained panelists. [b] Average NIF GC−O responses of orange juice data presented in Figure 1 using 10 panelists.

Table 2. Odor Threshold and Content (ng/L) and log OAV Values of 2-Methyl-3-furanthiol and Methional in Fresh, Pasteurized, and Stored Shamuti Oranges Juice

odorant	threshold[a]	fresh	pasteurized	stored
2-methyl-3-furanthiol	5	1.8 (−0.45)[b]	1.9 (−0.44)	270 (1.73)
methional	50	550 (1.04)	830 (1.22)	11550 (2.36)

[a] Threshold values are retronasal and were taken from refs *19*, *29*, and *30*. [b] Numbers in parentheses are log OAV values.

space. Calibration curves were obtained (*13*) employing the described SPME procedure. Each of the calibration mixtures (1 mL) was applied using the same GC system and operating conditions. The same 12-member panel that took part in the Shamuti orange juice trial was used to determine the calibration curves. Panelists were asked to record their odor sensation from RI 755 to 969 and from 1211 to 1599 in the very same manner as for the orange juice. Each panelist sniffed each of the calibration mixtures once (resulting in four aromagrams per panelist) containing the concentrations indicated above. Data recorded from all 12 panelists were summed.

Sensory Similarity Experiments. Shamuti orange juice samples were treated and extracted as detailed above. The resulting juice was then pasteurized in a microwave oven (1000 W) for 4 min (1 min at 100 °C from an initial 20 °C, 500-mL aliquots) and immediately cooled on ice. The combined pasteurized juice (5200 mL) was supplemented with 500 ppm sodium benzoate. A 1000-mL portion of this juice was placed in two 550-mL amber glass bottles, sealed, and stored for 21 days at 35 °C. The remaining pasteurized juice was immediately transferred to −80 °C storage.

Aliquots (25 mL) of juice from stored and pasteurized (after thawing) samples were placed in 120-mL amber glass bottles with Teflon-lined caps (Wheaton, Millville, NJ.) Aroma-similarity, cluster hierarchical tree structure (HTS) (*15*, *16*) analyses were then performed. Ten students, 20 to 25 years of age, were trained to evaluate the aroma similarity of five orange juice treatments. Bottles containing the various juice samples were brought to room temperature prior to each aroma test. During a 2-h aroma session, four 15-min aroma similarity tests were conducted. In each test, on a verbal signal from the experimenter, a panelist opened the bottles (after shaking) of two samples at 15-s intervals, smelled them, and rated the similarity level of their aromas on a scale of 1 to 20 (1 for no similarity, 20 for identical, and numbers between for other ratings). Seven sample pairs were presented in coded, randomized order per test, resulting in 28 pairs tested during each aroma session.

Data Processing. Summing NIF (%) per RI of all 12 individual aromagrams (EXCEL 97, Microsoft Co., Seattle, WA) produced the combined aromagram for each juice treatment. Probit values were calculated using the following EXCEL functions:

$$\text{probits of NIFs} = NORMSNIV\,(NIF\,\%) + 5$$

$$\text{probits of SNIFs} = \sum [probit(NIF)_t\, dt]$$

where $dt = 1$ RI unit, from the start to the end time of the GC−O peak, and $(NIF)_t$ = the instant NIF value (%) at time t. Calibration curves and confidence intervals were produced with JMP statistical software version 3.1.6.2 (SAS Institute Inc., Cary, NC). Juice aromagrams were analyzed for significance between treatments using one- and two-way analysis of variance (ANOVA). Because the smell data were derived from 12 panelists, Friedman and Wilcoxon nonparametric statistical tests were also performed (*17*) to further explore statistical differences. Significance was set at least at the level of $p < 0.05$. Odor activity values (OAV) (odor concentration/odor threshold) were expressed after logarithmic transformation.

A data matrix representing the aroma similarity results was obtained, in which each cell in the matrix represents the mean similarity for all panelists for the corresponding comparison. This proximity matrix was then analyzed by the clustering program ADDTREE (*16*), yielding a tree structure of branches and subdivisions with aromas located at the ends of the branches.

RESULTS AND DISCUSSION

Identification of 2-Methyl-3-furanthiol, Methional, and β-Damascenone in Orange Juice. The presence of methional (*8*) in fresh orange juice, and possible presence of β-damascenone (*18*) in heated orange juice, has been previously proposed. Because of the extremely low concentrations of potent odorants, their direct identification in the headspace by means of physical methods such as GC−MS is sometimes difficult (*8*). After SPME, FID peaks for 2-methyl-3-furanthiol, methional, and β-damascenone could not be detected (e.g., at the ppt/ppb levels). Thus, FID RI values of authentic markers of the three compounds, together with RI values obtained from the time−intensity GC−O analyses including the NIF/SNIF procedure, were then used to identify selected potent odorants in fresh, pasteurized, and stored orange juice. Time−intensity GC−O experiments suggested putative 2-methyl-3-furanthiol, methional, and β-damascenone peaks in the Kovats RI ranges indicated in Material and Methods. The obtained FID RI values for authentic markers in two different capillary GC columns (DB-5 and DB-WAX) were actually identical with those of GC−O using trained and untrained panelists, resulting in identical odor descriptions (Table 1). The level of 2-methyl-3-furanthiol found in stored juice was marginal but sufficient (about 270 ppt, Table 2) to enable its identification, although not enough for quantification by GC−MS as compared with an authentic sample of 2-methyl-3-furanthiol (*m*/*z*: 114 [M^+], 85, 69, 45, 43). Purge and trap (Tenax as adsor-

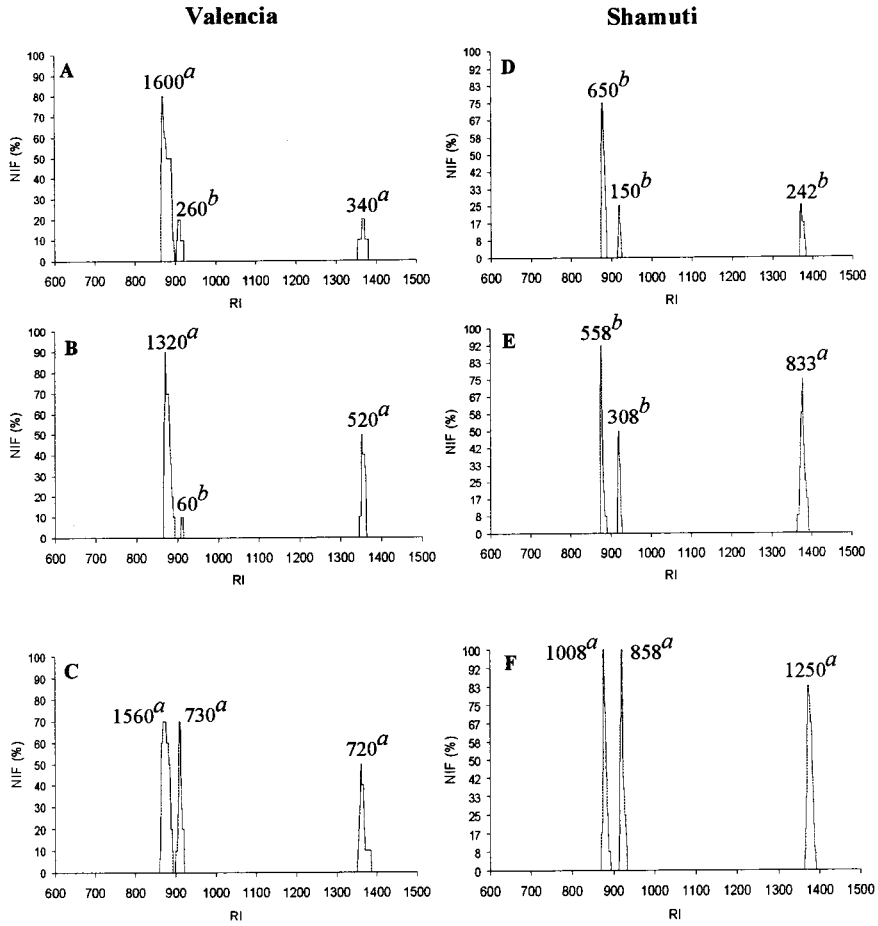

Figure 1. NIF and SNIF responses for selected RI values in the headspace of Valencia and Shamuti orange juices. Results are derived from 10 panelists in the Valencia experiment and 12 panelists in the Shamuti experiment. Numbers at the top of each peak represent SNIF values. Panels A and D, B and E, and C and F designate fresh, pasteurized, and stored juices, respectively. Peaks at RI 877, 918, and 1366 are, putatively 2-methyl-3-furanthiol, methional, and β-damascenone, respectively. Different superscript letters within each odor peak indicate significantly (at least $p < 0.03$) different SNIF responses between juice treatments.

bant) coupled to GC−MS did not result in any MS signals for the three compounds under the experimental conditions. To the best of our knowledge, the potent odorant 2-methyl-3-furanthiol has not been previously reported in citrus juice. Together, these results suggest the identification of 2-methyl-3-furanthiol, methional, and β-damascenone in the treated orange juice.

In addition to the contribution of the NIF/SNIF procedure to the identification (as other GC−O procedures), the actual raw NIF/SNIF values may serve as an indication of quantitative changes in odorant contents. Furthermore, obtaining a calibration curve and achievement of linearization (transformation of SNIF values into probits against log concentration) can lead to quantitative results. In Figure 1 we provide the NIF/SNIF values for Valencia and Shamuti orange juices, whereas quantification was performed for var. Shamuti only (Table 2).

Relative Levels of 2-Methyl-3-furanthiol, Methional, and β-Damascenone in Valencia Orange Juice as Determined by GC−O Analysis. In the fresh juice, two out of 10 panelists (NIF = 20) identified methional- and β-damascenone-like odors at Kovats RI values of 917 and 1368, respectively (Figure 1A), whereas eight out of 10 panelists (NIF = 80) identified a 2-methyl-3-furanthiol-like odor at an RI value of 875. After pasteurization, NIF values were 90, 10, and 50 for 2-methyl-3-furanthiol, methional, and β-damascenone-like odors, respectively. Following storage, NIF values were 70, 70, and 50 for 2-methyl-3-furanthiol, methional, and β-damascenone-like odors, respectively. These results appear to indicate that the detection frequency (NIF) due to juice treatment did not change for 2-methyl-3-furanthiol, increased for methional during storage, and increased for β-damascenone during pasteurization (remained the same for storage). Statistical analyses performed on the raw SNIF values (peak areas) indicate a significant increase only for methional following storage.

No quantitative determinations were performed with the Valencia orange juice, but the relative SNIF values (as indicated by the peak areas) essentially followed the NIF responses, supporting the increase in content of the β-damascenone-like odor during pasteurization and of the methional-like odor during storage.

Quantification of 2-Methyl-3-furanthiol, Methional, and β-Damascenone in Shamuti Orange Juice as Determined by GC−O Analysis. Although a different sniffing panel was used for the Shamuti var., the NIF/SNIF results were qualitatively the same as those for Valencia (Figure 1). This may support the reliability of the obtained results. In Shamuti, SNIF values for both 2-methyl-3-furanthiol and methional did not change during pasteurization (compared with fresh juice), but increased following storage (Figure 1D, E, and F). SNIF values for β-damascenone increased significantly during pasteurization; the increase during storage was not statistically significant (Figure 1E and F).

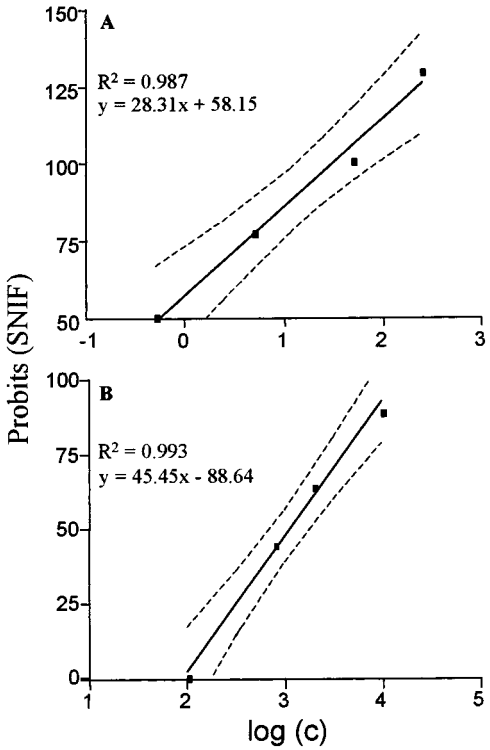

Figure 2. Calibration curves for 2-methyl-3-furanthiol (A) and methional (B) as expressed by probit transformations of SNIF responses. Odorant concentrations are indicated in Materials and Methods section. The same 12 panelists participating in the Shamuti experiment (Figure 1 D, E, and F) were used to obtain the calibration curves.

As discussed above,the SNIF values are derivatives of the NIF responses but include the time during which panelists were able to detect a given odor, and therefore, may be used for quantification (*13*). In general, the nose cannot be considered an objective instrument for quantification. However, the "subjective" problem can be overcome by using more subjects, as has been elegantly shown previously (*12*). Unlike other GC−O procedures, it is not the subject as an individual which is considered the measurement, but the sniffing panel as a whole. The use of the SNIF procedure for quantification lacks empirical proof of an odor's persistence being correlated with its intensity. However, the present results (e.g., the increases in the concentrations of 2-methyl-3-furanthiol and methional, which were highly correlated with SNIF responses, see below) clearly suggest high reliability of the SNIF experiments, at least under the present experimental conditions. With the current difficulties in quantifying ppt levels of various odorants by physical instruments, use of the SNIF procedure is a relevant choice.

The SNIF (peak areas) data were used to quantify the actual content of each compound. The same panelists who participated in obtaining the experimental data shown in Figure 1 (D, E, and F) were used to determine a calibration curve for each of the three compounds (Figure 2). A saturation curve was obtained. After the probit data transformation vs log concentration, a linear dependence with high correlation (r^2 = 0.99 with relatively small 95% confidence intervals) between the SNIF values and the concentrations of either 2-methyl-3-furanthiol or methional (Figure 2) was obtained. The logarithmic transformations using probit results in a SNIF-odor concentration dependence in which the increase in relative content of each odorant is much greater than the corresponding increase in the raw SNIF values. Using the same computations, no clear dependence was found between SNIF values and β-damascenone concentrations (correlation was less than 0.3). The reasons for this lack of correlation are not known. It is possible that the very high potency of β-damascenone (a concentration of 10 ppt resulted in a 100% NIF value using the GC−O) produced difficulties for the sniffing panel in terms of indicating various signals which could result in a normal calibration curve (e.g., for each increase in concentration, obtaining the appropriate increase in SNIF value). Therefore, although the increased formation of β-damascenone during pasteurization and storage was evident, a quantitative determination of this odorant in each juice treatment remains to be measured.

Results obtained from parametric (ANOVA) tests applied to the raw SNIF data were in agreement with the Friedman and Wilcoxon analyses performed to verify the statistical significance between treatments. These quantitative analyses indicated the content of 2-methyl-3-furanthiol in stored juice to be about 130-fold higher (resulting in a log OAV of 1.73) than that in fresh orange juice (Table 2). Pasteurization produced no change in 2-methyl-3-furanthiol content. OAV values for 2-methyl-3-furanthiol are based on a 5 ppt threshold value (*19*). The use of this threshold value resulted in some negative OAVs, but one should note that the threshold value for 2-methyl-3-furanthiol ranged from 0.5 to 7 ppt (*20*, *21*). Under the same conditions, pasteurization increased methional content by about 50% (resulting in a log OAV of 1.22) compared with that in fresh juice, whereas storage induced a 21-fold increase (resulting in a log OAV of 2.36) in methional content relative to that of fresh juice.

Sensory Similarity Experiments. The OAV values indicated in Table 2 suggest that 2-methyl-3-furanthiol and methional are impact odorants in stored orange juice. However, a strong GC−O peak for a potent odorant does not necessarily mean that this odorant plays a significant role in the overall aroma quality of a given product (*22*). It is the composition of a mixture of odorants in the food system, rather than individual odorants alone, that leads to specific sensations and hedonics. The objective of this experiment was, therefore, to assess the significance of 2-methyl-3-furanthiol and methional as storage off-flavors in orange juice. Pasteurized orange juice samples were fortified with 2-methyl-3-furanthiol and methional at the exact doses found in stored orange juice (Table 2), and their aroma qualities were tested for their similarity to the aroma of actual stored orange juice. In the aroma-similarity space diagram (Figure 3), the proportion of variance explained was 99.2% (r^2 = 0.996), indicating that distances in the clustering structure are highly correlated with the original aroma similarity. Sets of aromas located at the ends of branches and connected to the same node at relatively short (horizontal) distances from each other are highly similar. Thus, there are two criteria to evaluate similarity between treatments. The first is whether such treatments are connected to the same cluster and more specifically to the same node: location on the same node indicates high similarity. The second criterion is the horizontal distance between such treatments. A short distance indicates a high degree of similarity. For example, the relative distance value of 15.4 (arbitrary units) between

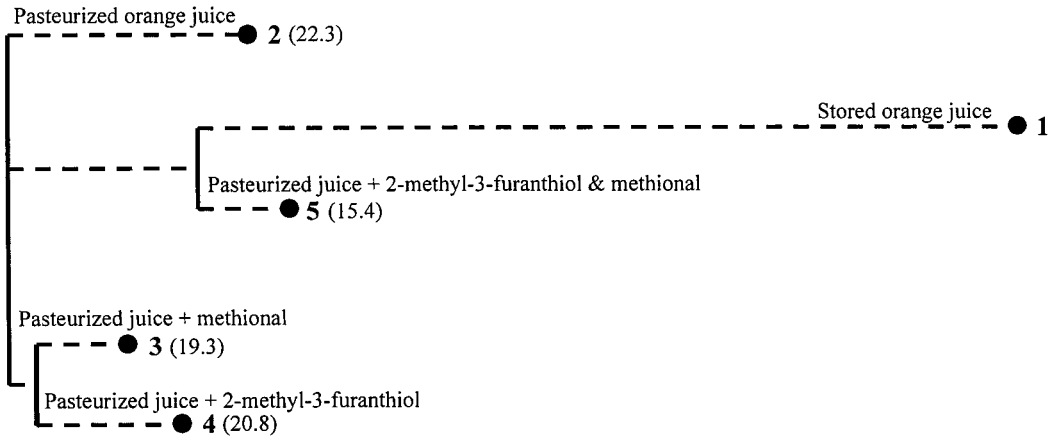

Figure 3. Cluster analysis by ADDTREE of aroma-similarity values. Branches: 1, stored orange juice (35 °C for 21 days); 2, pasteurized orange juice; 3, pasteurized juice fortified with 11.5 ppb methional; 4, pasteurized juice fortified with 0.27 ppb 2-methyl-3-furanthiol; and 5, pasteurized juice fortified with both 11.5 ppb methional and 0.27 ppb 2-methyl-3-furanthiol. Each value in the original matrix represents the mean of 10 panelists (each tested twice). Bold dots represent the location of each aroma in the hierarchical tree structure. Numbers in parentheses are horizontal distances from branch 1 (stored orange juice).

branches 1 and 5 (Figure 3) is the sum of the dashed segment of branch 1 and the dashed segment of branch 5. The relative distance value of 22.3 between branches 1 and 2 is the sum of the dashed segment of branch 1, the dashed segment of the main cluster (holding branches 1 and 5), and the dashed segment of branch 2. Three major clusters were found in the space diagram described in Figure 3, two of which were further divided into two branches. Branch 1 (stored orange juice) and branch 2 (pasteurized juice) were located in two different clusters and with the relatively longest horizontal distance (22.3) from each other compared to other aromas, indicating that these two aromas were the most disparate. Fortification of pasteurized juice with methional (branch 3) and 2-methyl-3-furanthiol (branch 4) resulted in a different cluster than either pasteurized (branch 2) or stored (branch 1) juice. The distances of branches 3 and 4 from branch 1 were shorter (19.3 and 20.8 for methional and 2-methyl-3-furanthiol, respectively) than that between branches 1 and 2. This result suggests that fortification of pasteurized orange juice with either 2-methyl-3-furanthiol or methional produces aromas which are closer in similarity to the aroma of stored juice than that of the unfortified pasteurized juice. Most importantly, fortification of pasteurized orange juice with both 2-methyl-3-furanthiol and methional at doses found in stored juice produced an aroma (branch 5) which was located on the same cluster as that of stored juice (branch 1) and with the shortest horizontal distance between them (15.4), indicating high similarity.

ANOVA of the raw data in the original matrix derived from the results presented in Figure 3 was further conducted to directly compare the similarity ratings between stored juice (branch 1) and other treatments. Here, higher rating values indicated higher similarity. These direct comparisons indicated a similarity rating of 7.8 ± 1.1 for the comparison between branch 1 and branch 5, 5.4 ± 1.0 for the comparison between branch 4 and branch 1, 4.95 ± 1.1 for the comparison between branch 3 and branch 1, and 4.35 ± 0.6 for the comparison between branch 2 and branch 1. These comparisons indicated that the similarity rating given for the comparison between the aromas of stored orange juice and pasteurized juice fortified with both 2-methyl-3-furanthiol and methional was significantly ($p < 0.002$) higher than the similarity rating given to the comparison between the aromas of unfortified pasteurized juice and stored juice.

These results suggest that pasteurized orange juice fortified with both 2-methyl-3-furanthiol and methional produces an aroma which is much more similar to that of stored juice compared to that of other treatments, and thus, suggest that methional and 2-methyl-3-furanthiol at doses found in stored orange juice are possible storage off-flavors. Among the previously reported storage off-flavors (4-vinylguaiacol, Furaneol, and α-terpineol) (*3*), hedonic sensory experiments (*1, 15*) suggest that 4-vinylguaiacol contributes significantly to the reduced acceptability of orange juice, whereas GC−O analyses suggested that α-terpineol and Furaneol may be less significant (*7, 23*). Nevertheless, the aroma similarity experiments suggest that, in addition to 2-methyl-3-furanthiol and methional, the previously proposed off-flavors and possibly β-damascenone, as well as additional unidentified compounds, should be added to the pasteurized juice to produce an aroma identical to that of stored orange juice.

In summary, GC-MS analysis provided additional evidence for the occurrence of 2-methyl-3-furanthiol in orange juice. The presence of methional (which was previously identified in fresh orange juice), and β-damascenone is also supported. Although these volatiles occur in fresh juice, their content increases significantly during pasteurization and/or storage. The precursors for these ppt and ppb levels of such volatiles in orange juice remain to be investigated. The presence of methional in fresh fruits such as tomatoes, grapefruits and oranges already has been proposed (*24, 25*) and may involve unknown biosynthetic pathways. To the best of our knowledge, 2-methyl-3-furanthiol can be produced in fruit after fermentation (e.g., grapes (*20*)) but has not been identified in fresh fruits. 2-Methyl-3-furanthiol can be produced by the hydrolysis of thiamine under acidic conditions (*26*). β-Damascenone may be released enzymatically from putative glycosides, as in apples (*27*). Evidently, these compounds are further produced during pasteurization and storage by chemical means. Methional can be produced from methionine by Strecker degradation (*28*), whereas β-damascenone can be released by acid hydrolysis of glycosides (which is also expected to increase with heat). Thus, the acidic condi-

tions inherent to citrus juice may favor both 2-methyl-3-furanthiol and β-damascenone formation. On the other hand, one may assume a lower likelihood of methional formation via Strecker degradation of methionine under the relatively mild pasteurization and storage conditions of citrus products. Nevertheless, the minor (ppb and ppt) levels found in this study can probably be formed, even under such unfavorable conditions. At the level found, only 2-methyl-3-furanthiol could be identified by GC−MS under the experimental conditions. In fact, the NIF/SNIF procedure has been proposed to be more sensitive than MS/MS analysis (*13*).

The present results strongly support the suggestion that the NIF/SNIF procedure is an important tool for both identification and quantitative determination of selected aroma compounds that are found in minor amounts in a food system, but which nevertheless play a role in its acceptance. However, quantification may not always be possible (e.g., β-damascenone in the present study). Most significantly, the aroma-similarity experiments clearly indicate that 2-methyl-3-furanthiol and methional, at the levels found in stored orange juice, are very likely storage off-flavors.

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