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FINAL REPORT

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Characterization of Unidentified Potent Flavor Changes during Processing and Storage of Orange and Grapefruit Juices

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Cover Page**

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GC = gas chromatography

GC-O = GC-olfactometry

MS = mass spectrometry

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
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Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
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Description of Cooperation: This project is the cumulation of a long series of cooperative work. The investigators have over 20 joint publications in recent years. Extensive use of e-mail has been made to exchange text, graphs and spreadsheet data, questions, editing manuscripts, sources for supplies, experimental protocols etc. We have visited each other's lab frequently (many not funded by BARD) and try to meet at national/ international meetings whenever possible. The trip to Israel by Rouseff in June, 1999 and the follow up visit by Naim to Lake Alfred in March 2000, allowed for the technology transfer necessary for the first modern GC-O unit in Israel to become functional. In addition to the mechanical assembly of the component parts the initial visit was used to train local personnel on the use of the equipment and software needed to make proper interpretation of the data. The degree of cooperation between the investigators is exceptionally high based upon years of trust established from a long history of mutually beneficial experiences and complimentary expertise.

Patent Summary: none

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ABSTRACT

Citrus juice flavor quality traditionally diminishes after thermal processing and continuously during storage. Our prior studies found that four of the five most potent off-aromas formed during orange juice storage had not been identified. The *primary* emphasis of this project was to characterize and identify those potent flavor degrading aroma volatiles so that methods to control them could be developed and final flavor quality improved. Our original objectives included:

1. Isolate and characterize the most important unidentified aroma impact compounds formed or lost during pasteurization and storage.
2. Determination of thiamine and carotenoid thermal decomposition and Strecker degradation pathways in model solutions as possible precursors for the unidentified off-flavors.
3. Evaluate the effectiveness of an Aelectronic nose® to differentiate the headspace aromas of from untreated and heat pasteurized orange and grapefruit juices.
4. Use model systems of citrus juices to investigate the three possible precursor pathways (from 2) for flavor impact compounds formed or lost during pasteurization or storage.

RESULTS – The components responsible for citrus storage off flavors and their putative precursors have now been identified. Certain carotenoids (β -carotene) can thermally degrade to produce β -ionone and β -damascenone which are floral and tobacco smelling respectively. Our GC-O and sensory experiments indicated that β -damascenone is a potential storage off-flavor in orange juice. Thiamine (Vitamin B1) degradation produces 2-methyl-3-furan thiol, MFT, and its dimer bis(2-methyl-3-furyl) disulfide which both produce meaty, savory aromas. GC-O and sensory studies indicated that MFT is another storage off-flavor. Methional (potato aroma) is another off flavor produced primarily from the reaction of the native amino acid, methionine, and oxidized ascorbic acid (vitamin C). This is a newly discovered pathway for the production of methional and is more dominant in juices than the classic Maillard reaction. These newly identified off flavors diminish the flavor quality of citrus juices as they distort the flavor balance and introduce non-typical aromas to the juice flavor profile. In addition, we have demonstrated that some of the poor flavor quality citrus juice found in the market place is not only from the production of these and other off flavors but also due to the absence of desirable flavor components including several potent aldehydes and a few esters. The absence of these compounds appears to be due to incomplete flavor volatile restoration after the making of juice concentrates. We are the first to demonstrate that not all flavor volatiles are removed along with water in the production of juice concentrate. In the case of grapefruit juice we have documented which flavor volatiles are completely removed, which are partially removed and which actually increase because of the thermal process. Since more than half of all citrus juices is made into concentrate, this information will allow producers to more accurately restore the original flavor components and produce a juice with a more natural flavor.

IMPLICATIONS - We have shown that the aroma of citrus juices is controlled by only 1-2% of the total volatiles. The vast majority of other volatiles have little to no direct aroma activity. The critical volatiles have now been identified. The ability to produce high quality citrus juices requires that manufacturers know which chemical components control aroma and flavor. In addition to identifying the critical flavor components (both positive and negative), we have also identified several precursors. The behavior of these key aroma compounds and their precursors during common manufacturing and storage conditions has been documented so manufacturers in Israel and the US can alter production practices to minimize the negative ones and maximize the positive ones.

BARD Final Scientific Report ACHIEVEMENTS

This is probably the most complete study of citrus aroma volatiles every reported. There have been numerous quantitative reports of total volatiles in citrus juices (1;2;3;4;5;6;7;8), but none measure aroma activity. It is becoming increasingly well known that most food volatiles do not have aroma activity. Using GC-olfactometry we demonstrated (pub. #1) that 95-99% of citrus volatiles have little to no direct aroma impact. Furthermore, we identified those trace compounds which possess aroma activity and impact juice flavor either positively or negatively. Others have used GC-O to study aroma active compounds and packaging interactions (9), aroma active composition of hand squeezed juices (10;11;12), and aroma changes in juices from concentrate (13). None of these studies examined aroma precursors and for the most part did not identify storage off flavors.

Significance of main scientific achievements or innovations.

The remaining undiscovered storage off flavors and probable precursors have now been identified. This was the primary objective of the current study (pubs. #2,3,5 and appendix Studies 1A-1F). β -damascenone, MFT and methional are the additional potent storage off aromas. The dimer of MFT and β -ionone which we have shown to be produced from the thermal degradation of thiamine and β -carotene respectively, may also degrade juice flavor. Furthermore, our studies with commercial juices from concentrate indicate that poor flavor found in market place juices is associated with incomplete aroma restoration. Earlier reports (14) had indicated that 4-vinyl guaiacol, Furaneol and α -terpineol were the primary flavor degrading compounds formed during orange juice storage. Our studies, using the recently developed GC-O technology, have confirmed the importance of the first two, but found that α -terpineol completely lacked aroma activity and should not be considered a storage off flavor. Although the concentration of α -terpineol was found to increase with increasing storage time and temperature (and thus could be used as a non causative indicator), it never directly produced aroma activity (pub #3).

Potential impact on agriculture, industry, environment, policy, legislation

The primary benefactors of this information will be the citrus processing industries in Israel and the US. Juice quality can now be improved by reducing or eliminating those volatiles which negatively impact flavor. Knowing which volatiles reduce juice flavor will now allow quality

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control tests to be developed. The procedures we have developed to measure these off flavors can be modified for quality control purposes. Thus fruit selection, juice extraction conditions, packaging and storage conditions can now be optimized to control these off flavors. Furthermore, by controlling the *precursors* of these off flavors as well as the conditions that are favorable for their conversion to potent off flavors, a fresher tasting juice can be produced. Consumers throughout the world have shown a decided preference for the taste of fresh juice.

Details of cooperation

As indicated in the summary and appendix of this report, we believe that the present project significantly upgrades the ability to produce high quality citrus juices and the flavor degrading components have been identified. By monitoring these compounds, juice producers in Israel and the US can alter their manufacturing, packaging and storage processes to minimize these off flavors. Prior to our study, these compounds could often be detected in the headspace by the human nose but rarely by physical instruments. This is why the introduction of GC-O analyses to the plan of our original proposal was so significant. However, introduction of the GC-O technique (a relative new technique which is still under development), although very useful, is complex and requires a special expertise to gain reliable information. When this project was initiated, a GC-O apparatus was available in Lake Alfred but not in Rehovot. In fact, such an instrument with the appropriate software was not available in other institutes in Israel. It was the collaboration with Rouseff (frequent correspondence by e-mail), his visit to Rehovot in the summer of 1999 and the visit by Naim to Lake Alfred in March 2000, which contributed significantly to the proper use of various GC-O procedures in Rehovot. As before, the advanced analytical tools available in Lake Alfred at Rouseff's laboratory and the expertise of various sensory experiments available at Naim's laboratory in Rehovot are combined into a single project, contributing to the new results reported here.

Publications from Project

1. Bazemore, R.; Rouseff, R. L.; Naim, M. Linalool in Orange Juice: Origin and Thermal Stability. *J Agric Food Chem* **2002**, *Accepted for publication, copy in appendix.*
2. Lin, J. M.; Rouseff, R. L.; Barros, S.; Naim, M. Aroma composition changes in early season grapefruit juice produced from thermal concentration *J. Agric. Food Chem.* **2002**, *50*, 813-

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819.

3. Bezman, Y.; Rouseff, R. L.; Naim, M. 2-methyl-3-furanthiol and methional are possible off-flavors in stored orange juice: Aroma-similarity, NIF/SNIF GC-O, and GC analyses *J. Agric. Food Chem.* **2001**, *49*, 5425-5432.
4. Rouseff, R.; Bazemore, R.; Goodner, K.; Naim, M.; GC-olfactometry with solid phase microextraction of aroma volatiles from heated and unheated orange juice; In *Adv.Exp.Med.Biol.*; Rouseff, R., Cadwallader, K., Eds.: New York, **2001**; Vol. 488; pp 101-112.
5. Rouseff, R.; Naim, M. Citrus flavor stability. In *Flavor Chemistry: Industrial and Academic Research*; C. T. H. S. Risch, Ed.; American Chemical Society: Washington, D.C., **2000**; pp 101-121.
6. Haleva-Toledo, E.; Naim, M.; Zehavi, U.; Rouseff, R. L. Effects of L-cysteine and N-acetyl-L-cysteine on 4-hydroxy-2,5- dimethyl-3(2H)-furanone (Furaneol), 5-(Hydroxymethyl) furfural, and 5-methylfurfural formation and browning in buffer solutions containing either rhamnose or glucose and arginine *J. Agric. Food Chem.* **1999**, *47*, 4140-4145.
7. Bazemore, R.; Rouseff, R. Discrimination of thermally treated orange juices by an electronic nose equipped with organic polymer sensors. *Seminars in Food Analysis* **1998**, *3*, 59-66.

APPENDIX:

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EXPERIMENT 1 - Isolate and characterize the most important unidentified aroma impact compounds formed or lost during pasteurization and storage.	
Study 1A. <i>Linalool in Orange Juice: Origin and Thermal Stability.</i> In the first paper published on aroma active components in orange juice (9), linalool was described as the most intense aroma component in commercially processed juice. Even though commercial and hand squeezed juices had similar total GC-O intensities, linalool was not found in hand squeezed juice. We noted that their hand squeezed juices had not been heated but the mechanically	

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squeezed juices had been thermally pasteurized so the two samples differed both in the method of juice extraction, but also differed in their heat treatments. Therefore, we examined the differences between squeezing (juice extraction) and heating effects independently in order to determine the source and fate of this most potent aroma.

(Paper accepted for publication in the Journal of Agricultural and Food Chemistry and included at the end of the appendix –summary presented below).

Approximately 80 % of total juice linalool content comes from peel oil linalool when using reamer design mechanical juice extractor and 96% is associated with the more common peel cutting/macerating extractor. Since all commercial juices are mechanically extracted using one of the two major designs tested, the vast majority of juice linalool in commercial orange juice originates in the peel and not juice vesicle cytoplasm. Thermal processing did not substantially alter juice linalool levels. Juice peel oil concentration is highly correlated with linalool content. Linalool concentrations were determined in juice from three groups of 60 Valencia oranges using pentane: ether extraction and high resolution capillary GC. One group was peeled, the other two remained unpeeled. Juice was extricated from the first two groups from the halved fruit using a mechanical reamer. A peel cutting/macerating juice extractor was used for the last group. Linalool concentrations in peeled fruit juice was 0.004 mg/L whereas juice from peel intact fruit ranged from 0.020 to 0.11 mg/L depending on extractor type. Juice from peeled fruit contained significantly ($p<.05$) less linalool than peel intact juice.

Study 1B - Aroma Volatile Changes Associated with Thermal Pasteurization in Orange Juice

A new static head space technique was used to examine the changes in aroma volatiles of two types of orange juice. Eighteen aroma components were common to both the heated and unheated juice. However, flavor differences were due to six aroma components observed only on the fresh juice and five aroma components were observed only in the heated juices. Furthermore, α -terpineol which had been considered to be a major off flavor (14), was found to lack aroma activity even at the highest concentrations found in the most heat abused juices.

Summary below – complete article at the end of the appendix.

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Rouseff, R.; Bazemore, R.; Goodner, K.; Naim, M.; GC-olfactometry with solid phase microextraction of aroma volatiles from heated and unheated orange juice; In *Adv. Exp. Med. Biol.*; Rouseff, R., Cadwallader, K., Eds.: New York, **2001**; Vol. 488; pp 101-112.

The solventless headspace volatile extraction technique, SPME, solid phase micro extraction, was combined with the unique capability of GC-olfactometry (GCO) to examine the changes in aroma active volatiles when orange juice was heated. One portion of commercially extracted orange (*Citrus sinensis*, Hamlin cultivar) juice received no heat treatment whereas the remainder was excessively heated (240s at 96°C). Juice volatiles extracted from the static headspace using SPME (carboxen-polydimethylsiloxane fiber) were compared to liquid-liquid diethyl ether extracts. Aroma active volatiles were evaluated by three trained panelists using OSME, the GCO time-intensity procedure. Eighteen aroma active peaks were common to both heat treated and untreated juice headspace extracts whereas six peaks were observed only in unheated extracts and five were found only in extracts from heated juices. The SPME extract contained a greater proportion of terpenes and less early eluting alcohols and aldehydes compared to pentane-ether liquid-liquid extracts. Relative amounts of acetaldehyde decreased with increasing headspace exposure time and elevated desorption temperatures.

Study 1C - *Aroma Composition Changes from Thermal Concentration in Grapefruit*

juice This study was undertaken to determine the effects of thermal concentration had on total volatile and total aroma retention since the majority of citrus juices sold in the US and the world undergo this process. It had been assumed since almost all volatiles were removed that essentially all aroma activity would also be removed. However, it has become increasingly clear that the aroma compounds in foods constitute less than 5% of the total volatiles (15). Even though more than 95% of total grapefruit juice volatiles were lost during the concentration process, the majority of the aroma compounds (57%) remained. It was further demonstrated that certain types of aroma compounds were greatly reduced whereas others were completely retained. This fundamental finding has tremendous commercial significance because it demonstrates that only certain aroma compounds need be added back to the concentrate to restore flavor and not the entire original mixture of

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aroma compounds. Much of the highly esteemed flavor of citrus juices is due to a complex blend of aroma compounds in certain proportions. This paper provides information to restore flavor volatiles in the correct proportion by adding back only those compounds that are needed.

Lin, J. M.; Rouseff, R. L.; Barros, S.; Naim, M. Aroma composition changes in early season grapefruit juice produced from thermal concentration. *Journal of Agricultural and Food Chemistry* **2002**, *50*, 813-819.

Differences in aroma components and total volatiles between a single unpasteurized Marsh grapefruit juice and its 65 °Brix concentrate reconstituted to 10 °Brix were examined using GC-olfactometry (GC-O) and GC-FID. Total volatiles (FID) in the reconstituted concentrate were reduced to less than 5% of initial values, but 57% of total aroma (GC-O) remained. Forty-one aroma-active compounds were observed in unpasteurized single strength juice, whereas 27 components were found in the unflavored reconstituted concentrate. Aroma-active compounds were classified into grapefruit/sulfury, sweet/fruity, fresh/citrusy, green/fatty/metallic, and cooked/meaty groups. Five of six components in the sweet/fruity and 14 of 18 green/fatty/metallic components survived thermal concentration. However, only 4-mercapto-4-methyl-2-pentanone in the grapefruit/sulfury group, and linalool and nootkatone from the fresh/citrusy group, were found in the reconstituted concentrate. Methional was the only aroma compound in the cooked/meaty category found in both juice types. α -Damascenone and 1- p-menthen-8-thiol were found only in the reconstituted concentrate. 4-Mercapto-4-methyl-2-pentanol was found for the first time in grapefruit juice.

Study 1D – Comparison of Aroma Volatiles in Good and Poor Flavor Quality Orange Juice using GC-olfactometry

The purpose of this study was to determine which aroma compounds were responsible for the large differences in flavor quality of orange juice found in the market place, especially juices reconstituted from concentrate. The complete manuscript is found in the appendix.

Rouseff, R. L.; Lin, J.; Dreher, J. G.; Naim, M. Comparison of Aroma Volatiles in Good and Poor Flavor Quality Orange Juice using GC-olfactometry. *J. Flavour and Fragrances* **2002**, *draft in preparation*.

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Good quality and poor quality from concentrate commercial orange juices were compared using time intensity GC-O (OSME) with simultaneous FID and olfactometric detection. It appears that some of the flavor differences are due to incomplete flavor restoration especially the low molecular weight aldehydes such as octanal, 4Z-octenal, nonanal and decanal as well as the presence of thermally generated off flavors such as methional, 4-vinyl guaiacol and Furaneol due to excessive heating or storage temperatures.

Study 1E – *Orange Juice Aroma Volatile changes during 12 Weeks storage at 4 and 35°C*

This study was undertaken to examine the off flavor components during long term storage at recommended refrigerated temperatures and 35°C. The later temperature was the same temperature used by Tatum and coworkers (14), but glass containers were used in this study rather than tin coated steel because at the present time, little orange juice is packaged in steel cans compared to glass, plastic or multilayer cartons. Container differences can have a profound effect on the type of storage volatiles formed as the tin coated steel would rapidly react with any dissolved oxygen to convert metallic tin into tin ions. Glass is a highly effective oxygen barrier, but any dissolved oxygen would react with juice components.

Materials and Methods

Orange Juice Samples. Commercial orange juice from concentrate was obtained from a Florida manufacturer packed in 473 mL (16 fluid oz.) glass containers. Half the samples were then transferred to PET containers by method of sterile transfer. The PET bottles were dipped into a 190°F water bath and filled with juice from the glass container. Both glass and PET samples were stored, at the following temperatures: 4, 25, and 35°C, for up to 16 weeks.

Sample Preparation. Extraction of volatiles was done in a similar method to Parliment (16) and modified by Klim and Nagy (17) and Jella and coworkers (18). Liquid-liquid extracts were obtained using 1:1 pentane: diethyl ether. 10 mL of 1:1 pentane: diethyl ether was added to 10 mL of single strength orange juice from concentrate and vigorously mixed by forcing between syringes connected with a 3 way valve. After mixing, samples were centrifuged at 4000 rpm for 10 minutes. The solvent layer was drawn off and the aqueous layer re-extracted with an additional 10 mL of 1:1 pentane: diethyl ether and recentrifuged. Solvent layers were combined, and then dried over

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anhydrous sodium sulfate. 25 μ L of 4000 ppm 2-heptadecanone in 1:1 pentane: diethyl ether was added as an internal standard. Samples were concentrated to 100 μ L under a gentle stream of dry N₂ and stored in a septum sealed vial in a freezer until later analysis.

Gas Chromatography-Olfactometry Conditions. A HP-5890A GC (Agilent Technologies, Palo Alto, CA) with a standard FID detector was used to separate the orange juice extracts with the following fused silica capillary columns: DB-Wax (30 m $\times$ 0.32 mm id, film thickness 0.5 μ m) and DB-5 (30m 0.32 mm id, film thickness 0.5 μ m). Column oven temperature was programmed from 40 to 240°C at a linear rate of 7°C/min. with no hold. Column injection volume was 0.5 μ L and splitless. Injector and detector temperatures were 225°C and 275°C respectively. A Gerstel (Baltimore, MD) column splitter was used to split the effluent with a ratio of 2:1 between the olfactometry and FID detectors respectively. The olfactometer used in this study is similar to that described by Acree (19). The hot effluent from the capillary column was combined with a large stream of humidified air in a 1 cm diameter stainless tube. The air was purified by passing through activated charcoal, Drierite, and molecular sieve 5A (Alltech, Deerfield, IL). The purified air was then humidified by bubbling through a temperature controlled, water filled round-bottomed flask. Airflow to the stainless tube was adjusted to 1 L/min. Panelists sniffed the effluent as it exited the stainless steel tubing, describing the aroma of the effluent as well as rating the intensity of the volatiles on a 10 cm linear potentiostat (0-1.0 V output). Data was collected and recorded using Chromperfect (Justice Innovations, Inc., Mountain View, CA) software.

Time-Intensity Analysis. The olfactometry panel consisted of two trained panelists, 1 male and 1 female. Panelists were trained in a manner similar to Rouseff and co-workers (20), 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.

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Gas Chromatography-Mass Spectrometry Analysis. Sample separation was performed on a Finnigan GCQ Plus system (Finnigan Corp., San Jose, CA), using a J&W Scientific DB-5 column (60m x 0.25mm i.d. x 0.25µm film thickness, (Folsom, CA)). Samples were concentrated by liquid-liquid extraction previously described. Initial column oven temperature was 40°C and increased at 7°C/min to a final temperature of 275°C, with an injector temperature of 250°C. Helium, at a rate of 1 mL/min was used as the carrier gas. The mass spectrometer was set in the positive EI mode and scanned from 40.0 to 300.0 mass at 0.5 sec/scan.

Comparison of Juice Aroma Profiles - The juice stored for 112 days at 35°C was darkened indicating the existence of non enzymatic browning. The same juice stored at 4°C was not darkened but the aroma quality was markedly reduced from the original juice. The overall aroma characteristics of the two juices were profoundly different from each other. The juice stored at 4°C was acceptable but of low quality compared to fresh juice. The juice stored at 35°C was unacceptable, with distinct heavy cooked, caramel, non-orange storage aromas. In figure 1 it should be noted that there seemed to be more aroma activity associated with the higher boiling, late eluting compounds rather than the more volatile, early eluting compounds. The most intense aromas are due to vanillin, ethyl vanillin, wine lactone, 4-

Figure 1 -GC-O aroma intensity vs commercial OJ reconstituted from concentrate and stored at either 4 or 35°C for 112 days. Aromagrams were obtained using a carbowax column. Experimental conditions in text.

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vinylguaiacol, Furaneol, β -ionone, linalool and 3E-hexenol. Two of these, 4-vinylguaiacol and Furaneol, had been noted in the earlier canned juice study (14). However, as noted in our earlier publications (21), α -terpineol was not an off flavor aroma compound. Even though elevated levels of this compound were found in the high temperature storage juice, no aroma activity was observed. Therefore, α -terpineol can be used as a marker compound for thermal abuse, but it is not a causative compound. 4-Vinylguaiacol has a clove, spice aroma and Furaneol smells like burnt sugar or cotton candy. In a previous study (20), we reported that 2-methyl-3-furanthiol, MFT, was formed during heating of fresh juices. However, in this long storage study, no MFT was observed, but the dimerized form bis (2-methyl-3-furfuryl) disulfide was observed as a mid intensity aroma compound that was more intense in the high temperature storage juice. Both MFT and its dimer produce similar beefy aromas, which would contribute negatively in orange juice. Apparently, the MFT dimerized after being formed during 112 days storage. Linalool was found to be one of the most intense aromas, but not as intense as reported in the earliest GC-O studies (9). Its aroma intensity was also slightly reduced in the high temperature storage juice, which is not unexpected as linalool is known to undergo acid catalyzed hydrations to produce a variety of products (22).

Also notable is the complete loss of a few significant aroma compounds in the high temperature storage juice. 3Z-hexenal is a key loss as its green, grassy aroma is also associated with freshness at low levels. Octanal and 4Z-octenal are additional key aroma losses which are absent in the high temperature storage juice. The citrusy aroma of octanal and the fruity, sweet aroma of 4Z-octenal are likely a key factors in the aroma differences between the two juices. Ethyl butyrate has also been considered a key quality factor in orange juice (23). However, the aroma intensities of this compound were identical in the two juices.

Thus, the loss of orange flavor quality is the combination of several processes. The first process represents the loss of certain critical aroma components such as 3Z-hexenal, octanal and 4Z-octenal. Concurrently, non-citrus storage flavors are being formed or increasing sufficiently to alter the flavor balance. Aroma compounds which degrade aroma quality include, methional (potato), 2-MFT or its dimer (beefy), 4-vinylguaiacol (clove, spicy), vanillin and ethyl vanillin (vanilla). Furaneol (burnt sugar, cotton candy) has been found in freshly squeezed juice (10). At low concentrations it may add a certain pleasant background sweetness but it rapidly accumulates

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with elevated temperature and length of storage to the point where its contribution is negative.

Study 1F - Thermal treatment of xanthophyll-containing neoxanthin fractions from orange juice may lead to β -damascenone production

The carotenoid-derived potent odor, β -damascenone is present in fresh orange juice, and its content increase during storage (20). In analogy to other fruits (24), the presence of β -damascenone in orange juice may be due to unidentified glycosides that may release appropriate precursors of β -damascenone under the acidic conditions, especially after thermal treatment. Alternatively, we are testing the hypothesis that β -damascenone is produced non-enzymatically from neoxanthin (25), which is present in orange juice (26;27). If this is so, both the acidic conditions in processed citrus juices and the thermal treatment may favor such a pathway.

Carotenoid fractions were isolated from orange juice by HPLC (27). We collected 13 xanthophyll fractions which were mixed with linoleic acid (28), citrate buffer (pH 3.0) and 2,2'-azobis(2-methyl propion amidine).2HCl (radical solution) to initiate xanthophyll degradation. Test tubes were then placed in a boiling water bath for 3.5 min, cooled to room temperature, and stored at -20°C until GC-O analysis. A two mL aliquot from each original xanthophyll fraction was kept as a control.

Co-oxidation of these buffer solutions was evident as color was almost completely bleached. GC-O/FID (recorded simultaneously using DB-Wax and DB-5 columns) following SPME (15 min at 36°C) revealed the formation of β -damascenone-like odor, especially in fraction no. 7 (as detected by 3 trained panelists). These responses were detected at the corresponding RT values of the β -damascenone marker which was treated in the same manner. Control samples did not produce any detectable β -damascenone-like odor. The suggestion that neoxanthin is present in fraction no. 7 was supported by an R_f value in TLC compatible with that reported for neoxanthin in the literature, and by a UV spectrum of fraction 7 which was very close to that indicated in the literature for neoxanthin (UV_{max} 415, 437.5, 467 nm).

EXPERIMENT 2. - Evaluate the relative contribution of the above compounds to sensory acceptance using GC-O and advanced sensory evaluation techniques.

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Study 2A: Since it was very difficult to concentrate the potent aromas which are formed during processing and storage a recently developed GC-O procedure was employed to identify and quantify three potent aroma compounds. Methional smells like cooked potato, β -damascenone smells sweet, like honey and 2-methyl-3-furanthiol smells meaty. These compounds were quantified in fresh and stored orange juice using nasal impact frequency (NIF). Advanced sensory techniques indicated that when methional and 2-methyl-3-furanthiol were added to fresh juice at the levels they were found in stored juices, that the fresh juices tasted much like the stored juices.

GC-O analysis using the nasal impact frequency (NIF) and surface of nasal impact frequency (SNIF) to identify off-odors in citrus juice – Published -- complete article in appendix with summary presented below.

Bezman, Y.; Rouseff, R.; Naim, M. 2-Methyl-3-furanthiol and methional are storage off-flavors in orange juice: Aroma similarity, NIF/SNIF GC-O and GC analyses. *J Agric Food Chem.* 2001, 49, 5425-5432.

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, 30, 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.

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EXPERIMENT 3. - Evaluate the effectiveness of an “electronic nose” to differentiate the headspace aromas of from untreated and heat pasteurized orange and grapefruit juices.

Study 3A - Discrimination of thermally treated orange juices by an electronic nose equipped with organic polymer sensors Multielement solid state sensor arrays coupled with multivariate statistic packages can be used to quickly classify citrus juices within certain categories defined by a training set for each category. However, it is easy to overfit the data, producing an algorithm that has little validity outside the original training set. To complicate matters more the sensors tend to drift with time, requiring the training sets to be constantly re-run. The technology has yet to live up to its potential.

Bazemore, R.; Rouseff, R. Discrimination of thermally treated orange juices by an electronic nose equipped with organic polymer sensors. *Seminars in Food Analysis* 1998, 3, 59-66. reprint in appendix.

An electronic nose was used to discriminate between juices from the most popular and economically important orange cultivars in Florida. Early-mid season (Hamlin, Pineapple and Parson Brown) and late season (Valencia) orange juice samples were divided into four groups according to the following time-temperature treatments (pasteurization): no heat (unpasteurized), 8s at 96°C, 120s at 96°C, and 180s at 96°C (severely pasteurized). An electronic nose equipped with 12 organic polymer sensors was able to discriminate between early-mid season juice exposed to different time-temperature treatments and also between early-mid and late season cultivars. Discrimination between late season juice samples exposed to different heat treatments was less successful. The development of sensors specific for flavor impact volatiles should improve this technology.

EXPERIMENT 4. - Use model systems of citrus juices to investigate three possible precursor pathways for flavor impact compounds formed or lost during pasteurization or storage.

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Study 4A. Precursors for Bread-like Aroma. Furaneol (2,5 dimethyl-4-hydroxy-3-(2H)-furanone) is an important flavor compound, having a caramel-like burnt-pineapple flavor in various fruits (Rodin et al., 1965; Pickenhagen et al., 1981; Wilson et al., 1988; Pisarnitskii et al., 1992). However, Furaneol can be objectionable in citrus juices (Tatum et al., 1975), it is not present in fresh juice but rather formed during processing and storage (Haleva et al., 1997). Its presence in stored orange juice was also evident in the GC-Olfactometry (GC-O) chromatogram (see p. 17, our BARD proposal of this project). Under acidic conditions of citrus juices, Furaneol is formed from 6-deoxy-sugars (rhamnose), but not from hexoses, in the presence of an amino acid such as arginine (Haleva-Toledo et al., 1997). Thiols such as the amino acids L-cysteine (Cys) and N-acetyl-L-cysteine (AcCys) and glutathione inhibited browning and ascorbic acid degradation, and improved the acceptance of orange juice stored under moderate conditions (Naim et al., 1997). These thiols inhibited the formation of a variety of compounds related to browning, such as hydroxymethyl furfural (HMF) and methyl furfural (MF), as well as the accumulation of citrus juice off-flavor compounds, such as p-vinylguaiacol (PVG) and Furaneol (Naim et al., 1993, 1997). Following the above results and since cysteine occurs in orange juice (though in minor amounts) and may react directly with Furaneol to produce aroma of roasted food (Shu and Ho, 1988), this study was designed to quantify the effect of Cys and AcCys on Furaneol formation in buffer solutions used as a model. *Study published and summary provided below:*

Haleva-Toledo, E.; Naim, M.; Zehavi, U.; Rouseff, R. L. Effects of L-cysteine and N-acetyl-L-cysteine on 4-hydroxy-2,5- dimethyl-3(2H)-furanone (Furaneol), 5-(Hydroxymethyl)furfural, and 5-methylfurfural formation and browning in buffer solutions containing either rhamnose or glucose and arginine *J. Agric. Food Chem.* 1999, 47, 4140-4145.

Solutions of L-cysteine (Cys) and N-acetyl-L-cysteine (AcCys), containing glucose or rhamnose, with or without arginine, were buffered to pH 3, 5, and 7 and incubated at 70 °C for 48 h. Cys and AcCys

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inhibited the formation of (hydroxymethyl)furfural (HMF) from glucose and methylfurfural (MF) from rhamnose under acidic conditions. AcCys inhibited the accumulation of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (DMHF, Furaneol) from rhamnose, but Cys, under our experimental conditions, enhanced Furaneol accumulation from rhamnose. Cys and AcCys reacted directly with Furaneol but not with HMF or MF. Both Cys and AcCys inhibited nonenzymatic browning at pH 7. At pH 3, however, Cys reacted with both glucose and rhamnose to produce unidentified compounds that increased the visible absorbency.

Study 4B -- Degradation of thiamine in model solutions of orange juice: conditions adjusted to simulate actual storage of citrus products:

As indicated in our original proposal, thiamine (vitamin B1) is the second most abundant water soluble vitamin in orange juice after ascorbic acid (Ting, 1980). The role of thiamine as a precursor of significant aroma components in foods such as meat products has been well established, and in fact, thiamine is a precursor of one of the most potent aroma compound ever found bis(2-methyl-3-furyl)-disulfide (Buttery et al., 1984). Processing of citrus juices (e.g., pasteurization) and storage are expected to induce degradation of thiamine (vitamin B1), leading to aroma components which are, unlike meat products, objectionable compounds in citrus. In the following experiment we used model solutions of orange juice to monitor thiamine degradation.

Materials and Methods

Preparation and storage: Model orange juice solutions (MOJ) were prepared according to Peleg et al., (1992) with slight modifications. MOJ contained in 100 g (w/w); 5.0 sucrose, 2.5 glucose, 2.5 fructose, 1.2 citric acid, 0.6 tri-potassium citrate, 0.3 arginine, 0.3 GABA and 0.06 ascorbic acid in double distilled water (DDW). MOJ was fortified with either 0.5, 1.0 and 2.0 mg/kg of thiamine-HCl and 500 mg/kg sodium benzoate. A solution of $K_3Fe(CN)_6$ (30%, w/w, Sigma Co.) was prepared in DDW and 1 mL of this solution was mixed with 24 mL of 3.75 M NaOH to form the "oxidation solution". Sixteen mL aliquot of MOJ were transferred to 28-mL brown vials (each sealed with a septum). Nitrogen atmosphere was produced by 1 min nitrogen flowing into the MOJ containing vials. Samples were then stored at 4, 25 and 35°C for 2 or 6 weeks. Following storage, samples were kept at -20°C until analyzed. For each thiamine concentration (including samples

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containing zero thiamine), a matched sample was kept at -20°C throughout the entire experiment, assuming no thiamine degradation at this temperature.

Chemical analysis: Derivatization of thiamine was made in the tested samples for spectro-fluorescence analysis of thiamine following the method described by Ting and Rouseff (1980) with modifications. One hundred μL from each of the incubated samples was transferred to a 5 mL test tube kept at a room temperature. Fifty five μL of 1% (w/v) ascorbic acid solution was added and vortexed. Oxidation of thiamine to thiochrome was started by the addition of 300 μL of the above oxidation solution and stopped 1 min later by adding 900 μL of 1 M HCl. Volume was made up to 2 mL by DDW and pH was adjusted to 7 by 3.75 M NaOH. Quantitative determinations were then conducted in the same day. A Merck-Hitachi L-7100 HPLC equipped with a LiChrosphere 100 RP-18 column (5 μm , 250mm x 4mm, Merck) and a RP-18 pre-column was used for chromatographic separation. Twenty μL aliquot of each sample was injected and the column was eluted isocratically with

acetonitrile – DDW (50:50 v/v)

at a flow rate of 1 mL/min, at room temperature. The separated chromatographic peaks were identified and quantified by F-1050 Merck-Hitachi fluorescent detector (excitation 366 nm, emission 435 nm).

Data analyses: Results of chemical analyses were tested by one or two-way analyses of variance using SAS-statistical-package programs. A Tukey

multiple range test was performed for comparisons among the means.

Results and Discussion

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Figure 2. Thiamine degradation in MOJ fortified with 0.5, 1.0 and 2.0 mg/kg thiamine and stored at 4 (open bars), 25 (hatched bars) or 35°C (crossed-hatched bars) for two weeks. Control samples were kept at -20°C . Values are the means and SEM of two samples for each data point, each injected 3 times to HPLC. Different superscript letters within each block of thiamine concentration indicate significant differences ($p<0.05$).

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Degradation of thiamine was already evident after 2-weeks storage of MOJ solutions at 35°C (Fig. 4). A clear trend (which was significant for 2 mg/kg thiamine in MOJ) toward reduced thiamine was observed in the 25°C-stored samples. Under these conditions, degradation of thiamine was amount to about 20% in storage at 25°C and 50% at 35°C. In general, the rate of degradation was independent of the initial concentrations of thiamine. Storage at 4°C did not induce any degradation of thiamine.

The results of the 6-week storage (Fig. 5) showed a similar pattern of thiamine degradation observed in the 2-week period. Under these conditions, degradation was more evident at both 25 and 35°C, indicating a loss of 50% of thiamine in samples stored at 25°C and 65% at 35°C. As with the 2-week storage, thiamine degradation was essentially independent of the initial thiamine concentrations.

To the best of our knowledge, no information is available on thiamine degradation in citrus products during storage or the thiamine status in other foods stored at less than 40°C. The reported

Figure 3 Thiamine degradation in MOJ fortified with 0.5, 1.0 and 2.0 mg/kg thiamine and stored at 4 (open bars), 25 (hatched bars) or 35°C (crossed-hatched bars) for six weeks. Values are the means and SEM of two samples for each data point, each injected 3 times to HPLC. Different superscript letters within each block of thiamine concentration indicate significant differences ($p < 0.05$).

content of total (free and bound) thiamine in orange and grapefruit juices is between 0.5-1 mg/kg product (Fellers et al., 1990). Thus, in the present experiments, concentration of thiamine in MOJ are compatible with those in citrus products. If the degradation values observed here with MOJ are also true for citrus juices stored under similar conditions, then the amount of the resulted degradation products of thiamine in MOJ is significant and evidently may either have odor activity of themselves, or being the potential

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precursors for off-flavors in citrus products. For example, the 50% degradation of the 1 ppm thiamine occurred in MOJ under moderated storage conditions may result in a concentration of 0.5 ppm (500 ppb) degradation products. In view of the fact that the taste (odor) threshold of important aroma components formed from thermal degradation of thiamine (meaty, cabbage, burnt) is in the range of 1-100 part per trillion (Guentert et al., 1994), the potential of producing active aroma components (most likely off-flavors) from thiamine in our MOJ is high. We will soon apply our GC-O system to test the possible occurrence of these putative odorants in MOJ and in actual stored citrus products. We will further determine their formation during pasteurization which is another important processing stage where thermal treatment may be relevant.

Study 4C – Evaluation of Aroma Active Thermal decomposition products from thiamine (vitamin B1) using GC-O

In the previous section, 4B, thiamine degradation is demonstrated by measuring the loss of thiamine under specific conditions. In this study, we have examined the aroma active products formed from the decomposition of thiamine. Special emphasis is placed on the identification and quantitation of MFT and its dimer. They are identified using GC-O techniques as well as using sulfur specific GC detectors and MS. Their concentration changes during 12 weeks storage is reported. *A summary is provided below.*

Dreher, J. G.; Rouseff, R. L.; Naim, M. Characterization of aroma volatiles from the thermal degradation of thiamine in model orange juice using GC-O. *J Agric Food Chem* 2002, *in prep.*

Thiamine dissolved in model orange juice solutions was stored for up to 12 weeks at 35°C. Solutions were evaluated on a regular basis using GC-O and a sulfur specific detector. Both 2-methyl-3-furanthiol, MFT, and its dimer, bis-(2-methyl-3-furyl)disulfide are thiamine degradation products. Up to 11 aroma active components were noted, but the two most intense were due to MFT and its dimer. MFT was detected on day seven. Its concentration peaked at day 45, then declined. The dimer was not detected until day 14 and its concentration plateaued at 30 days. MFT appears to be the precursor for the dimer and the

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dimer is the more stable form. The identities of both compounds were confirmed by MS as well as LRI data on two columns.

Study 4D - Strecker production of methional from methionine with dehydroascorbic acid: Effect of temperature, pH and dehydroascorbic acid concentration

Methional, a sulfur based aldehyde, is one of the potent aromas which degrade the flavor of citrus juices. It is an extremely potent aroma impact compound whose baked potato aroma diminishes the “fresh juice” flavor and probably contributes to cooked or processed flavors observed in commercial juices. This component can be smelled by the human nose long before it can be detected with typical FID or MS instrumentation. It was reported as a potent aroma component in tomato (29) with a threshold of 0.2 μ L. We had originally characterized this odorant as “bread” when the proposal was submitted in 1995 and again in 1997. It was subsequently found in hand squeezed orange juice (10). Askar (30) implicated methional in the unfavorable flavor changes of vegetable juices after thermal processing and storage. He hypothesized that methional is formed from the Strecker degradation of methionine with Maillard α -dicarbonyl reaction products. We have demonstrated that its concentration increases with increasing storage time-temperature, and it is formed primarily via the Strecker of methionine with dehydroascorbic acid, not a Maillard product. The primary source of the required α -dicarbonyl is oxidized ascorbic acid. Ascorbic acid is relatively abundant in citrus juices and readily reacts with dissolved oxygen to produce dehydroascorbic acid. This mechanism satisfactorily explains the presence of Strecker aldehydes in citrus juices because the Maillard reaction which normally produces the required α -dicarbonyl reactants typically requires much higher temperatures ($>200^{\circ}\text{C}$) and lower water activities. The purpose of this study was to determine if the primary source of α -dicarbonyl used to produce methional was from the classic Maillard reaction or whether it was from the oxidation of ascorbic acid (dehydroascorbic acid).

Material and Methods:

Model orange juice (MOJ) solutions contained deionized water, sucrose, glucose, fructose, citric and malic acids and tripotassium citrate. After the dissolution of all substances, MOJ was filtered

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using a 0.45 micron membrane and stored at 4°C. Methionine (0.15 mM/L) and dehydroascorbic acid were prepared just prior to use. The MOJ solutions were prepared according to a central composite design resulting in 18 experiments. The independent variables were temperature (90, 75, 60°C), pH (4.5, 3.5, 2.5) and dehydroascorbic acid concentration (0.5, 1.0, 1.5mM). Dehydroascorbic acid and methionine were added prior to heating treatments. Heating time was 1 hour for all treatments. Data was analyzed using response surface methodology (RSM).

Methional quantification

Extraction – An aliquot of 10 mL of MOJ was transferred to a 40 mL bottle containing a micro stirring bar and placed on a water bath at 40°C. After an equilibrium time of 45 min, a SPME (Supelco Co., Bellefonte, PA) fiber (50/30 µm DVB/Carboxen/PDMS) was manually inserted into the headspace of the sample bottle. Exposure time was 30 min, after that the SPME was injected into the GC injection port at 200°C and kept for 3 min for desorption.

Gas Chromatography - Volatiles constituents were separated using a HP-5890A GC (Palo Alto, CA) with a OI Analytical Model 5380 Pulsed Flame Photometric Detector (PFPD) (OI Analytical, College Station, TX) and a 30m x 0.32 mm x 0.50 µm film thickness low bleed Zebron ZB-5 column (Phenomenex-Torrance, CA). Column oven temperature was programmed from 40 to 250°C at 7°C/min with holding time of 5 min. Injector temperature was maintained at 200°C and detector temperature at 250°C. The carbon gate time was set to 1-3 mseconds and the sulphur gate time was 6-24.9 mseconds. Chromatograms were recorded and integrated using Chrom Perfect version 5.0.0, Justice Laboratory Software (Palo Alto, CA, USA). Peaks were identified using standardized retention indices (*3I*) calculated using retention time data from a series of alkane standards (C5-C25) run under identical chromatographic conditions.

Results and Discussion

The results of two accelerated storage studies are shown in the following figure. Samples are model orange juices at pH 3.5 which have been heated at 75°C for one hour. The upper trace is from the model orange juice containing all the major amino acids and sugars that typically undergo Maillard reactions to produce α -dicarbonyl compounds which take part in the classic Strecker degradation of methionine. However, under these relatively mild conditions, no methional could be

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detected. The lower chromatogram corresponds to the same model orange juice solution but it contains 1 mM dehydroascorbic acid and methionine is the only amino acid present. It is apparent

Figure 4 - Comparative chromatograms from PFPD of model orange juice solutions at pH 3.5 both heated at 75°C for 1 hr. The upper chromatogram contained all the major amino acids + methional, but no dehydroascorbic acid. The lower model solution contained methional + dehydroascorbic acid only. DMS = dimethyl sulfide, DMDS = dimethyl disulfide, DMTS = that dehydroascorbic acid is the preferred source of α -dicarbonyl (as it is a tri-carbonyl) which takes place in the Strecker degradation of methionine to produce methional. It is also interesting to note that methionine thermally decomposes to produce dimethyl sulfide and higher dimers and trimers. Furthermore, more of these compounds appear to be formed in the absence of a ready source of α -dicarbonyl.

Effects of pH, temperature and dehydroascorbic acid concentrations on methional formation

Figure 5 - Relationship between temperature and DHAA on methional formation at pH 3.5

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Since methional is preferentially formed in the presence of dehydroascorbic acid, DHAA, experiments were conducted to determine how the rate of methional was affected by pH, temperature, DHAA concentrations using the response surface methodology. As indicated in the methods section, the range of values studied was fairly limited. The results comparing the effect of temperature and DHAA concentration on methional formation is shown in figure 5. It is apparent that methional concentrations increase in the presence of higher DHAA concentrations and higher temperatures.

Figure 6 - Effect of pH and DHAA on methional formation at 75°C

In figure 6, the effects of pH and DHAA on methional formation are compared. It is clearly shown that rates of methional formation increase with either increasing or decreasing pH. pH 3.5 appears to be the least favorable pH for the formation of methional from the Strecker degradation of methionine with DHAA. The pH of citrus juices typically range from pH 3.5 – 3.8. Therefore, even though the reaction of methionine with DHAA is not favored

at citrus juice pH's, it is still the dominant pathway by which the off flavor methional is formed.

Study 4E - Potent aroma volatiles from the thermal decomposition of β -carotene

Carotenoids are large (C40), non-volatile molecules which are responsible for the color of orange juice. They are too large to be volatile but they can be degraded by enzyme, chemical and thermal reactions. Some of their smaller decomposition products such as norisoprenoids (C9-C13 : β -ionone, β -damascenone etc.) have intense aroma activity. Carotenoids-derived aroma compounds have been identified in tobacco, tea, grapes, tomato, saffron etc. and in some cases produce the characteristic aromas of the respective food. Certain citrus carotenoids (β -carotene, neoxanthin and β -cryptoxanthin) have the structural potential to generate norisoprenoids with intense aroma activity. Therefore, juice carotenoids may be the source of some aroma changes associated with flavor changes during thermal processing of citrus juice. The objective of this study was to

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determine if aroma active compounds are produced from the thermal decomposition of β -carotene. Identify aroma active compounds using the combination of SPME and GC-Olfactometry.

Materials and Methods

β -carotene was first recrystallized by petroleum ether and ethanol. One milligram of recrystallized β -carotene was dissolved in acetone and diluted to 100 ml citric acid buffered to pH 3.8. Ten milliliters of the model solution was added to a 40 ml vial and heated at $98 \pm 2^\circ\text{C}$ for 1, 2 and 3 h. in a water bath. Headspace volatiles were collected using SPME (Carboxen/PDMS) at 40°C with agitation. Separation was accomplished with a HP-5890 GC using either a DB-wax or DB-5 column. The effluent was split between an olfactometer or flame ionization detector, FID. Column oven temperature (for DB-wax) was 40 to 240°C (but 40 to 265°C for DB-5) at $7^\circ\text{C}/\text{min}$ with a 5 min hold. Degradation products were tentatively identified from their aroma description and retention characteristics on two column types and identification confirmed by comparison with standards. Fresh Valencia orange juice was pasteurized using UHT/HTST lab Microthermics Inc. Model 25 at temperature 195°F , held for 12 second and filled at 41°F . Headspace volatiles of unpasteurized, pasteurized and unflavored reconstituted concentrate orange juice were determined by SPME and GC-O. Ten milliliters of the juice plus 1 gram of NaCl was added to 40 ml vial and headspace volatiles were collected using SPME at 40°C with agitation.

Results and Discussion

This study was conducted because β -damascenone was detected as a possible thermal degradation

Figure 7 - Chromatogram (upper) and superimposed aromagram (lower) of headspace from purified β -carotene in model orange juice solution heated for two hours at $98 \pm 2^\circ\text{C}$

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product in our earlier SNIFF studies (Study 2A). Preliminary results found that even with the best β -carotene standards, additional aroma impact compounds were present even before heating. Therefore, careful recrystallization was required. The results from recrystallized β -carotene are shown in figure 7. The more complex FID chromatogram indicates that many volatile products are formed from the thermal decomposition.

Figure 8 - Behavior of aroma active components formed during the thermal decomposition of β -carotene as a

Conclusion

Three aroma active volatiles (β -cyclocitral, β -damascenone, and β -ionone) were detected in model orange juice solutions containing β -carotene after heating. Concentrations of these compounds increased with increased heating time. There was no significant difference in these volatiles between helium sparged and control samples, suggesting that low levels of oxygen did not alter the thermal

degradation pathway of β -carotene. These studies have demonstrated that the thermal degradation of β -carotene is a possible pathway in the formation of three potent aroma impact compounds found in citrus. However, the extreme temperature and times are not typically experienced in orange juice production so if this pathway is valid at lower temperatures, it would take a much longer times.

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