PHOTOCHEMICALLY INDUCED FLAVOR CHANGES IN ORANGE JUICE EXPOSED TO LIGHT IN GLASS AND POLYETHYLENE TEREPHTHALATE AT 4°C

By

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by

Kristin Ann Nelson

I would like to thank my husband Jared, for his support and encouragement these past two years. He has been there to offer advice when things did not go as planned and to cheer me on when things did. I would also like to thank my parents who have been there since day one. No matter where my travels take me, they have always been ready with bags packed to rush to my aid when they were needed. Without my family, I would not be where I am today and therefore I dedicate this project to them.

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Pasteurized Valencia orange juice was stored in glass and polyethylene terephthalate containers and exposed to fluorescent light at 4°C for twelve weeks. The flavor, color and ascorbic acid concentrations of juices exposed to light were appreciably different than control samples covered with aluminum foil. Light exposed juices became darker, as indicated by significant ($p<0.01$) decreases in "L" values. Light exposed orange juices lost 21% and 68% more ascorbic acid than unexposed controls when stored in plastic and glass containers respectively.

Using a defined 15 point flavor quality scale, a trained sensory panel judged the light exposed juices to be of lower quality than controls. Juices exposed to light for twelve weeks had an average rating of 3.8 whereas control juices had an average rating of 6.8. In addition to overall flavor evaluations, individual aroma components were evaluated using GC-olfactometry (GC-O). Light exposed samples contained less β-

myrcene, and more carvone, 1,8-cineole, *p*-cymene, vanillin, and Furaneol. Extracted ion chromatogram GC-MS data indicated that light exposed juices had on average 85% more *p*-cymene than those that were not exposed. Vanillin, Furaneol, and some sulfur compounds typically form in juice due to thermal degradation and thermally induced non-enzymatic browning. However, all juices were stored at 4°C which is well below the minimum temperatures needed to produce thermal degradation by classical chemical means, suggesting these compounds were products of photochemical reactions. The thermally induced off-flavors α-terpineol and 4-vinylguaiacol did not increase in light exposed samples, indicating that these two degradation products were not catalyzed by light-exposure.

Two tentatively identified sulfur compounds were observed in juice samples that were exposed to light. The compound 4-mercapto-4-methylpentan-1-ol had an onion-like, moldy, or soured aroma and 3-mercapto-hexen-1-ol had a moldy or soured aroma. In addition, two sulfur smelling compounds (R.I. of 806 and 900) were produced only in light exposed juices during an accelerated storage study. The appearance of these off-aromas in only light exposed juices partially explains why the overall orange juice character was degraded.

CHAPTER 1
INTRODUCTION

Citrus is one of the most important agricultural crops in Florida with annual orange juice sales topping three billion dollars in 2004. However, in recent years there has been a steady decline in orange juice consumption and industry profits (*1*). Manufacturers have found that using different packaging may increase sales. A recent trend is to employ clear containers in order to attract consumers with the fresh, bright color of citrus juices (*2*). Glass has been used for this purpose; however, this material is expensive, heavy, and prone to breakage. An alternative is to utilize clear plastic such as polyethylene terephthalate (PET). The plastic is lightweight, robust, and inexpensive. Limitations include low oxygen and light barrier properties.

There have been numerous reports concerning the influence of light, and container oxygen permeability on color changes and Vitamin C losses (*3-5*). Solomon et al. (*3*) reported that light had no effect on ascorbic acid content and an insignificant effect on browning on orange juices stored for 52 days at 8°C whereas Ahmed and coworkers (*5*) reported a 20% loss of ascorbic acid in only six days at a similar storage temperature. Sensory panels have also compared light exposed orange juices with controls and found significant differences. Although overall flavor changes have been reported, the individual flavor compounds responsible for these changes have yet to be identified. Light and oxygen studies conducted using orange oil and lemon oil aqueous emulsions at room temperature and slightly elevated temperatures have shown that changes in flavor compounds are due to specific chemical and photochemical reaction products (*6-9*).

Since orange juice has many compounds in common with orange and lemon oils, it is hypothesized that similar photochemical reactions might occur in orange juice. However the rate at which these products would form at 4°C is uncertain.

CHAPTER 2
LITERATURE REVIEW

Orange Juice Volatiles and Oxidation Reactions

The aroma of orange juice consists of a combination of volatile compounds in specific proportions. The classes that make up orange juice flavor are terpenes, aldehydes, esters, alcohols and sulfur compounds. Terpenes make up the largest percentage of orange volatiles, with the main terpenes being d-limonene, myrcene, and valencene. Although most terpenes do not play a direct role in orange juice flavor, they may work as carriers for other oil-soluble volatiles. The aldehydes that are thought to make the largest contribution to flavor are acetaldehyde, citral, octanal, nonanal, decanal, and sinensal. According to Shaw (*10*) ethyl butanoate, ethyl 2-methylbutanoate, ethyl propionate, methyl butanoate, and ethyl 3-hydroxyhexanoate are the major esters in orange juice and are responsible for the fruity, "top note" aroma in fresh juice. Shaw (*10*) has also indicated that alcohols such as ethanol, E-2-hexenol, Z-3-hexenol, linalool, and α-terpineol are present in orange juice, but few make a significant contribution to flavor. Finally, it has been suggested that various sulfur compounds that are present at very low concentrations in the juice make a large contribution to the overall flavor. Some of these compounds are hydrogen sulfide, methanethiol, and dimethyl sulfide. (*10*)

"Off-flavor" is defined as a flavor that is not natural or normally present in fresh foods resulting from deterioration or contamination. Off-flavors in orange juice are primarily formed due to reactions with oxygen. Oxidation reactions decrease ascorbic

acid levels, and induce terpene oxidation. Aerobic microbiological growth can also produce off-flavors in orange juice (*11*).

These situations occur when oxygen is dissolved in the product, through contact with oxygen in the headspace and from oxygen diffusion through the container material. Decomposition of residual hydrogen peroxide can also produce oxygen and oxidation reactions in those cases where it has been used as a package sanitizer. Dissolved oxygen in the initial juice can be decreased by deaeration, and oxygen in the headspace can be reduced by filling the container completely or by flushing it with nitrogen. However, the only way to reduce oxygen permeation through the package is by changing the barrier properties of the container.

Packaging Materials and Interactions

Orange juice is packed in a wide variety of materials including metal cans, paperboard cartons, plastic containers, and glass bottles. Although these materials are designed to protect the juice, packaging materials can also affect the juice's flavor in one of at least three ways. The three primary flavor altering processes associated with packaging are flavor scalping, flavor leaching, and permeation of compounds through the package.

Scalping

Scalping refers to the absorption of one or more compounds from the orange juice into the packaging material. This process has been observed primarily in plastic containers. Even though it is generally accepted that volatile composition is altered as a result of scalping, there were conflicting reports as to whether the loss of certain volatile compounds influenced the juice's taste or aroma.

In 1987, Kwapong and Hotchkiss studied citrus essential oil solutions stored in low density polyethylene (LDPE) and two polyethylene ionomers (*12*). Orange oil components were not sorbed equally. Benzaldehyde and ethyl butyrate were sorbed to at approximately equal levels with K_e values ranging from 2-7, where $K_e = C_{(plastic)}/C_{(aq\ solution)}$. Neral and geranial were moderately sorbed with K_e values ranging from 15 to 23 and 22 to 40 respectively. Limonene was heavily sorbed especially in LDPE with a K_e value of 4700. Ten untrained panelists detected significant differences ($p<0.05$) in the aroma of the citrus samples using the triangle test. Also in 1987, Manheim, Miltz, and Letzter compared orange and grapefruit juices stored in laminated cartons and glass jars at 35°C for 10-12 weeks (*13*). They found a 25% loss of limonene in both orange and grapefruit juice stored in cartons within 14 days of storage. A panel of twelve to fifteen experienced tasters detected a significant difference ($p<0.05$) in the juices flavor. In 1992, Marin and colleagues observed an 80% loss of orange juice limonene into LDPE within 24 hours at 25°C(*14*). They used gas chromatography-olfactometry (GC-O) to determine that limonene has relatively low aroma activity and thus contributes little if any to overall orange juice flavor and aroma. Also in 1992, Pieper and colleagues compared preference scores for orange juices stored at 4°C for 24 weeks in glass and LDPE cartons (*15*). They found that although the plastic cartons absorbed 50% of the limonene, no significant difference was observed between the hedonic scores of any of the juices. Ethyl butyrate (which was thought to be an important aroma component) was not absorbed to a measurable extent by the plastic container. In 1997, Sadler and colleagues examined sorption of orange juice volatiles into LDPE, polyethylene terephthalate (PET), polyamide (PA), and ethylene (co-)vinyl alcohol (*16*). The juice

was maintained at 4.5°C while the polymer strips were exposed for three weeks. Juices were stored in sterile Erlenmeyer flasks in contact with the different polymer strips at a surface to volume ratio that was twice that which is used commercially. No significant flavor difference was detected in between any polymer treated and control juices using triangle tests with 15-22 experienced panelists.

Leaching

Leaching refers to the migration of compounds from the container into the orange juice. This can be caused by residual monomers, plasticizers, processing aids, and solvents from printing inks and adhesives (*17*). In the orange juice industry, this problem includes off-flavors caused by juice stored in metal cans. In 2000, Takahashi and colleagues concluded that plated tin inside the can reacted with dissolved oxygen to cause unwanted reactions in fresh mandarin orange juice (*18*).

Permeation through Package

Permeation relates to movement of flavor compounds through the package. This includes flavor compounds leaving the juice, and unwanted flavors entering the juice from outside the container. The greatest problem with permeation in orange juice is oxygen being transferred into the container and negatively impacting the juice inside.

Light and Oxygen Effects

When packaging materials do not provide an adequate barrier to light and oxygen, the juice's quality can be affected. The most common problems involve browning of the juice, loss of ascorbic acid, and changes in the overall flavor of the juice.

Browning

Non-enzymatic browning, or the Maillard reaction, occurs when reducing sugars such as sucrose, glucose, and fructose react with proteins, peptides, amino acids or

amines (*19*). The reaction is favored by higher temperatures, lower water activities, and during extended storage. Non-enzymatic browning is an undesirable reaction that occurs in orange juice when heated during pasteurization or storage. The reaction causes a loss of essential amino acids and leads to the formation of brown pigments known as melanoidins that cause juice colors to darken. Compounds such as furaneol, norfuraneol, furfural, 5-hydroxymethylfurfural, and sotolone are produced and contribute to flavor changes in the juice (*20*). The greatest driving force of these reactions is increased temperature, and the presence of these compounds has been correlated to elevated temperature storage.

Several studies have investigated the effects of light and oxygen on browning in orange juice. In 1995, Solomon et al. conducted research on pasteurized orange juice stored for fifty-two days at 8°C (*3*). The juice was stored in glass containers with glass, polyethylene and paper closures. They found that browning was significantly ($p<0.001$) correlated with the amount of dissolved oxygen in the juice which occurred to the greatest extent in paper capped bottles because they had the greatest oxygen permeability. However, the difference in the extent of juice browning due to light-exposure was found to be insignificant ($p<0.05$).

In 1986, Trammell and colleagues conducted an experiment on single-strength orange juice with varying dissolved oxygen levels (*21*). Juice was stored for five months at 22°C. It was again found that greater amounts of oxygen in the juice led to increased browning. However, a sensory evaluation did not detect changes in the juice flavor ($p<0.05$).

Ascorbic Acid Loss

The majority of research has investigated the effect of light and oxygen on ascorbic acid, also known as Vitamin C, in the juice because of its nutritional value. In 1976, Ahmed and colleagues investigated the effects of fluorescent light on flavor changes and ascorbic acid loss in reconstituted orange juice and orange drinks (*5*). The juices were divided into plastic, glass, and paperboard containers and were placed in a light chamber at 6°C for 6 days. It was found that, when exposed to light, the orange juice lost 20% of its ascorbic acid and the orange drink lost 40-90%. A trained taste panel of 10-12 women performed a hedonic scale rating that indicated the juice in the paperboard containers tasted significantly better ($p<0.05$) than juice in the light exposed plastic and glass bottles.

In 1992, Kennedy and colleagues studied commercial single-strength orange juice in TetraBrik cartons (*22*). The juices were stored at 4, 20, 37, 76, and 105°C for sixty days. They found that juices with lower initial dissolved oxygen (1.70ppm compared to 4.30ppm) had a slower rate of ascorbic acid loss (6.5mg/L*day compared to 25.5mg/L*day). It was also found that the temperature the juice was stored at plays the greatest role in deterioration in that the higher the temperature, the more ascorbic acid was lost.

Sattar and colleagues performed similar experiments in 1989 using pasteurized orange drink in clear, green and amber glass bottles as well as in a wax laminated paper (TetraPak) carton (*4*). The containers were stored at room temperature for thirty-two days. Ascorbic acid losses were 60.6%, 54.6%, 51.0%, and 45.5% in clear glass, green glass, TetraPak laminated paper, and amber glass respectively. This shows that greater light-exposure resulted in significantly greater ($p<0.05$) ascorbic acid loss. Also, the loss

in TetraPak cartons was greater than in amber bottles because of the higher oxygen permeability. A ten member panel performed hedonic ratings based on color, taste, and flavor and reported higher preference ratings for juices stored in amber bottles.

Photochemical Reactions

Several previous studies have investigated the changes in flavor compounds in lemon oil as a result of exposure to oxygen and light. In 1988, Schieberle and Grosch studied lemon oil in an aqueous citric acid emulsion (*7*). The samples were left for thirty days at 37°C. The team found that neral, geranial, and linalool decreased, with a corresponding increase in *p*-methylacetophenone, *p*-cresol, fenchyl alcohol, *p*-cymene, and 1-terpinen-4-ol. They believe this change in composition is responsible for the deterioration of lemon oil flavor over time.

In 1997, Iwanami and colleagues also investigated the effects of ultraviolet light on lemon oil (*6*). The team exposed a mixture of lemon oil, a phosphate buffer, and ethanol to UV-light (≤ 400nm) for four days at 30°C. They found that citral (a combination of neral and geranial), limonene, terpinolene, and nonanal decreased, while the decomposition product, *p*-cymene, increased. The finding that citral was the most unstable component and that *p*-cymene was produced mirrored the results of Schieberle and Grosch.

In 1991, Ziegler and colleagues studied the changes in flavor compounds in orange oil after exposure to ultra-violet light (*9*). The orange oil was dissolved in ethanol, acidified with citric acid and homogenized to form an emulsion. The emulsions were exposed to ultraviolet light for fifty minutes at 20°C. Ziegler found a significant increase in carvone, isopulegol, isomers of carveol, limonene oxide, and linalool oxide with a corresponding decrease in neral, geranial, and cintronellal. In addition, several new

compounds formed during the study including *p*-mentha-1,8,dien-4-ol, α-cyclocitral, photocitral A, iso(iso)pulegol, carvonecamphor, methone, isomenthone, isomers of *p*-menth-1(7),8-dien-2-ol and isopiperitenol.

Extracting and Concentrating Flavor Volatiles

Volatiles in orange juice are in very low concentrations and are part of a complex matrix of insoluble and nonvolatile compounds that cannot be injected into a gas chromatograph. For these reasons, the volatiles in the orange juice must be isolated and concentrated before analysis can occur.

Sample Extraction

Two common extraction techniques are headspace analysis (either static or dynamic) and liquid-liquid extraction. These techniques have various advantages and limitations and the method performed depends on the compounds of interest. Regardless of the isolation technique, the goal remains to make the extraction representative of the original sample.

Liquid-liquid extraction relies on the differences in polarity to extract the desired compounds from the overall sample. The solvent chosen for the extraction determines which compounds will be isolated. Compounds that are non-polar will be extracted to a greater extent by a non-polar solvent such as pentane. The juice and extracting solvent are thoroughly mixed, and centrifuged to separate aqueous and organic layers. The organic layer is retained, while the aqueous layer may be discarded or can be extracted with the same solvent or another solvent repeatedly. However, the more solvent that is used for extraction, the further the sample must be concentrated before analysis, and thus the more volatiles may be lost.

One advantage of this extraction method is that the solvent comes in direct contact with the juice and extracts the volatiles from the juice matrix, whereas in headspace analysis the volatiles must partition from the pulp into the aqueous phase, come to equilibrium with the headspace, and finally adhere to the fiber coating. Liquid-liquid extraction allows for a more accurate quantification of a wider range of volatiles, as competition for headspace and fiber coating is eliminated. One drawback is that it also isolates some non-volatile material such as carotenoids and lipids that can degrade in the GC injector and form artifacts.

It is important to note that not all chemical compounds have the same extraction efficiencies, and thus are not in the same proportions in the extract as they were in the original juice. This can be overcome by adding internal standards to the sample mixture. Internal standards should be chosen such that the standard and the compound of interest have similar structures and physical properties. Ideally, for GC-MS analysis, a deuterated isomer of the desired compound should be used for comparison, since it will have the same extraction efficiencies and evaporation loses. Knowing the concentration of the internal standard can allow for back calculation to find the concentration of compounds in the original sample.

Sample Concentration

Concentration can be achieved by a variety of methods. The nitrogen blowdown method is commonly used. In this method, a stream of nitrogen gas is gently blown across the surface of the extract. This action increases the speed at which the solvent evaporates. The nitrogen blowdown method must be performed slowly so that the volatiles of interest are not lost with the solvent. Also, it is important to use a solvent that has much lower boiling point from that of the volatiles to reduce loss. After sample

preparation, the juice extract can then be injected into an analytical tool such as the gas chromatograph.

Gas Chromatography-Olfactometry History and Methods

Gas chromatography is a method that employs a capillary column of varying materials in order to separate chemical compounds based on their polarity and affinity for the column material. The temperature inside the column is steadily increased, causing the compounds to elute from the column at different retention times. Various instruments such as a flame ionization detector (FID) or a mass spectrometer (MS) can be used for compound detection.

In 1964, Fuller, Steltenkamp, and Tisserand first reported the use of an olfactometer (*23*). This device consisted of a sniff port attached to the GC, parallel to the detector. A human assessor could sit at the sniff port and observe the eluting aromas. This new technique helped researchers determine compound identities and also to establish which compounds were aroma active. Based on the intensity of the aromas, researchers could also determine how much each compound contributed to the overall aroma profile. This was a significant advancement in gas chromatography, in that some highly aroma active compounds that occurred at very low concentrations in orange juice and were previously overlooked, now received greater focus.

There are three main types of GC-O analysis: Dilution Analysis, Time-Intensity Analysis, and Detection Frequency. In Dilution Analysis, a sample is repeatedly reduced in concentration and analyzed on the GC-O. It is noted which compounds are detected at each dilution level. This information can be used to determine each chemical compound's threshold. The threshold is then divided by the concentration of that compound in the original sample in order to determine the compound's aroma activity.

The higher the activity, the more that compound contributes to the sample's overall aroma. The two types of Dilution Analysis are Aroma Extract Dilution Analysis (AEDA) which measures the peak height (*24*), and Combined Hedonic Aroma Response Measurement (CHARM) which measures the peak area (*25*). Time-Intensity Analysis consists of only running the sample once on the GC-O with no subsequent dilutions. During the GC run, the aroma intensity of each compound is noted by sliding a lever across a sectioned bar. The bar contains various intensity descriptors such as slight, moderate, and strong, and is attached to a computer that records the subject's inputs. The amount a compound contributes to the sample's overall aroma is then based on the height or area (OSME analysis) over which the subject moved the lever (*26*). The third method, Detection Frequency, consists of measuring what percentage of assessors detects a given aroma at various concentrations and is measured by Surface of Nasal Impact Frequency (SNIF) (*27*). In this study time-intensity GC-O was employed.

Purpose

The purpose of this study was to determine the effect of fluorescent light on ascorbic acid, browning and flavor of orange juices stored in glass and PET. Since both chemical and photochemical reactions occur during a storage study, this study will be conducted at temperatures just above freezing (4°C) to minimize chemical reactions. Therefore if any major changes occur, they would be due primarily to photochemical reactions. This study will also have practical significance as these conditions are more similar to retail or market place conditions than any previous study. This will also be the first study to examine the individual volatile components in light exposed orange juice.

CHAPTER 3
MATERIALS

Light Chamber

The light chamber was built to maximize the light-exposure the bottles would receive. A diagram and picture of the light chamber can be seen in Figures 1 and 2 respectively. In Figure 2, the side door is open for viewing.

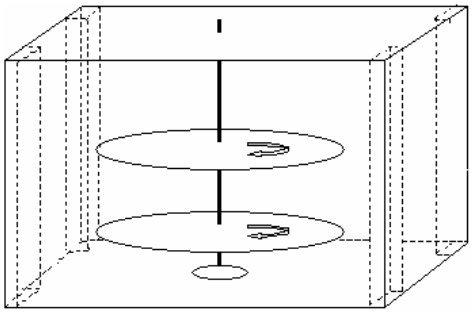

Figure 1: Diagram of Light Chamber

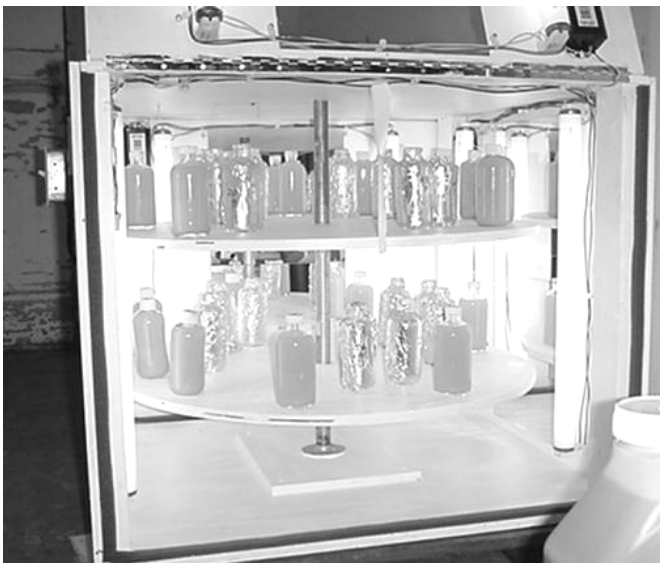

Figure 2: Picture of Light Chamber in Cold Storage

A steel pipe was secured to the center of two circular tables. The pipe rotated freely on its axis to allow the tables to be turned daily. This design ensured that each

container received an equal amount of light-exposure during storage. The chamber was 32”x 32”x 26”, supported by four 2”x 4” studs, and consisted of 5/8”plywood on the top, bottom, and two support sides, and 3/8” plywood on the two opening doors. It was equipped with eight 20W Phillips cool fluorescent light bulbs (Royal Philips Electronics USA, Somerset, New Jersey) that provided an average intensity of 1750 lux as measured on a Lambda Instruments LI-185 light meter (Lambda Instruments Corporation, Lincoln, Nebraska). These bulbs were chosen in order to simulate the wavelength of light that the juice would normally experience in a supermarket setting. The manufacturer’s specification of the light’s spectrum can be seen in Figure 3.

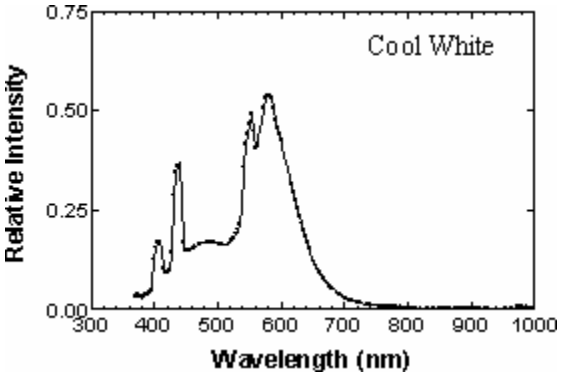

Figure 3: Wavelength Spectrum for Philips Cool White Fluorescent Lights

The lights were installed vertically to ensure that the top and bottom platforms would receive equal lighting and to reduce shadows. Eight reflective mirrors were placed on the sides of the chamber to reflect and increase the light intensity. Finally, two fans were installed in the sides of the chamber in order to increase airflow through the chamber and keep temperatures evenly distributed.

Orange Juice

The orange juice was from late season Valencias, was not concentrated, and did not have pulp or flavors added. The juice was pasteurized at 212°F for 10 seconds and then cooled to refrigeration temperature at a local orange juice processing plant before being

filled into five-gallon aseptic Scholle® bags. These were transported in coolers in order to assure that the juice did not undergo temperature abuse. The juice was transferred aseptically into sterilized plastic and glass bottles and placed into the storage chamber. The total time the juice spent between leaving the processing facility and being placed into the storage chamber was about one hour.

Storage Containers

The bottles used in this experiment were eight-ounce "Boston round" bottles obtained from Lerman Container Corporation (Lerman Container Co., Naugatuck, CT). The container materials were polyethylene terephthalate (PET) and glass and were similar dimensions. A graph of the light transmission characteristics for the two materials can be seen below in Figure 4. Both materials had similar light transmission characteristics and most of the light emitted by the fluorescent lights was in the wavelength range that the materials transmitted. Each bottle held 250 mL of juice.

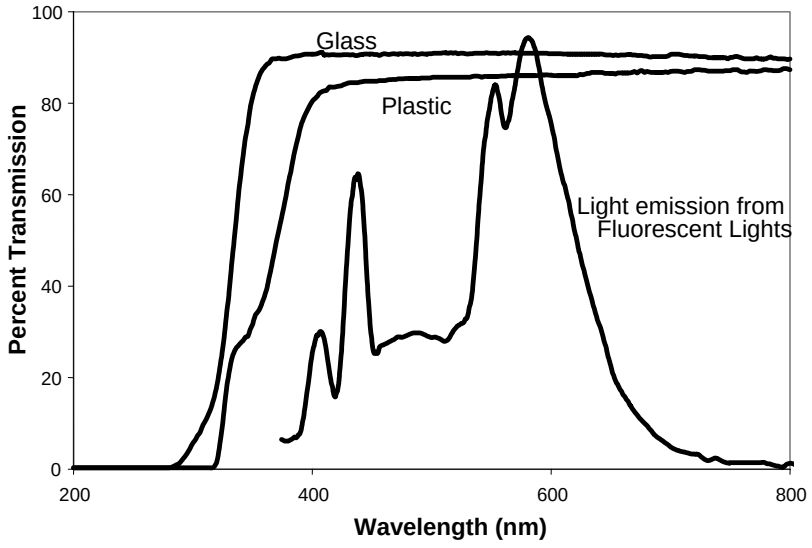

Figure 4: Light Transmission Through Glass and Plastic Containers

CHAPTER 4
METHODS

Initial Juice Measurements

Several tests were performed in order to document starting conditions. The °Brix was determined as a measurement of the soluble solids in a juice. One drop of the juice was placed onto a digital refractometer. This device related the juice's refractive index to °Brix (*28*).

The percent oil in the juice was measured using the Scott oil test (*29*). This test consisted of a titration based on the chemical reaction between d-limonene (the main component in orange oil) and bromine. The sample was prepared by mixing it with alcohol and heating it until the alcohol and oil evaporated. The vapor was condensed and collected for analysis. Bromine was added dro*p*-wise until it completely reacted with all the unsaturated compounds in the oil. Knowing the concentration of the bromine and the amount added to reach the stoichiometric endpoint allowed for the calculation of d-limonene content. This is normally expressed as % by volume in 11.8 °Brix juice.

The total titratable acidity of the sample was determined using a titration procedure (*28*). Sodium hydroxide was added to the acidic juice until a pH of 8.2 was achieved. This pH is used in industry because that is the endpoint of phenolphthalein indicator, which was used before electronic titration devices were available. Using this endpoint allows for comparison of new values to those determined using the old method. Knowing the amount of juice, and the amount and concentration of sodium hydroxide used allowed for the calculation of the acidity of the orange juice.

Storage Conditions

The juice was divided with half going into plastic bottles and the other half into glass bottles. For each container type, there were three replications performed. For each replication there was several bottles filled such that a new bottle could be open, analyzed, and frozen on each of the test dates. A second group of identical bottles were also placed in the light chamber. However, this group was wrapped in aluminum foil in order to insure that the juice was not exposed to light during storage. This combination of container type and aluminum foil allowed for comparison of compounds in juices exposed to light in plastic, light in glass, no light in plastic, and no light in glass. The light chamber was kept in cold storage at 4°C. A temperature probe on the inside of the storage chamber recorded any temperature deviations.

Storage Studies

There were two storage studies conducted during this experiment. The first study consisted of storing the juices in the fluorescent light chamber for twelve weeks. In this experiment, juices were tested on the first day, as well as after 4 weeks, 8 weeks, and 12 weeks. This experiment was designed to monitor changes in juice over an extended amount of time. This should minimize thermally induced chemical reactions and maximize the possibility of photochemical reactions. The study also had the added advantage in that it would be very similar to what juices might experience under the best of conditions in the market place. The second study was an accelerated storage study. At the beginning of the experiment oxygen was bubbled through each of the juices in order to increase the dissolved oxygen content and thus any oxidation reactions that may occur over time. The juices were then stored for two weeks and sampled after Week 1 and

Week 2. In this experiment all juices were stored in glass bottles and the bottles wrapped in foil were considered "control".

Sample Preparation

Orange juice samples were prepared for analysis using liquid-liquid extraction. Twenty-five milliliters of juice were mixed thoroughly with 10 mL of n-pentane solvent in a 50 ml glass syringe. The mixture was then placed into a centrifuge for 10 minutes to allow the aqueous and organic layers to separate. The organic top layer was drawn off and retained, while the aqueous bottom layer was placed back into the syringe and extracted with 10 mL of ethyl ether. The same process was repeated and this organic layer was combined with the first. A small amount of sodium sulfate was added to the extract to remove any remaining aqueous material. The extract was drawn from the salt and placed into a clean vial. Two internal standards were added to the solution. Fifty microliters of a 2000 ppm solution of ethyl valerate was added to mimic conditions experienced by low molecular weight compounds, and 50 μL of a 2000 ppm solution of 4-heptadecanone was added to mimic those compounds with higher molecular weights. A nitrogen blowdown method was employed in order to slowly concentrate the sample to 0.1 mL.

Sensory and Analytical Tests

Sensory Analysis

Sensory analysis was performed by five trained panelists. The panelists were all members of a group trained to rate orange juice for a study conducted by Elston, Rouseff, and (publication pending). Juices were rated on a fifteen point overall flavor quality scale that reflected attributes such as aroma strength, orange juice character, peel oil, fatty/metallic/green, fruity/floral, cooked/heated/processed, sweetness, sourness,

bitterness. In this scale juices ranked between 10 and 15 were considered superior quality, juices ranked between 5 to 10 were good quality juices and juices ranked 5 and below were considered to be of poor quality. These quality scores were not a reflection of hedonic rating or preference. The juice was presented at around 17°C in an open room under white lighting.

Juice Color Measurements

The juice was measured on the first day of the experiment as well as after twelve weeks on a Gretag MacBeth Color-EYE 3000 spectrophotometer (Gretag MacBeth, Regensdorf, Switzerland). This device emits a flash of light from a pulsed xenon arc lamp and measures the light reflection from the juice. Samples were evaluated using the International Commission of Illumination's *L, *a, and *b standard color space specification as outlined by Lee and Chen in 1998 (*30*). The value "L" measures relative lightness or darkness of the juices where L= 0 would correspond to black (total absence of reflected light) and L=100 would correspond to white (total reflection of incident light). The value "a" is a measure of green to red, with negative numbers indicating more green, a value of 0 being neutral, and positive numbers indicating more red. The value "b" is a measure of blue to yellow, with negative values indicating more blue, a value of 0 being neutral, and positive numbers indicating more yellow.

Ascorbic Acid Measurement

Ascorbic acid measurements were performed using capillary electrophoresis by Yehong Xu at the Florida Department of Citrus using published methods (*31*). The sample was prepared by placing 4 ml of juice into a capillary electrophoresis tube and adding 12 ml of 0.1% ethylenediamine tetraacetic acid (EDTA) solution and 100 µl of ferulic acid as an internal standard. The sample was filtered. Injection volume was set at

10 µl. The run time was 30 minutes and the running buffer was 35mM sodium borate and 5 % acetonitrile at a pH of 9.3. The column was uncoated fused silica capillary with dimensions 50 µm x 70 cm with a temperature of 23-25°C. Voltage applied was 21 kv, and scanning was performed between 200 and 360 nm using a Photodiode Array (PDA) detector.

Gas Chromatograph-Flame Ionization Detector / Olfactometer

The gas chromatograph used in this study was a HP 5890A (Agilent, Palo Alto, CA) with a Datu (Geneva, NY) high volume olfactometer and described in detail by Bazemore and coworkers (*32*). There were two columns used during analysis. The first was a thirty-meter ZB-5 column (Zebron, Torrance, CA) with a 0.32 mm inner diameter, and 0.50 µm film thickness. The GC was run in splitless mode with an injector temperature of 220°C, a detector temperature of 250°C, an initial oven temperature of 40°C with a 7°C/min ramp up to a final oven temperature of 265°C for a 5 minute hold time. The other column was a 30 meter DB-Wax column (J&W Scientific, Folsom, CA). The column's inner diameter, film thickness, injection temperature, detector temperature, initial oven temperature, and temperature ramp were the same as previously stated. However, the DB-Wax column utilized a final oven temperature of 240°C. All injection volumes were 0.2µl. Chromperfect Spirit 5 version 5.0.0 software was used to record data and integrate the resulting chromatograms.

Gas Chromatography – Mass Spectrometry

The GC-MS used was a Finnigan GCQ Plus system (Finnigan, San Jose, CA) with a DB-5 column (J&W Scientific, Folsom, CA). The column was 60 m long, had an internal diameter of 0.25 mm., and a film thickness 0.25 □m. Helium (99.999% purity) was used as the carrier gas. Samples were injected using the AI/AS 3000 autosampler in

the splitless mode with the injector temperature at 200°C. Oven temperature was 40°C, and was increased at a rate of 7°C/min to 275°C and held for 5 min. Column head pressure was maintained at 14.5 psi. Transfer line and ion source temperatures were 275°C and 200°C, respectively. The mass spectrometer detector scanned at *m/z* 40-300. The ionization energy was set at 70 eV. Xcalibur version 1.3 software was used to record and integrate mass spectrometer chromatograms and spectra.

Microbiological Analyses

On the first day and after one month, microbial tests were performed to insure that off-flavors were not the results of microbiological activity. Samples from each of the four material and light-exposure combinations were plated and incubated for 48 hours at 30°C. Potato Dextrose Agar (Difco Laboratories, Detroit, MI) with 10% tartaric acid was used to test for yeasts and molds, while Orange Serum Agar (Difco) was used to test for bacteria and yeast (*33*).

Statistical Analysis

Statistical analysis was performed using Minitab Statistical Software version 13.32. Tests for statistical significance were calculated using an independent two-sample t-test at a 99% confidence interval. A hypothesis of equality was assigned and Minitab was used to calculate t and p values. If the p value was less than the alpha value of 0.01, then the hypothesis was rejected and the two samples were not equal.

CHAPTER 5
RESULTS AND DISCUSSION

At the end of the twelve week and the accelerated storage studies, several data trends were observed. The results listed below are a combination of trends seen in both experiments. The averages and standard deviations are based on triplicate analysis from a singe juice container per condition. Although it may be more precise to have multiple juice containers for each condition, the size of the storage chamber and scope of this experiment limited the amount of containers used. Container to container variations are usually due to problems along container seems and closures as seen in metal cans and paperboard cartons. Since all containers used in this experiment were blow molded plastic and glass with no seams, the container to container variation should be low, and the results from each sample should be an accurate representation of each storage condition.

Orange Juice Properties

Initial juice properties can be seen in Table 1.

Table 1: Initial Orange Juice Properties

Property	Value
Brix	12.8
Percent Total Acid	0.724
Brix/acid ratio	17.7
Percent Oil	0.0246

Sensory Flavor Changes

After twelve weeks, the juices were removed from the storage chamber and frozen until sensory evaluations. Juice was also frozen on the first day of the experiment to be

used as a control. Shown in Table 2 are the cumulative sensory comments for both orthonasal aroma and flavor impressions for each juice from five trained panelists. Panelists also evaluated each juice for overall flavor quality based on a 15 point scale.

Table 2: Sensory Analysis of Juice after Twelve Weeks

Conditions	Aroma	Flavor	Quality Rating
Initial Juice	Good OJ character, cooked, apricot, citrusy, slight oxidized note	Processed, cooked, good sweet/sour balance, good overall flavor, very peely, somewhat musty, oxidized flavor,	8.8 ± 1.3
Light-Plastic	No OJ character, peppery, cooked vegetable, solventy, painty, sweet caramel notes, fermented	Almost no OJ character, apricot, candy sweetness, burning backend, processed, cotton candy flavor, lack of fruity notes	3.6 ± 1.5
No light-Plastic	Sulfury, over mature, fermented, weak OJ character, slightly peppery, fatty aroma, metallic	More OJ character, good sweet/sour balance, furaneol sweet, orange peel, bitter, fatty/musty off-flavor, no fruity notes	3.8 ± 0.4
Light-Glass	Terpeney, peely, week OJ character, slightly peppery, sweet/processed, painty off-flavor	Almost no OJ character, artificial candy sweet, sulfury vegetable, acidic, pineapple, bitter, cardboard-like, pronounced off-flavor	4.0 ± 1.6
No light-Glass	Musty, peely, terpeney, slightly fermented, peppery, bleach, some OJ character, no off-flavor	Floral, candy sweet, some OJ character, little sour, slightly cleaner like	6.4 ± 1.1

There was a considerable loss of flavor quality after 12 weeks storage at 4°C as noted from the difference in average flavor quality score of the control juice (8.8) compared to even the highest rated stored juice (6.4, no light – glass). Since all juices

(except control) were stored for the same time (12 weeks) and at the same temperature (4°C), any flavor changes must be due to container properties or exposure to light. Since juices exposed to light in either PET or glass had similar ratings, it appears that container material made no difference in overall flavor score. However, it appears that container oxygen permeability can influence juices protected from the light. Juices in plastic and not exposed to light had about the same flavor quality scores as light exposed juices. However, orange juice stored in glass and not exposed light had higher flavor quality scores than the similar juice stored in PET, suggesting that oxygen permeability also influences flavor even at 4°C. In general, juices exposed to light exhibited diminished orange juice character and rated lower than those that were not exposed. Therefore exposure to light appears to be a major factor in juice quality.

Color Changes

The color of the juices exposed to light during the twelve week storage study appeared darker than those unexposed. Juice color was evaluated instrumentally using a spectrophotometer to determine their respective L, a, and b values. The relative lightness, redness and yellowness of the stored juices and control are shown in Figures 5-7. Error bars represent one standard deviation above and below the average value.

Since L measures relative lightness or darkness of the juices, it can be readily seen from Figure 5 that juices exposed to light have darkened. The light exposed juice had a significantly lower L value ($p<0.01$) than the original juice (see Appendix A for statistical calculations). Those that were not exposed to light showed less change from the original juice. Non-enzymatic browning is typically responsible for darkening citrus juices during elevated temperature storage in the absence of light. A colored compound has been identified that is thought to be formed by the condensation of the norfuraneol with the

aldehyde group of furfural at elevated temperatures (*20*). The darkening of the juices in this study which occurred at 4°C indicates that browning can be induced by light as well as elevated storage temperature.

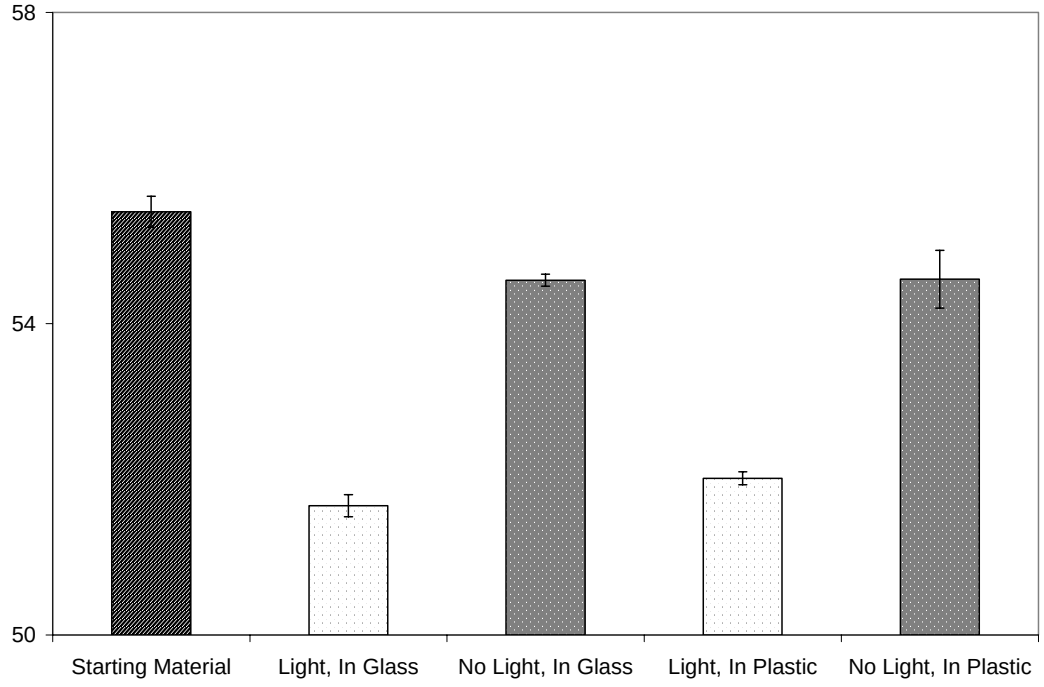

Figure 5: "L" Values for Juices after Twelve Week Storage Study

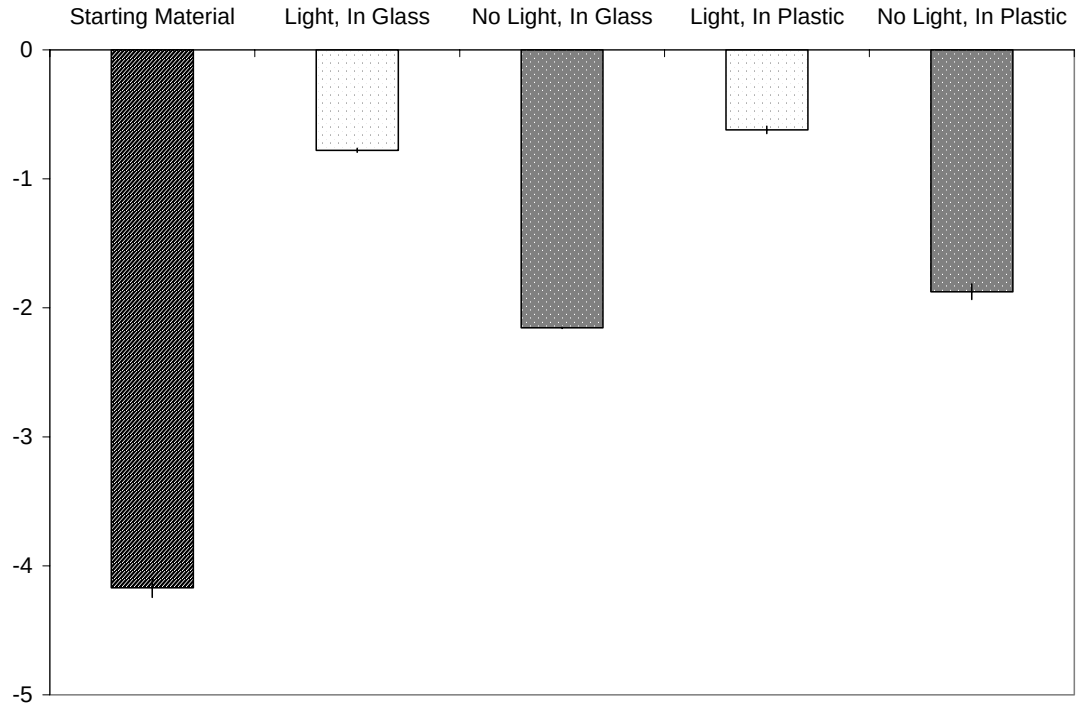

Figure 6: "a" Values for Juices after Twelve Week Storage Study

Since "a" is a measure of green to red, with negative numbers indicating more green, a value of 0 being neutral, and positive numbers indicating more red, it was found that juices that were exposed to light had significantly less ($p<0.05$) of a green color than the original juice (see Appendix B).

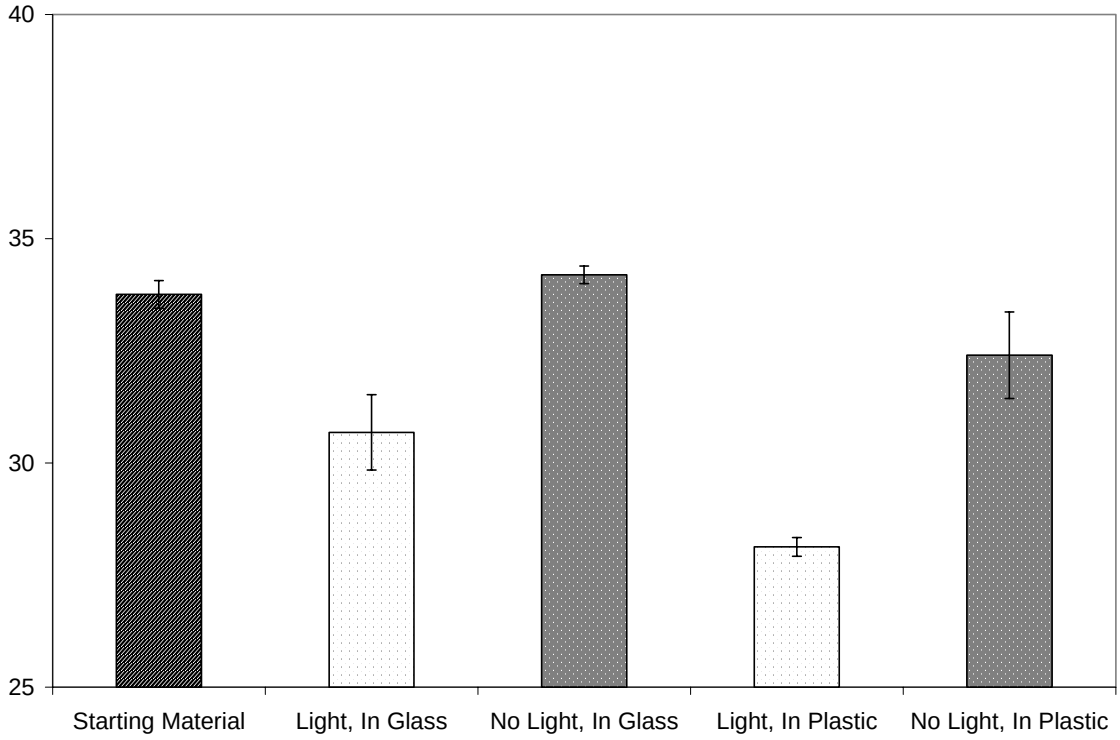

Figure 7: "b" Values for Juices after Twelve Week Storage Study

Since "b" values are a measure of blue to yellow, with negative values indicating more blue, a value of 0 being neutral, and positive numbers indicating more yellow, it was found that those juices that were exposed to light had significantly less of a yellow color than those that were protected (see Appendix C). These three measurements confirm that those juices exposed to light did become darker in color and more brown than light yellow or orange.

Ascorbic Acid Changes

During the twelve month study, ascorbic acids measurements were taken periodically in order to monitor any losses. The results of these measurements can be seen below in Table 3.

Table 3: Ascorbic Acid Loss During Storage

	Concentration Ascorbic Acid (µg/ml)
Initial	320
Light, In Plastic	36
No Light, In Plastic	87
Light, In Glass	45
No Light, In Glass	160

It is apparent that those samples that were exposed to light lost more vitamin C than those samples that were not exposed. Juice in plastic bottles lost 21.5% more vitamin C when exposed to light as compared to juice that was not exposed to light, and juice in glass lost 68.0% more. Also, samples that were stored in plastic bottles lost more ascorbic acid than those stored in glass.

The results found in this study correspond with those reported in earlier studies concerning ascorbic acid loss and sensory ratings. The increased loss of ascorbic acid in juices exposed to light and corresponding decrease in consumer acceptance (through hedonic ratings) is consistent with the findings of both Ahmed and colleagues in 1976 (*5*)and Sattar and colleagues in 1989 (*4*). Also the increased loss of ascorbic acid in juices stored in plastic and thus allowing more oxygen permeation is consistent with findings by Kennedy and colleagues (*22*).

Aroma Active Compounds (GC-O Studies)

Not all volatiles in orange juice are aroma active. Aroma activity for each volatile must be established using human assessors. Instruments detect only those volatiles present in highest concentration. Humans respond only to volatiles with particular functional groups and molecular shape. Table 4 contains a list of the aroma active

compounds that were identified in the initial juice by comparing GC-olfactometry descriptors and retention index from both DB-5 and Wax columns with published standards (*34*).

After storage in the light chamber for twelve weeks, some compounds decreased, some increased, and some new compounds formed. A comparison of the GC-O relative responses (peak area of compound divided by the peak area of the internal standard ethyl valerate) of samples exposed to light and not exposed to light during storage in plastic containers can be seen in Table 5. Likewise, a comparison of GC-O relative responses for samples stored in glass containers after twelve weeks can be seen in Table 6.

It is also important to note that some aroma active compounds occur at such low concentrations that they are below the detection limits of the flame ionization detector. Alternatively, some compounds that occur in large concentrations in orange juice have very low aroma activity and thus are not detected through the olfactometer. An example of this can be seen in Figure 8.

Table 4: Aroma Active Compounds Identified in Initial Valencia Orange Juice

LRI on DB-5	Identification	Sensory Description	LRI on Wax
805	ethyl butanoate	grassy, fruity	1041
825	sulfur smelling compound	skunk	
851	z-3-hexen-1-ol	fruity	1383
859	sulfur smelling compound	rotten fruit	
867	1-hexanol	sour fruit	1352
871	2-methyl-3-furanthiol	cooked grain	
900	ethyl valerate (I.S.)	fruity	1141
907	methional	baked potato	1468
924	2-acetyl-1-pyrroline	graham cracker	
935	α-pinene	pine tree	1024
942	4-mercapto-4-methylpentan-2-one	chicken, moldy	1376
982	1-octen-3-one	mushroom	1313
986	b-pinene	musty, soil	1106
992	β-myrcene	green	1171
1003	octanal	fruity, lemon	1309
1006	ethyl hexanoate	lemon	
1035	*p*-cymene	minty, fresh	1225
1041	limonene	licorice, minty	1211
1046	4-mercapto-4-methylpentan-2-ol	fruity	1545
1065	(E)-2-octenal	fruity, green	
1071	Furaneol	caramel	2038
1085	unknown	coffee, burnt, processed	
1099	linalool	lemon	1553
1100	nonanal	citrus, floral	1394
1105	fenchol	lemony, citrus	
1116	unknown	sweet, popcorn	
1134	2,6-nonadienal	roses, green, cucumber	1601
1207	decanal	lemon, sour, woody	1518
1233	neral	lemon, sweet, floral	
1252	carvone	minty	1754
1320	eugenol	balsamic, cloves	2176
1412	vanillin	vanilla, sweet	2565
1455	wine lactone	dill, crayons	2254
1494	b-ionone	raspberry	1955
1564	dodecanoic acid	musty	2500
1706	b-sinensal	marine, old house	2243
1755	a-sinenesal	marine, dusty	2420
1822	nookatone	green, spicy, fruity	co-elutes with vanillin

Table 5: Aroma Active Compounds in Juice Stored in Plastic for Twelve Weeks

Light Exposed	No Light-exposure	DB-5 LRI	Descriptor	Identity
0.79	0.54	805	grassy	ethyl butanoate
0.00	0.46	825	skunk	butyric acid
0.98	0.39	851	fruity	z-3-hexen-1-ol
0.00	0.27	856	rotten	unknown sulfur
0.00	0.69	867	sour fruit	1-hexanol
1.07	0.84	871	cooked grain	2-methyl-3-furanthiol
1.00	1.00	900	fruity	ethyl valerate
0.79	0.64	907	baked potato	methional
0.00	0.32	924	graham cracker	
1.33	0.68	935	pine tree	a-pinene
0.45	0.57	982	mushroom	1-octen-3-one
0.68	0.46	986	green,soil	b-myrcene
0.81	0.46	1006	lemon	ethyl hexanoate
1.04	0.44	1035	licorice,minty	1,8-cineole
0.63	0.89	1041	minty	limonene
1.25	0.40	1046	moldy	4-mercapto-4-methylpentan-2-ol
1.16	0.74	1071	caramel	furaneol
0.50	0.51	1085	fruity,sweet	tetramethyl-pyrazine
0.00	0.41	1099	lemon,burnt	linalool
1.52	0.56	1100	sweet,citrus	nonanal
0.77	0.00	1105	green	
0.00	0.48	1116	citrus,floral	unknown
0.92	0.00	1116	moldy	3-mercapto-hexen-1-ol*
0.56	0.58	1121	sweet,popcorn	
0.62	0.43	1134	sweet	ethyl 3-hydroxyhexanoate
0.93	0.00	1207	lemon,sour	unknown
0.58	0.39	1233	lemon	neral
0.84	0.50	1252	minty	carvone
0.90	0.00	1285	cloves,burnt	
1.31	0.83	1320	balsamic	4-vinyl guaiacol
0.87	0.38	1350	metallic	
1.25	1.29	1383	very metallic	
1.57	1.04	1412	vanilla	vanillin
0.73	0.58	1455	dill	wine lactone
0.82	0.77	1494	berries	b-ionone
1.31	0.67	1564	marine	b-sinensal
0.00	0.54	1584	burnt,spicy	
0.96	0.43	1706	marine,old house	a-sinenesal
0.00	0.47	1755	musty,dusty	
0.00	0.19	1822	green	nookatone

Table 6: Aroma Active Compounds in Juice Stored in Glass for Twelve Weeks

Light Exposed	No Light-exposure	DB-5 LRI	Descriptor	Identity
0.52	0.73	805	grassy	ethyl butanoate
0.62	0.54	825	skunk	butyric acid
0.41	0.39	851	fruity	z-3-hexen-1-ol
0.68	0.39	856	wet dog	unknown sulfur
0.36	0.57	867	fruity	1-hexanol
0.57	0.60	871	cooked grain	2-methyl-3-furanthiol
1.00	1.00	900	fruity	ethyl valerate
0.81	0.64	907	baked potato	methional
0.00	0.00	924	graham cracker	
0.52	0.55	935	pine	a-pinene
0.39	0.96	942	chicken	4-mercapto-4-methylpentan-2-one
0.42	0.41	982	mushroom	1-octen-3-one
0.75	0.51	986	green,dirt	b-myrcene
0.35	0.00	1003	fruity,lemon	octanal
0.67	0.00	1006	lemon	ethyl hexanoate
0.55	0.38	1035	licorice,minty	1,8-cineole
0.40	0.49	1041	minty	limonene
0.83	0.00	1046	moldy,soured	4-mercapto-4-methylpentan-2-ol
0.25	0.00	1065	fruity,green	(E)-2-octenal
0.87	0.46	1071	caramel	furaneol
0.87	0.62	1085	coffee	tetramethyl-pyrazine
0.50	0.51	1099	burnt,lemon	linalool
0.94	0.00	1100	sweet	nonanal
0.00	0.59	1116	lemon,citrus	unknown
0.47	0.00	1116	sweet,moldy	3-mercapto-hexen-1-ol*
0.91	0.57	1121	onion	
0.59	0.34	1134	sweet	ethyl 3-hydroxyhexanoate
0.54	0.28	1207	floral,sour	unknown
0.36	0.35	1233	lemon,woody	neral
0.49	0.37	1252	minty	carvone
0.91	0.64	1285	burnt	
0.71	0.63	1320	cloves	4-vinyl guaiacol
0.93	0.83	1383	metallic	
0.74	0.48	1412	vanilla	vanillin
0.76	0.79	1455	dill,old house	wine lactone
0.40	0.80	1494	berries	b-ionone
0.00	0.53	1564	marine	b-sinensal
0.60	0.38	1706	marine	a-sinenesal
0.46	0.48	1755	peppery	
0.00	0.29	1822	green	nookatone

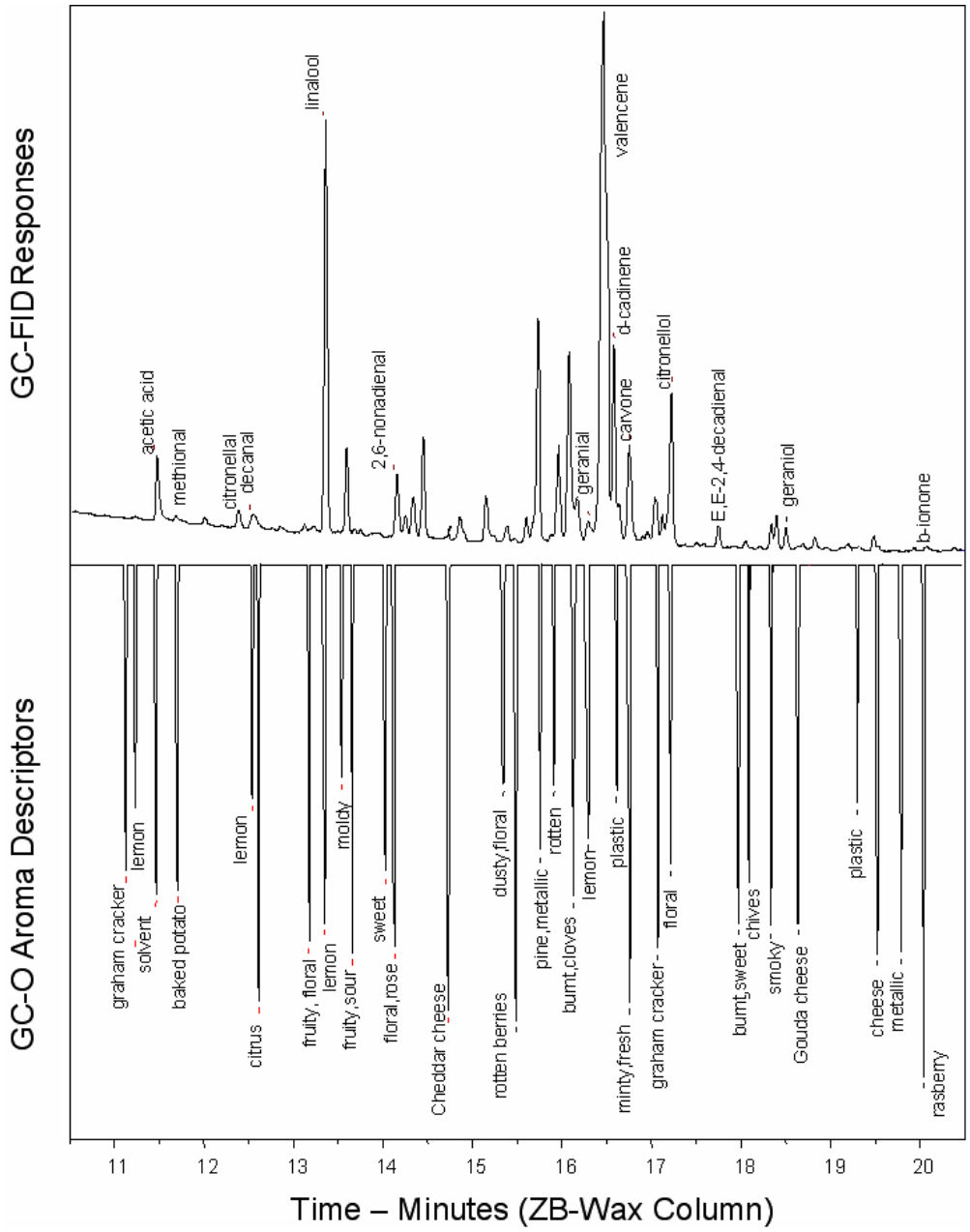

Figure 8: Comparison of Detector Responses

The compound valencene has a large peak on the FID, but is not present on the GC-O. Whereas the compound b-ionone has a large peak on the GC-O but is not present on the GC-FID. For this reason it is important to look for changes in the juice using both detection methods. However, the GC-O data will indicate which FID peaks are associated with aroma activity and which are not.

Qualitative Differences

In Figures 9 and 10, the average relative intensities of the aroma active components in light exposed and control juices in PET and glass are compared. The average aroma intensities are indicated by the bar height and are compared "head to tail" or "fishbone" by inverting the control juice data.

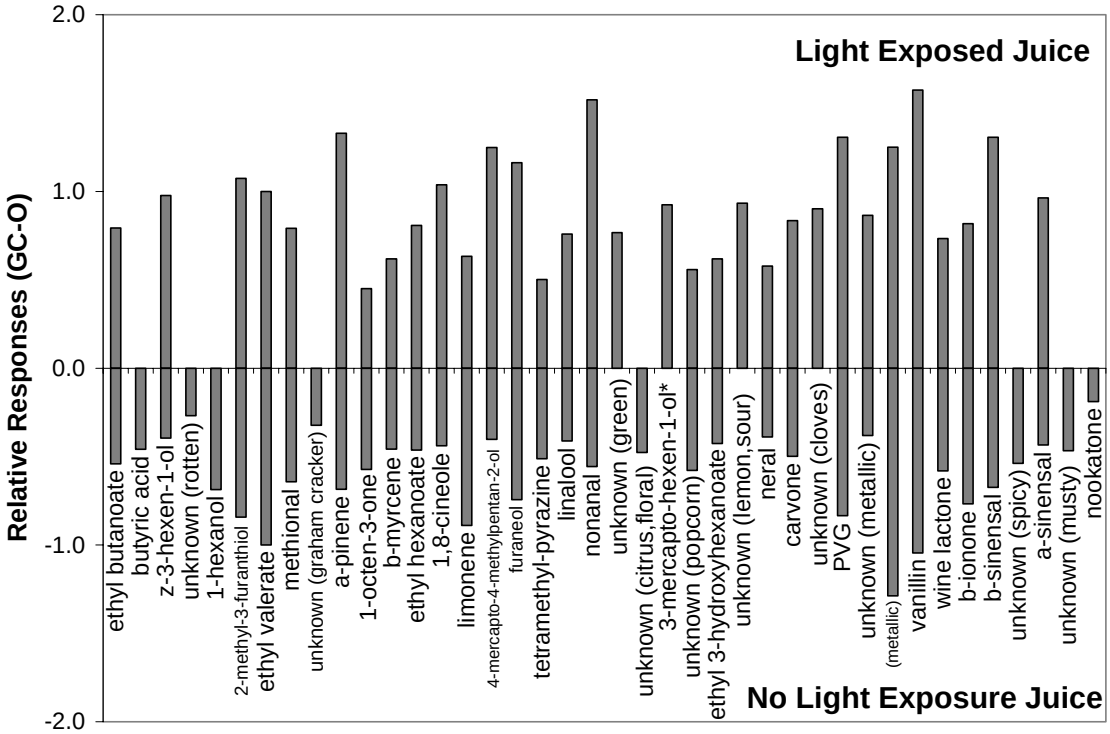

Figure 9: Aroma Active Compounds in Juices Stored in PET for Twelve Weeks. Note ethyl valerate was an aroma active internal standard.

In the case of plastic, 31 aroma active components were observed in juices exposed to light and 36 aroma active components were noted in the control juices. Twenty six aroma components were common to both juices. From a qualitative point of view, the primary effect of light was the loss of several aroma components. The loss of the aroma components unbalanced the orange flavor. A few negative aroma components

were formed from light-exposure, but since the overall sensory quality of these juices were similar, it appears that that their flavor reducing impact was minimal.

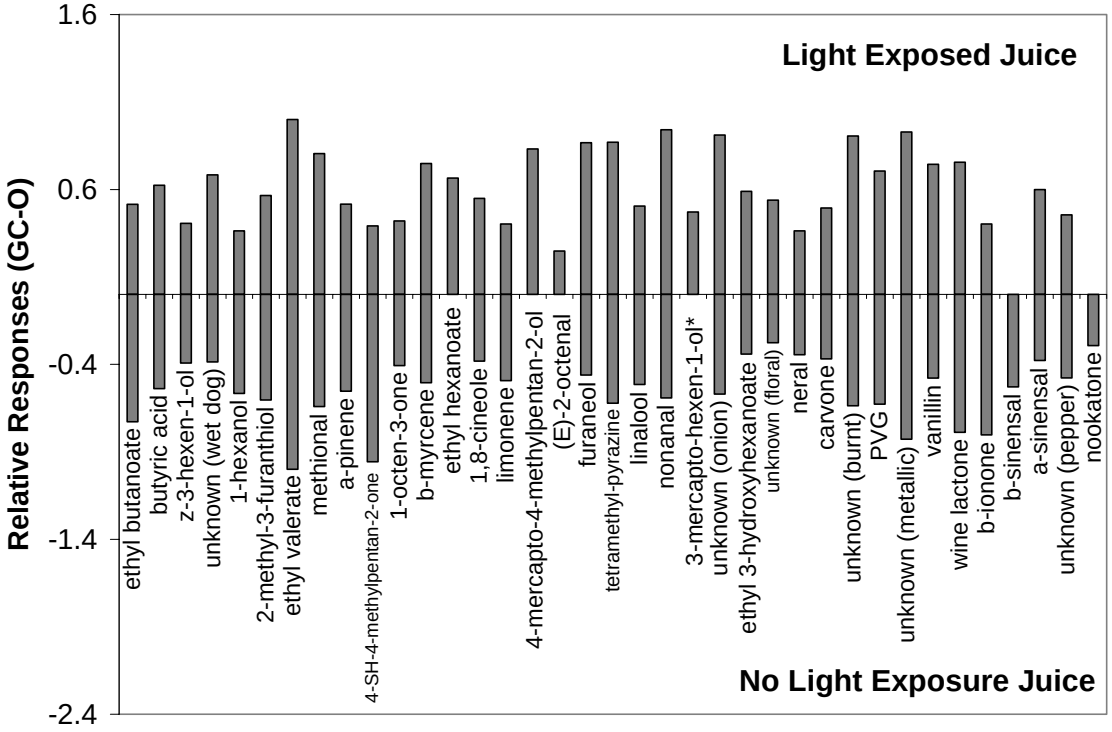

Figure 10: Aroma Active Compounds in Juices Stored in Glass for Twelve Weeks. Note ethyl valerate was an aroma active internal standard.

In Figure 11 the aroma components for light exposed and control juices are also compared. The number of aroma active components in both juice types were almost identical and the vast majority (30 aroma components) were common to both juice types. However, the major aroma difference appears to be due to the production of the extremely potent sulfur component, 4-mercapto-4-methyl-2-pentanol in the light exposed juice.

Quantitative Differences

At the end of storage the aroma active compound myrcene was found to decrease in concentration when exposed to light. The decrease in myrcene after twelve weeks of storage as measured on the GC-FID is shown in Figure 11.

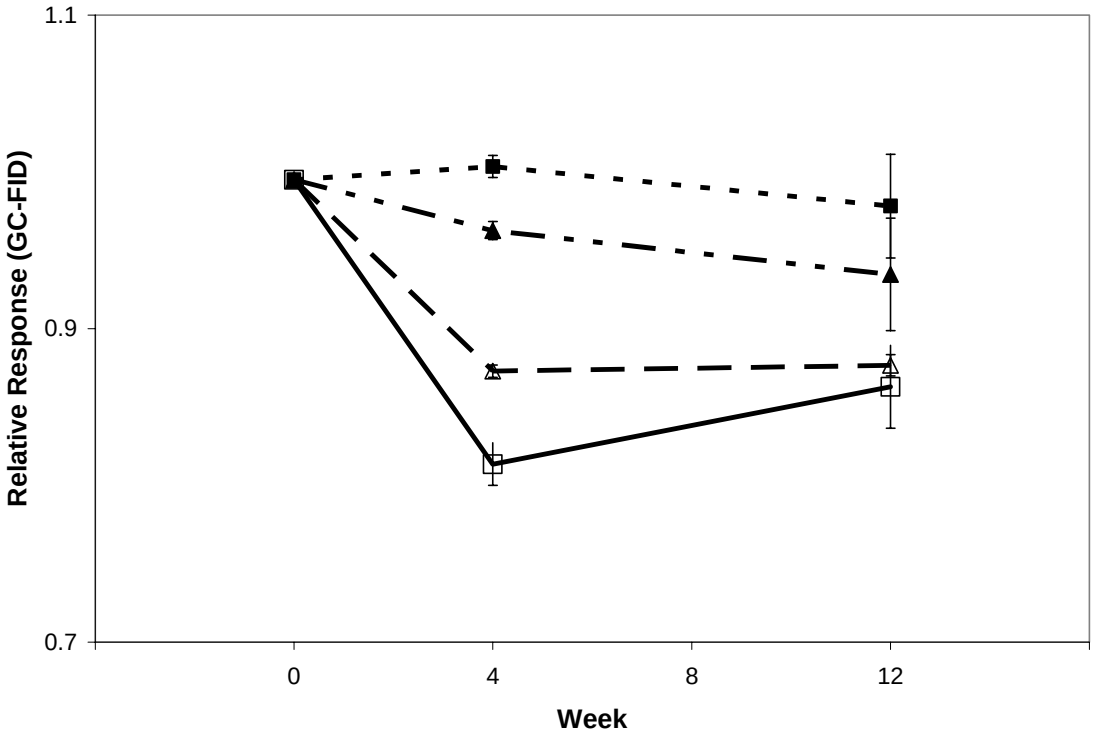

Figure 11: Decrease in Myrcene after Twelve Week Storage Study. □ represents juice in plastic containers exposed to light, ■ represents juice in plastic containers not exposed to light, Δ represents juice in glass containers exposed to light, and ▲ represents juice in glass containers not exposed to light

The amount of myrcene is shown as a measure of "relative response", the area of the GC-FID peak for myrcene divided by the area of the GC-FID peak of the internal standard, ethyl valerate. Myrcene decreased significantly ($p<0.01$) in those samples that were exposed to light, whereas the myrcene levels in protected samples did not change (see Appendix D). After twelve weeks the amount of myrcene decreased by 13.3% in juices in plastic and exposed to light, 1.7% in plastic and not exposed to light, 11.9% in

glass and exposed to light, and 6.1% in glass and not exposed to light. It should be noted that the sample that was stored in plastic and not exposed to light had the least amount of myrcene loss. Therefore the loss of this compound is mainly dictated by exposure to light and not by sorption into the plastic container.

The compound β-myrcene degrades in an acidic environment such as orange juice to form geraniol and its isomer nerol (*35*). This is perhaps why β-myrcene decreased initially and then leveled off. The increased amounts of myrcene lost in those samples that were exposed to light can be explained by light acting as a catalyst that increased the rate of reaction in those juices.

Normally, changes occurring in juice are produced by chemical reactions induced by heat. However, in the case of this experiment, all samples were kept at the same temperature, such that reactions that occurred to a greater extent after light-exposure must have been catalyzed by the energy from light. An example of the difference between heat and light catalyzed reactions can be seen below.

Energy of Activation from Heat:

$$A + B \xrightarrow{\Delta} AB$$

Energy of Activation from Light:

$$A + B \xrightarrow{h\nu} AB$$

The compound that showed the most dramatic increase during all three experiments was carvone. Figure 12 shows the increase in carvone after twelve weeks of storage as measured by the GC-FID.

It is apparent that the amount of carvone increased significantly ($p<0.01$) in samples that were exposed to light, whereas there was little change in those samples that were not exposed (see Appendix E). After twelve weeks of storage, carvone increased by

240.1% in plastic and exposed to light, by 66.6% in plastic not exposed to light, 368.0% in glass and exposed to light, and 27.7% in glass not exposed to light.

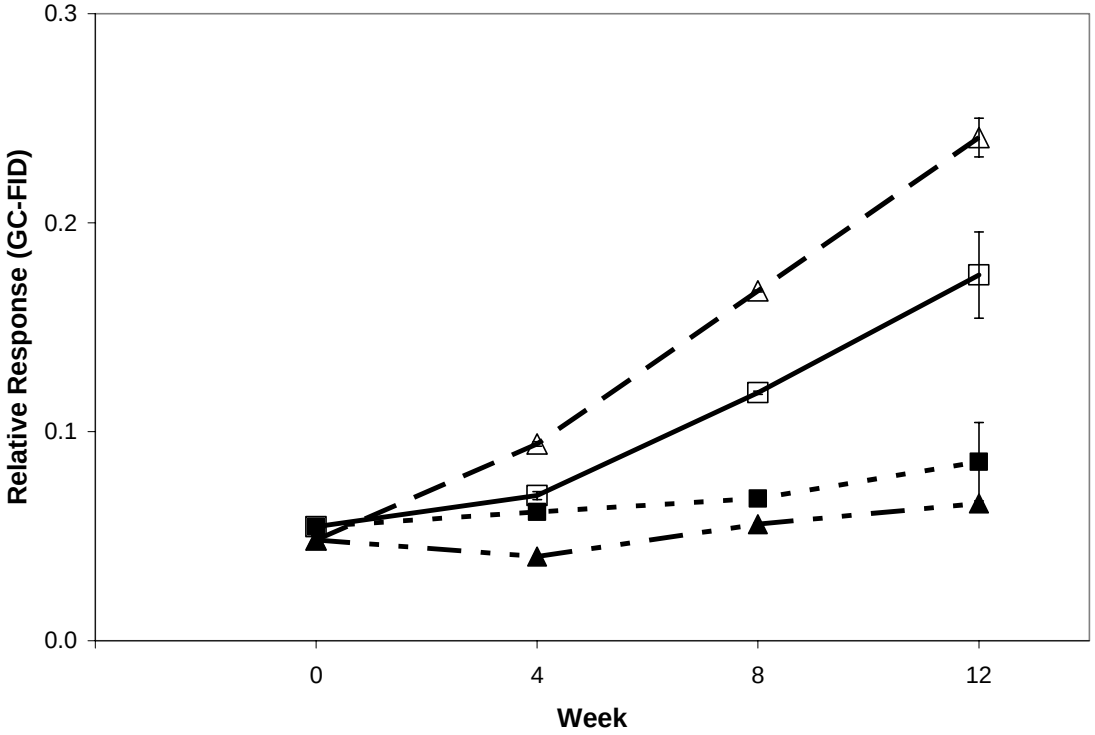

Figure 12: Increase in Carvone after Twelve Week Storage Study. □ represents juice in plastic containers exposed to light, ■ represents juice in plastic containers not exposed to light, Δ represents juice in glass containers exposed to light, and ▲ represents juice in glass containers not exposed to light

Carvone is an oxidation product of limonene (*36*). This oxidation of limonene is perhaps why juices that were stored in plastic with no light, and thus had a greater chance of oxygen exposure had a greater amount of carvone formation than those juices that were stored in glass. Also, increases in carvone, which has a minty aroma, and the subsequent decrease in limonene, which has very little aroma activity, could be a contributing factor to the overall change in aroma and flavor of the orange juice after storage.

Studies by Ziegler and colleagues conducted on orange oil also documented a significant increase in carvone (*9*). The changes in carvone in the orange juice used in this experiment occurred to a lesser extent than those changes in the orange oil. This is most likely due to the matrix the flavor compounds are suspended in. The compounds in the orange oil are in close proximity to one another and therefore have a greater chance of reacting with one another and with light induced oxidation. The compounds in the orange juice are separated by large quantities of water that decrease compound interaction and oxidation reactions. The insoluble material (cloud) in the juice may also work as a reflective material that blocks some of the entering light from contacting the flavor compounds. This difference between juice and oil may also explain why compounds that changed in orange and lemon oils (such as neral and geranial) did not show significant changes in this experiment.

The minty smelling compound 1,8-cineole also increased during storage. This compound co-elutes on a DB-5 column with limonene. Since the concentration of limonene is relatively large in orange juice, the resulting GC-FID peak is also large and therefore the peak for 1,8-cineole could not be quantified. Instead, the peak was quantified using results from the GC-O which was able to separate the two compounds. Figure 13 shows the increase in this oxidation product during storage.

Although initially, 1,8-cineole increased in juice not exposed to light, it leveled off after four weeks of storage. At this time, juice exposed to light showed an increase in this compound, with a greater increase in the juice that was stored in a plastic bottle and therefore had a greater possibility of oxygen content. After twelve weeks of storage, 1,8-cineole increased by 173.5% in plastic and exposed to light, by 15.6% in plastic not

exposed to light, by 44.7% in glass and exposed to light, and by 0.7% in glass not exposed to light.

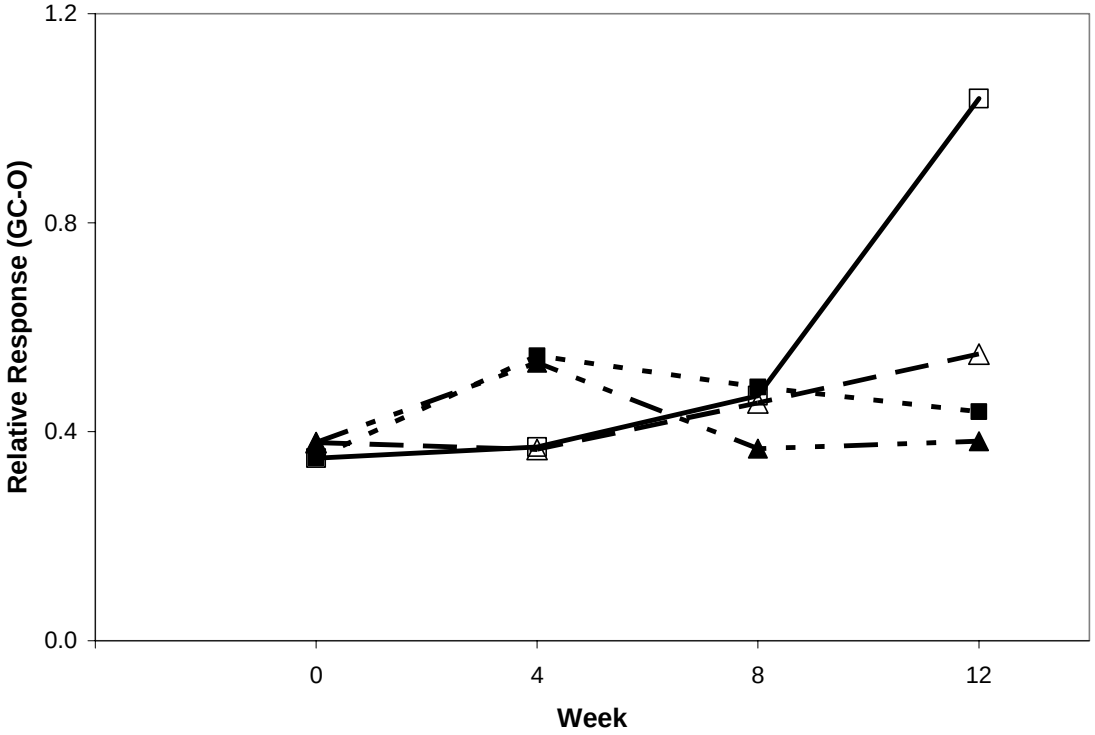

Figure 13: Increase in 1,8-Cineole after Twelve Week Storage Study. □ represents juice in plastic containers exposed to light, ■ represents juice in plastic containers not exposed to light, Δ represents juice in glass containers exposed to light, and ▲ represents juice in glass containers not exposed to light

The volatile 1,8-cineole is a decomposition product of limonene. It has been reported in past literature that 1,8-cineole only formed when stored at 23°C and not at 6°C (*37*). The orange juice in this study was stored at 4°C, and thus the increase of 1,8-cineole may be light induced as well as heat induced.

The volatile compound *p*-cymene was found to increase in samples that had been exposed to light. Since *p*-cymene has a similar retention time as limonene on the DB-5 column (LRI=1026 and LRI=1031 respectively), the two compounds coeluted and could not be quantified by GC-FID or GC-O. However, the polar wax column in the GC-MS

allowed for separation of the two compounds. The peak areas of *p*-cymene were measured based on an extracted ion chromatogram at m/z of 119. An example of how the peak area was integrated and the library standard mass spectra for *p*-cymene can be seen in Figure 14.

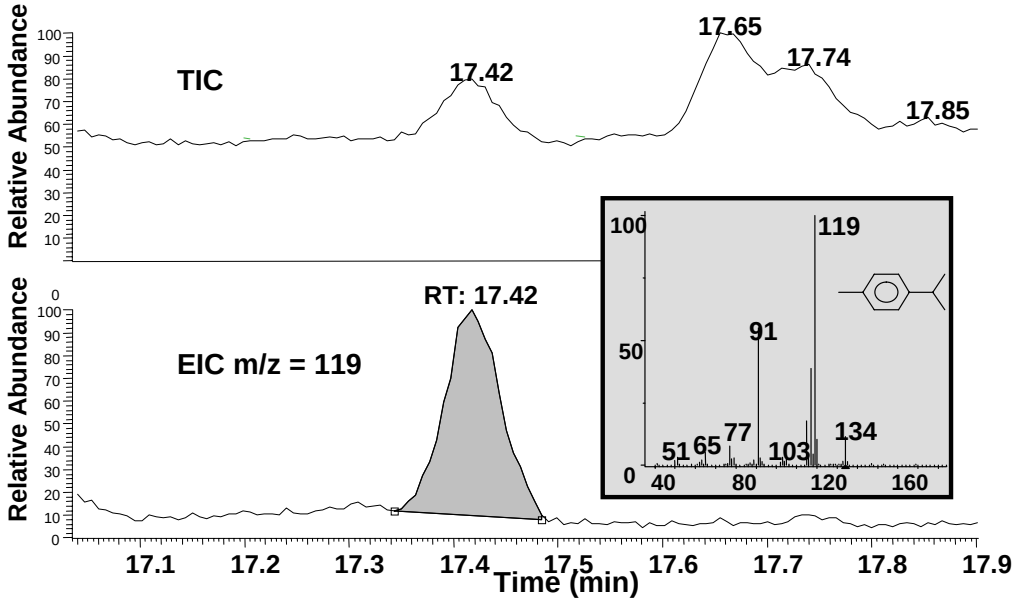

Figure 14: p-cymene Peak Area Measurement on GC-MS

The peak area of the internal standard was measured using the total ion chromatograph for all samples. The internal standard used in this comparison was 4-heptadecanone. The increase in *p*-cymene in those samples that were exposed to light can be seen in Table 7.

Table 7: *p*-cymene Comparison for Juice Stored Twelve Weeks

Sample	Peak Area Cymene	Peak Area IS_2	Ratio
Light, In Plastic	9.66x10^4	3.54x10^7	2.73
No Light, In Plastic	4.94x10^4	4.24x10^7	1.16
Light, In Glass	6.97x10^4	3.10x10^7	2.25
No Light, In Glass	2.04x10^4	1.33x10^7	1.53

The samples that were exposed to light have on average 85% more *p*-cymene than those that were not exposed. However, there was little difference in *p*-cymene content from juices stored in plastic or glass containers either light exposed or not, suggesting that difference in oxygen permeability of storage container materials was not a factor in *p*-cymene formation.

The compound *p*-cymene is formed from the acid catalyzed decomposition of citral (*38*). These results of increased *p*-cymene after exposure to fluorescent light mirror the findings of both Schieberle and Grosch and Iwanami and colleagues for *p*-cymene in lemon oil (*6;8*).

The compounds vanillin and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol) also showed a slight increase during storage. These compounds occurred at levels below the detection limits of the GC-FID and GC-MS and therefore the results reported are based on GC-O data. Figures 15 and 16 below show the increase in vanillin and Furaneol respectively.

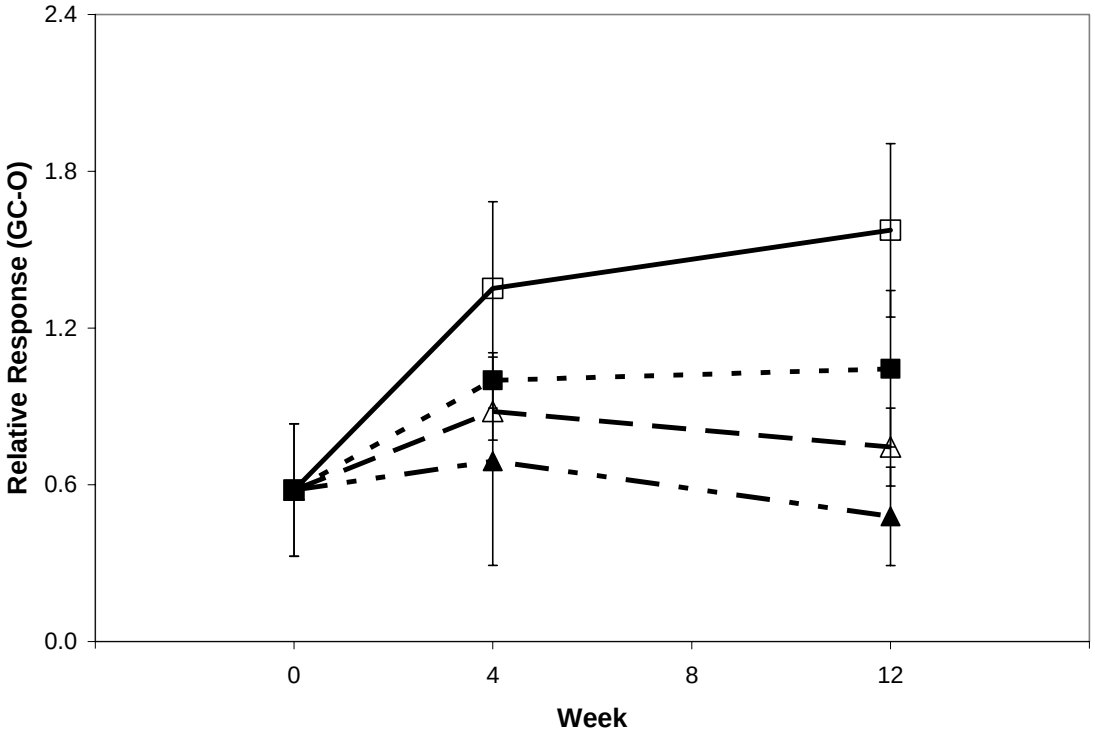

Figure 15: Increase in Vanillin During Twelve Week Storage Study. □ represents juice in plastic containers exposed to light, ■ represents juice in plastic containers not exposed to light, Δ represents juice in glass containers exposed to light, and ▲ represents juice in glass containers not exposed to light

There was an increase in vanillin in juice samples that were exposed to light. It is also evident that samples stored in plastic bottles had a greater increase of vanillin than those that were stored in glass. This may be caused by a greater amount of oxygen being present in the juice stored in plastic bottles. At the end of twelve weeks, vanillin increased by 171.4% in plastic and exposed to light, by 80.0% in plastic not exposed to light, by 28.3% in glass and exposed to light, and decreased by 17.4% in glass not exposed to light.

Vanillin has been reported to form from the thermal degradation of ferulic acid in citrus juices (*39*). In this experiment, all of the juices were kept at the same temperature, so thermal degradation should have occurred equally amongst all samples. However, the

juices that were exposed to light formed more vanillin that those that were protected. Therefore, light may have provided the energy source needed for ferulic acid decomposition in place of heat. It has also been noted that vanillin can further degrade to form phenols and cresols (*40*). This could explain why the amount of increase in vanillin levels off after four weeks and actually decreases in the case of juices stored in glass bottles.

Furaneol increased significantly in samples that were exposed to light during this study and increased to a lesser extent in those samples left unexposed.

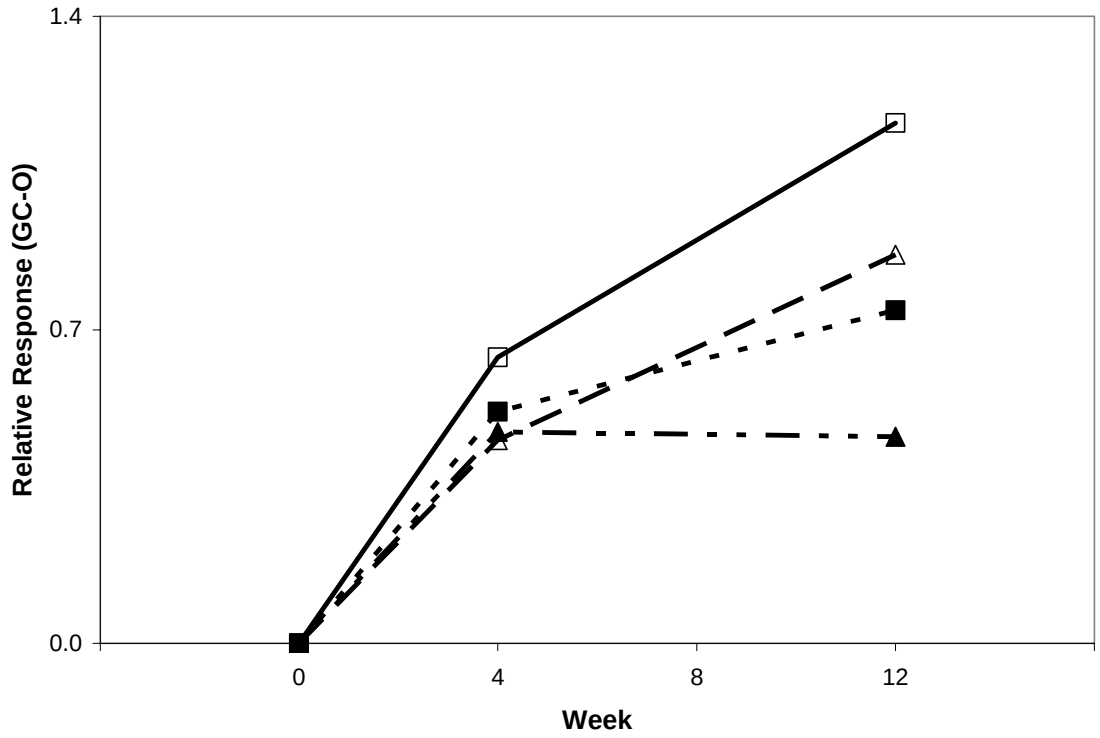

Figure 16: Increase in Furaneol During Twelve Week Storage Study. □ represents juice in plastic containers exposed to light, ■ represents juice in plastic containers not exposed to light, Δ represents juice in glass containers exposed to light, and ▲ represents juice in glass containers not exposed to light.

Also, juices that were stored in plastic containers again had a greater increase in Furaneol than juices stored in glass bottles. Furaneol is an undesirable product that is

formed through non-enzymatic browning during orange juice storage (*41*). It has been documented as occurring in juices that have been subjected to temperature abuse. Again, since all of the juices were under the same temperature conditions throughout storage, the increased amount of Furaneol in juice exposed to light indicates that light may be a possible energy source that catalyzes the reaction.

A compound that has been used as a marker for temperature abuse in orange juice is 4-vinyl guaiacol (*42*). Like vanillin, this compound forms from the thermal degradation of ferulic acid (*43*). Because extraction efficiencies are low, it is difficult to quantify 4-vinyl guaiacol (PVG) using GC-FID and GC-O. However, quantification can be achieved by measuring the peak area of an extracted ion chromatogram at m/z of 150. An example of the integration and the library standard mass spectra for PVG can be seen in Figure 17.

This peak area can be divided by the peak area of an internal standard in order to determine the relative response. The internal standard used for this comparison was 4-heptadecanone because of its similar retention time to PVG on a wax column (RT=33.89 and 34.89min respectively). It can be seen in Table 8 that the amount of PVG was essentially the same after twelve weeks of storage regardless of container type and light-exposure. Therefore, PVG, probably the most important off flavor formed from thermal abuse is not responsible for the flavor differences in the light exposed samples at 4°C.

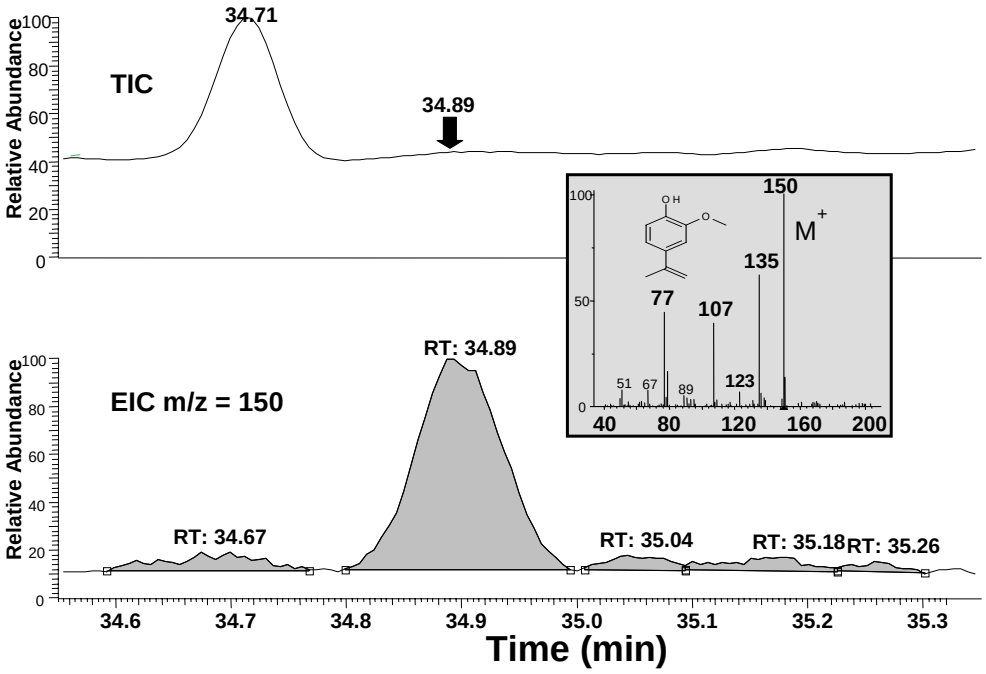

Figure 17: 4-Vinyl Guaiacol Peak Area Measurement on GC-MS

Table 8: Comparison of PVG after Twelve Weeks Storage Using GC-MS

Sample	Peak Area PVG	Peak Area IS_2	Ratio
Light, In Plastic	8.44x10^4	3.54x10^7	2.38
No Light, In Plastic	6.38x10^4	4.24x10^7	2.56
Light, In Glass	6.38x10^4	3.10x10^7	2.06
No Light, In Glass	8.29x10^4	1.33x10^7	2.47

Another compound that has been used as a marker for temperature abuse in orange juice is α-terpineol. This compound forms from acid catalyzed hydration of limonene and linalool in the presence of water at elevated temperatures (*44*). It is present in higher concentrations, and is therefore easier to identify and quantify than PVG (*45*). It can be seen in Table 9 that the amount of α-terpineol was the same after twelve weeks of storage regardless of container material and light-exposure.

Table 9: GC-FID Responses for α-terpineol In Juices Stored for Twelve Weeks

Samples	Relative Response of α-terpineol
Initial Juice	0.126
Light, In Plastic	0.449
No Light, In Plastic	0.497
Light, In Glass	0.464
No Light, In Glass	0.477

Although the samples increased in α-terpineol during the twelve weeks of storage, the formation was equivalent regardless of container material or light-exposure. Again this shows that changes in other compounds such as vanillin and Furaneol were caused by exposure to light and not due to thermally induced reactions.

Two additional compounds that are used as a marker for temperature abuse in orange juice are furfural and 5-hydroxymethyl furfural (*46*). However, due to their low extraction efficiencies, they were not identified or quantified using either GC or GC-MS. Their thresholds were too high to be aroma active and they were not detected using GC-O.

Sulfur Smelling Aroma Compounds

As seen in Figures 9 and 10, a total of five sulfur containing/smelling aroma compounds were observed in the juices after twelve weeks storage. Methional and 2-methyl-3-furnathiol levels were essentially the same in light exposed and control samples. Methional is a Strecker aldehyde formed from the decomposition of the sulfur-containing amino acid, methionine. The unknown sulfur compounds were higher in light exposed sample when stored in glass, but slight lower when stored in plastic, and did not seem to make a major contribution to the overall flavor. However, two thiol (mercapto) compounds (4-mercapto-4-methylpentan-2-ol and 3-mercapto-hexen-1-ol) were formed

only in light exposed juices in both glass and PET. The aroma of 4-mercapto-4-methylpentan-2-ol was described as moldy or soured, and 3-mercapto-hexen-1-ol was described as onions, moldy, or soured. It should be noted that the identity of 3-mercapto-hexen-1-ol must be considered tentative as it has been identified only on the basis of retention time matching. Therefore, the appearance of these profoundly negative aroma compounds in only the light exposed juices would explain in part the diminished flavor quality of these juices. As shown in Figure 18, 3-mercapto-hexen-1-ol increased during the twelve week storage study only in samples exposed to light. Also, the concentration increased at an earlier date and to a greater extent in juice that was stored in plastic bottles as compared to juice that was stored in glass.

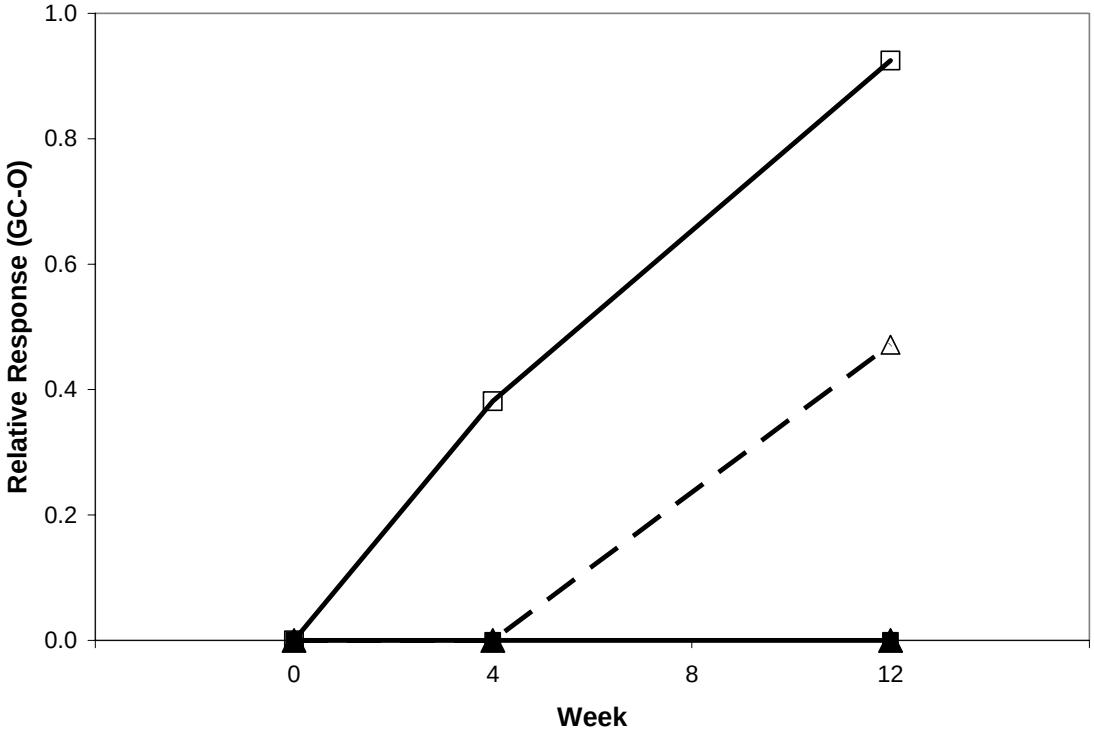

Figure 1: Increase in Sulfur Compound (LRI 1116) During Twelve Week Study. □ represents juice in plastic containers exposed to light, ■ represents juice in plastic containers not exposed to light, Δ represents juice in glass containers exposed to light, and ▲ represents juice in glass containers not exposed to light

Finally, the sulfur smelling compounds that formed or increased were probably caused by the degradation of known sulfur compounds such as the amino acid methionine or the vitamin thiamine which are both present in fresh orange juice (*47*). The appearance of these skunky, chicken-like, or onion-like aromas in only light exposed juices partially explains why the overall orange juice character was degraded.

Accelerated Study

At the beginning of the accelerated storage study, oxygen was bubbled through all juice samples. Control juices were wrapped in aluminum foil. After two weeks, two unidentified sulfur (skunk-like) smelling compounds increased. These compounds occurred at very low concentrations in the juice and thus were only detected on the GC-O. The linear retention indices were 806 and 900 on the DB-5 column. The peak at LRI 900 was observed just before the internal standard, ethyl valerate, eluted. The sulfur smelling compound (LRI 806) that increased in the accelerated study is not the same as the early eluting unknown sulfur (LRI 856) observed in the twelve week study. The sulfur smelling compound at R.I. 806 had a skunk like aroma, whereas the compound at R.I. 856 smelled like wet dog. As seen in Figure 19, the skunky aroma was at higher concentrations in the sample exposed to light.

The sulfur compound at R.I. 900 also had a skunk like aroma and formed during the accelerated storage study. As seen in Figure 20, this compound was not present in the original juice, and only formed in the juice that was exposed to light after two weeks.

The increase or formation of sulfur smelling compounds after light-exposure has been documented in previous literature. It has been found that the amino acid methionine degrades during ultraviolet light-exposure in the presence of riboflavin and oxygen to

form methional. Methional further degrades to form methanethiol, dimethyl sulfide, and dimethyl disulfide (*41*).

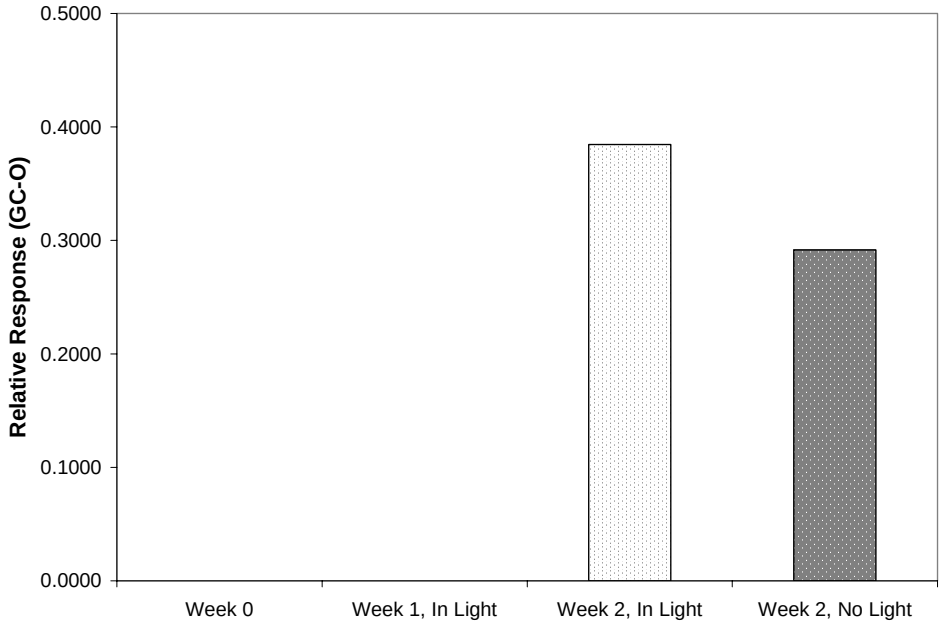

Figure 19: Formation of Sulfur Compound (LRI 806) after Accelerated Storage Study

Microbiological Evaluation

At the beginning of the study and after one month, microbial counts were performed. There was one colony formed on several of the orange serum agar plates before and after the study, however, this colony was identified as a bacillus strain that does not affect juice flavor. Therefore, essentially no microbial growth was observed in the orange juice before or during the experiment. Therefore any changes in juice flavor were not caused by microbial contamination.

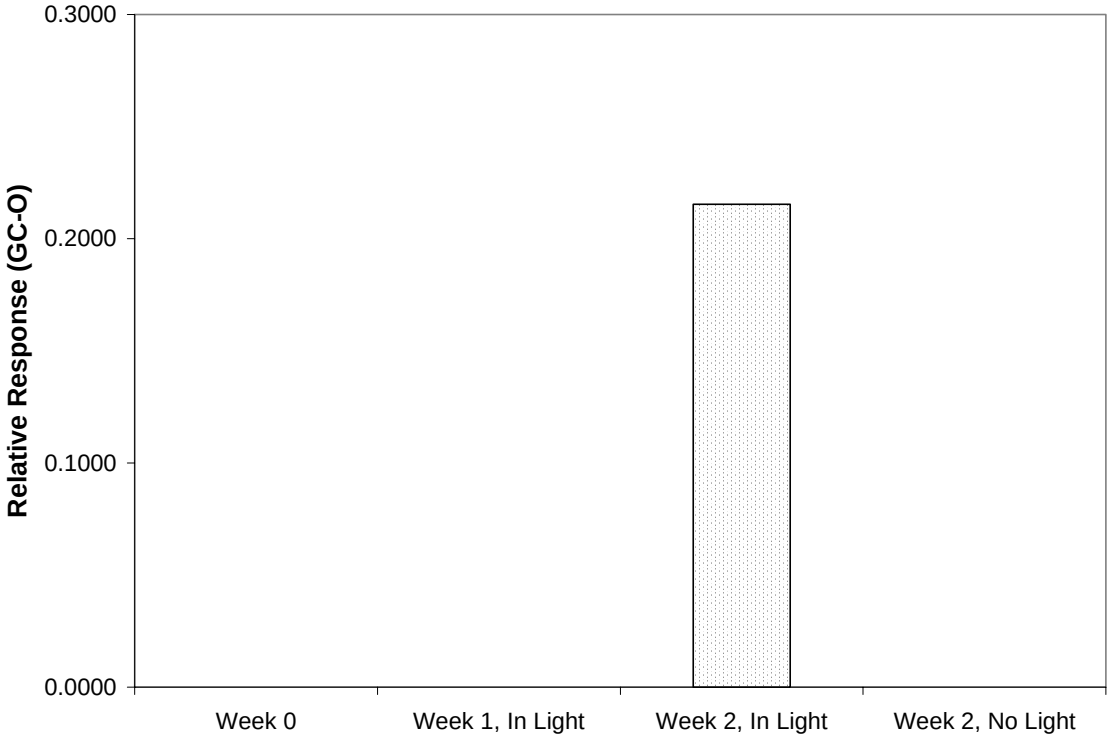

Figure 20: Increase in Sulfur Compound (LRI 900) after Accelerated Storage Study

CHAPTER 6
CONCLUSIONS

The decrease in β-myrcene, the increase in carvone, *p*-cymene, 1,8-cineole, vanillin, and Furaneol, and the formation and increase of various sulfur smelling compounds helps explain the overall changes in aroma and flavor of orange juice that has been exposed to light. It is these changes that upset the usual balance of flavor compounds in fresh orange juice.

The increase in vanillin, Furaneol, and sulfur smelling compounds in samples that were not subjected to increased temperature abuse indicates that light may also play a role in non-enzymatic browning and thermal degradation.

Changes in volatiles were not significant during the first month of storage. Therefore, orange juice exposed to light in a retail setting where there is a high turnover rate would not be affected to a measurable extent and no loss of overall quality should be detected. However, juices that are sold at a lower turnover rate are at a higher risk for developing these off-flavors and overall deterioration of quality.

It was shown in this experiment that in general juices that are stored in glass retained more orange juice character and had less photoxidation reactions occurring. This is probably due to less oxygen being able to permeate the container, and fewer oxidation reactions occurring in the juice. Retailers can therefore guard against some of these reactions by using glass containers or multilayer plastic materials with higher oxygen barrier properties. New packaging technology which utilizes higher oxygen

barrier materials or oxygen scavenging capability and shields the juice from light would protect orange juice quality and ensure consumer satisfaction.

APPENDIX A
STATISTICAL TEST FOR "L" SIGNIFICANCE

```
Two-sample T for Initial vs L-P

         N      Mean     StDev   SE Mean
Initial  8    55.437     0.199     0.070
L-P      2   52.0135    0.0841     0.059

Difference = mu Initial - mu L-P
Estimate for difference:  3.4234
95% CI for difference: (3.1678, 3.6789)
T-Test of difference = 0 (vs not =): T-Value = 37.19  P-Value = 0.000  DF = 4
```

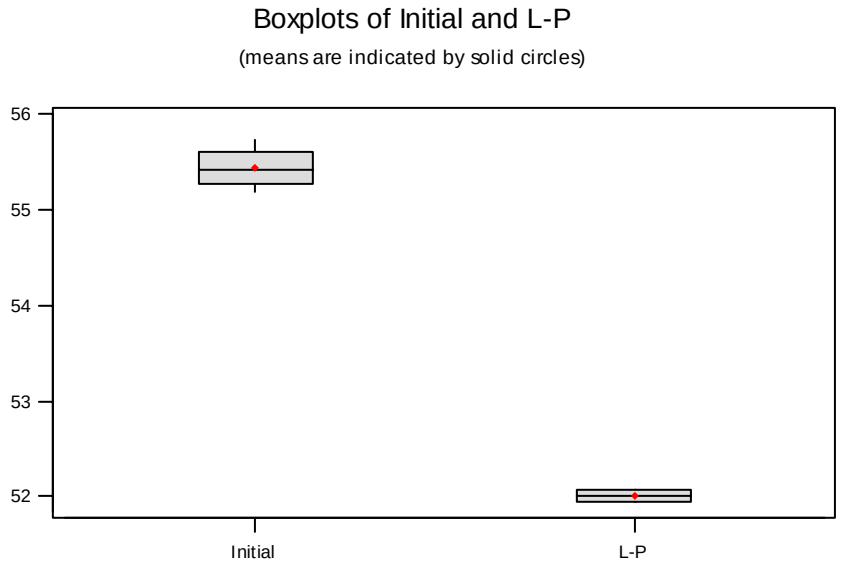

Figure 21: Significant Difference Between L Values

APPENDIX B
STATISTICAL TEST FOR “A” SIGNIFICANCE

```
Two-sample T for Initial vs L-P

         N      Mean     StDev   SE Mean
Initial  8   -4.1694    0.0754     0.027
L-P      2   -0.6200    0.0283     0.020

Difference = mu Initial - mu L-P
Estimate for difference:  -3.5494
95% CI for difference: (-3.6351, -3.4637)
T-Test of difference = 0 (vs not =): T-Value = -106.50  P-Value = 0.000  DF = 5
```

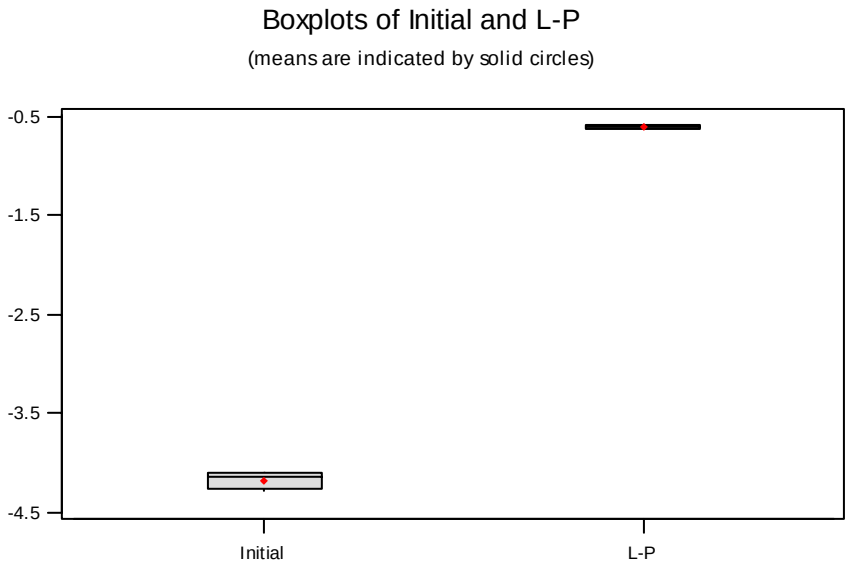

Figure 22: Significant Difference in "a" Values

Since the p value is 0.000, which is less than the alpha value of 0.01, we reject the null hypothesis. The “a” value of the initial juice is not equal to the “a” value of juice exposed to light in plastic.

APPENDIX C
STATISTICAL TEST FOR “B” SIGNIFICANCE

```
Two-sample T for Initial vs L-P

         N      Mean     StDev   SE Mean
Initial  8    33.760     0.308      0.11
L-P      2    28.129     0.210      0.15

Difference = mu Initial - mu L-P
Estimate for difference:  5.631
95% CI for difference: (4.839, 6.424)
T-Test of difference = 0 (vs not =): T-Value = 30.58  P-Value = 0.001  DF = 2
```

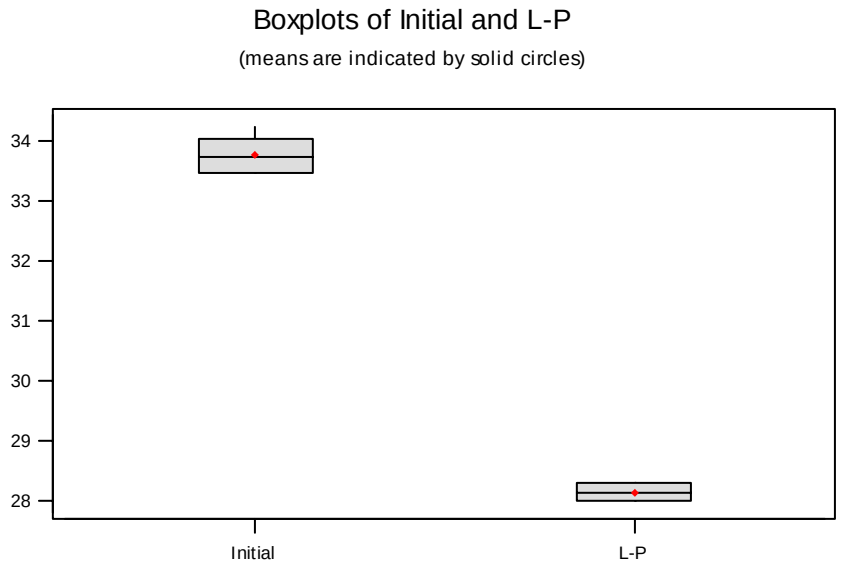

Figure 23: Significant Difference in "b" values

Since the p value is 0.001, which is less than the alpha value of 0.01, we reject the null hypothesis. The “b” value of the initial juice is not equal to the “b” value of juice exposed to light in plastic.

The Appendices in the *Guide for Preparing Theses and Dissertations* provided by the Graduate School's Editorial Office give numerous examples regarding the proper construction of an appendix. In most cases, the appendices will vary from dissertation to dissertation and may vary within a dissertation, depending on the content of the individual appendix. Obey the general guidelines given in the *Guide for Preparing Theses and Dissertations*.

Although the margins have been set throughout this document correctly, please pay close attention to the possibility of picture frames overlapping the margin. The base style to use is Normal.

The remainder of this text is extraneous. We included it in this version of the template so that appendix A can have page numbers on all its pages. The text that follows was copied directly from the Guide for Preparing Theses & Dissertations.

Candidates in the English department who author a collection of poems, short stories, or a novel for a thesis degree should consult the Editorial Office and not other theses as a guide to format. Typing, spacing, margin, heading,, numbering, and formatting requirements in this guide apply to all theses.

If a thesis consists of a collection of poems that are not grouped under headings, the first page of each poem has a 2-inch top margin. Each poem title is centered and in all capital letters. The first page of each poem is numbered bottom center with the rest of the pages of the poem numbered in the top margin. The poems may be double- or single-spaced but must conform to the other margin and formatting requirements in this guide.

APPENDIX D
STATISTICAL TEST FOR MYRCENE SIGNIFICANT DIFFERENCE

```
Two-sample T for Initial vs L-P

         N      Mean     StDev   SE Mean
Initial  2   1.03376   0.00165    0.0012
L-P      3    0.8629    0.0264     0.015

Difference = mu Initial - mu L-P
Estimate for difference:  0.1709
95% CI for difference: (0.1051, 0.2367)
T-Test of difference = 0 (vs not =): T-Value = 11.18  P-Value = 0.008  DF = 2
```

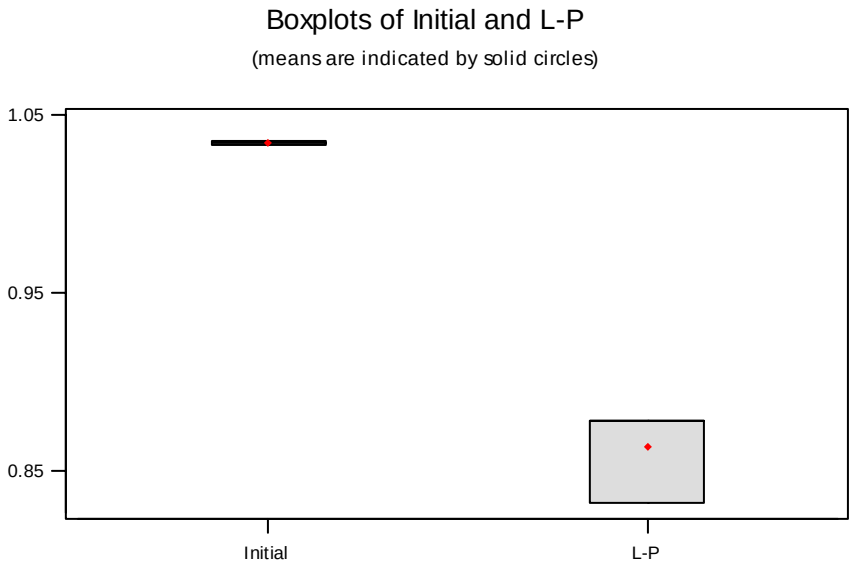

Figure 24: Significant Difference in Amount of Myrcene

Since the p value is 0.008, which is less than the alpha value of 0.01, we reject the null hypothesis. The amount of myrcene in the initial juice is not equal to the amount in the juice exposed to light in plastic.

```
Two-sample T for Initial vs D-P

         N      Mean     StDev   SE Mean
Initial  2   1.03376   0.00165    0.0012
D-P      3    0.9782    0.0331     0.019

Difference = mu Initial - mu D-P
Estimate for difference:  0.0556
95% CI for difference: (-0.0268, 0.1380)
T-Test of difference = 0 (vs not =): T-Value = 2.90  P-Value = 0.101  DF = 2
```

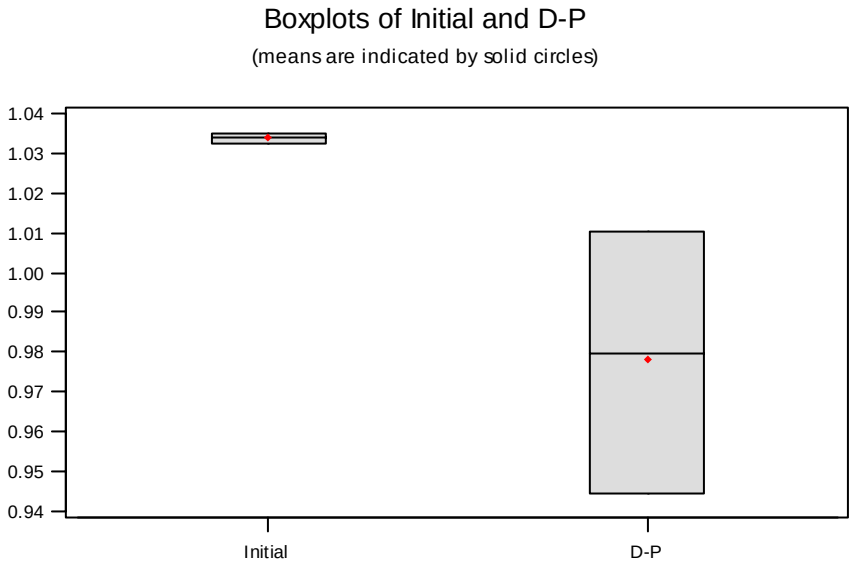

Figure 25: No Difference in Amount of Myrcene

Since the p value is 0.101, which is greater than the alpha value of 0.01, we fail to reject the null hypothesis. The amount of myrcene in the initial juice is equal to the amount in the juice protected from light in plastic.

APPENDIX E
STATISTICAL TEST FOR CARVONE SIGNICANT DIFERENCE

```
Two-sample T for Initial vs L-P

         N      Mean     StDev   SE Mean
Initial  2   0.04826   0.00196    0.0014
L-P      3    0.1750    0.0207     0.012

Difference = mu Initial - mu L-P
Estimate for difference:  -0.1267
95% CI for difference: (-0.1784, -0.0750)
T-Test of difference = 0 (vs not =): T-Value = -10.55  P-Value = 0.009  DF = 2
```

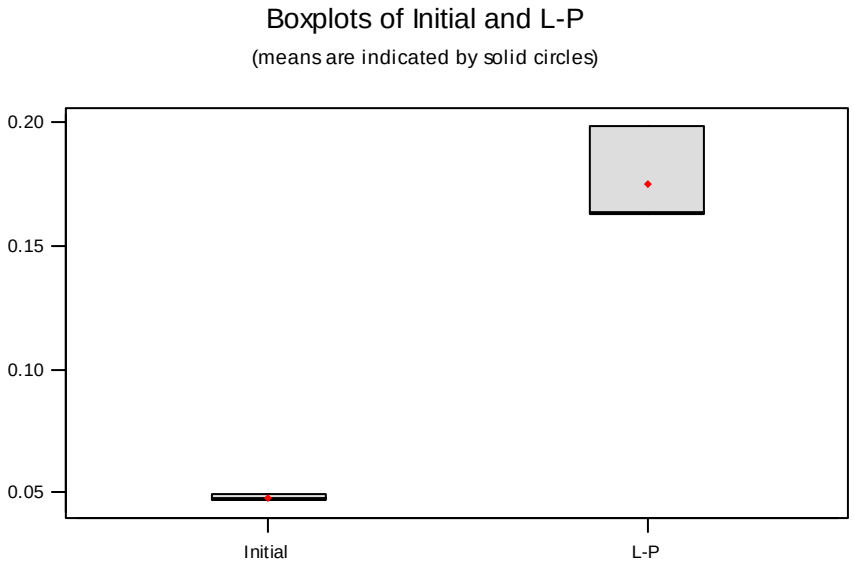

Figure 26: Significant Difference in Amount of Carvone

Since the p value is 0.009, which is less than the alpha value of 0.01, we reject the null hypothesis. The amount of carvone in the initial juice is not equal to the amount in the juice exposed to light in plastic.

```
Two-sample T for Initial vs D-P

         N      Mean     StDev   SE Mean
Initial  2   0.04826   0.00196    0.0014
D-P      3    0.0857    0.0187     0.011

Difference = mu Initial - mu D-P
Estimate for difference:  -0.0374
95% CI for difference: (-0.0843, 0.0095)
T-Test of difference = 0 (vs not =): T-Value = -3.43  P-Value = 0.076  DF = 2
```

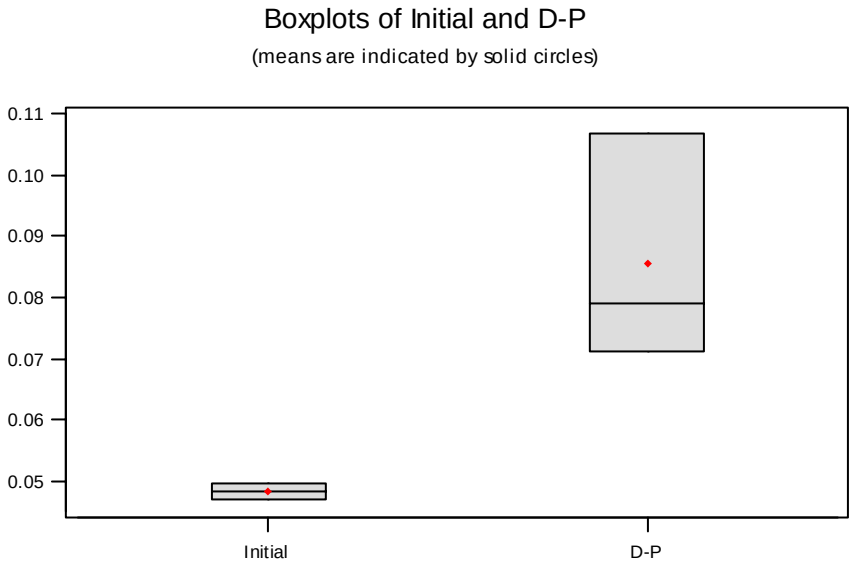

Figure 27: No Difference in Amount of Carvone

Since the p value is 0.076, which is greater than the alpha value of 0.01, we fail to reject the null hypothesis. The amount of carvone in the initial juice is equal to the amount in the juice protected from light in plastic.

APPENDIX F
GC-FID RESULTS FOR JUICES STORED IN PLASTIC

Light, In Plastic				No Light, In Plastic						
Week 0	Week 4	Week 8	Week 12	Week 0	Week 4	Week 8	Week 12			Identities
Ave Area	Ave Area	Ave Area	Ave Area	Ave Area	Ave Area	Ave Area	Ave Area	Ave Time	DB-5 LRI	
0.264	0.265	0.093	0.366	0.264	0.504	0.255	0.333	2.54	731	
0.060	0.054		0.089	0.060	0.089	0.051	0.124	3.27	777	
0.129	0.066	0.274	0.230	0.129	0.119	0.268	0.233	3.68	802	ethyl butanoate
1.000	1.000	0.400	1.000	1.000	1.000	0.400	1.000	5.55	901	ethyl valerate
0.306	0.260	0.285	0.272	0.306	0.294	0.305	0.277	6.29	936	a-pinene
0.995	0.814	0.892	0.863	0.995	1.003	1.052	0.978	7.54	993	b-myrcene
0.185	0.050	0.104		0.185	0.144	0.141		7.80	1005	octanal
0.038	0.039	0.017		0.038	0.036	0.019	0.034	8.00	1014	3-carene
54.076	44.197	47.998	47.173	54.076	51.387	53.610	50.653	8.64	1042	limonene
0.140	0.243	0.101	0.150	0.140	0.104	0.109	0.262	9.35	1073	p-cresol
0.553	0.461	0.513	0.615	0.553	0.549	0.591	0.674	10.03	1103	linalool
0.038	0.044	0.039	0.073	0.038	0.046	0.033	0.073	10.54	1126	trans-rose oxide
0.228	0.229	0.247	0.327	0.228	0.258	0.265	0.356	10.64	1130	ethyl 3-hydroxyhexanoat
	0.111	0.111	0.204	0.106	0.135	0.123	0.216	11.82	1183	
0.126	0.209	0.289	0.449	0.126	0.250	0.366	0.497	12.11	1197	a-terpineol
0.170	0.093	0.097	0.173	0.170	0.128	0.110	0.083	12.33	1207	
0.033	0.040	0.049	0.089	0.033	0.036	0.039	0.054	12.72	1225	nerol
0.028	0.022	0.013	0.038	0.028	0.030	0.031	0.055	12.86	1231	neral
0.055	0.069	0.119	0.175	0.055	0.062	0.068	0.086	13.25	1250	carvone
	0.027	0.018	0.096		0.027	0.040	0.058	14.73	1321	eugenol
0.045	0.055	0.069	0.137	0.045	0.053	0.057	0.107	15.27	1347	E-2-undecenal
0.032	0.026	0.038	0.064	0.032	0.033	0.030	0.071	16.51	1410	b-demascenone
0.062	0.053	0.065	0.084	0.062	0.065	0.073	0.098	16.93	1432	
0.052	0.047	0.060	0.074	0.052	0.062	0.070	0.095	17.49	1461	wine lactone
0.075	0.067	0.077	0.098	0.075	0.081	0.089	0.129	17.99	1488	
0.263	0.230	0.266	0.327	0.263	0.269	0.317	0.433	18.15	1497	
3.192	2.788	3.311	4.104	3.192	3.339	3.761	5.040	18.37	1508	b-ionone
0.124	0.146	0.149	0.182	0.124	0.138	0.150	0.317	18.53	1517	
0.217	0.214	0.244	0.314	0.217	0.237	0.283	0.386	18.81	1533	
0.032	0.027	0.017	0.069	0.032	0.035	0.038	0.083	19.41	1565	dodecanoic acid
0.030	0.043	0.048	0.082	0.030	0.051	0.052	0.124	21.26	1672	
0.031	0.023	0.021	0.034	0.031	0.023		0.041	21.73	1699	b-sinensal
0.071	0.063	0.094	0.153	0.071	0.128	0.113	0.215	22.76	1761	a-sinensal
0.233	0.196	0.256	0.393	0.233	0.232	0.300	0.529	23.78	1825	nootkatone
0.021	0.016		0.046	0.021	0.051		0.079	24.33	1859	
1.397	0.740	1.034	1.903	1.397	1.134	0.652	2.439	24.65	1880	
0.838	0.808	1.272	2.601	0.838	1.954	1.578	3.880	24.88	1895	
0.138	0.124	0.175	0.254	0.138	0.139	0.178	0.346	29.97	2251	
0.186	0.151	0.885	0.119	0.186	0.249	0.210	0.222	30.44	2286	
0.046	0.041	1.136	0.193	0.046	0.137	0.045	0.273	30.74	2308	
0.116	0.071	0.104	0.199	0.116	0.246	0.109	0.204	31.01	2329	
0.056	0.037	0.045		0.056	0.060	0.053	0.058	31.24	2346	
0.172	0.140	0.174	0.175	0.172	0.180	0.193	0.244	31.36	2356	
0.093	0.057	0.067	0.087	0.093	0.134	0.085	0.182	31.69	2381	

APPENDIX G
GC-FID RESULTS FOR JUICES STORED IN GLASS

Light, In Glass				No Light, In Glass						
Week 0	Week 4	Week 8	Week 12	Week 0	Week 4	Week 8	Week 12			Identities
Ave Area	Ave Area	Ave Area	Ave Area	Ave Area	Ave Area	Ave Area	Ave Area	Ave Time	DB-5 LRI	
0.223	0.505	0.310	0.650	0.223	0.271	0.273	0.872	2.54	731	
0.048	0.098	0.032	0.163	0.048	0.052	0.055	0.216	3.27	777	
0.117	0.114	0.223	0.299	0.117	0.068	0.207	0.183	3.68	802	ethyl butanoate
1.000	1.000	0.400	1.000	1.000	1.000	0.400	1.000	5.55	901	ethyl valerate
0.288	0.271	0.296	0.280	0.288	0.289	0.308	0.268	6.29	936	a-pinene
0.995	0.873	0.936	0.877	0.995	0.963	1.016	0.935	7.54	993	b-myrcene
0.167	0.118		0.061	0.167				7.80	1005	octanal
0.036	0.033			0.036	0.031	0.034		8.00	1014	3-carene
50.489	47.118	51.762	48.312	50.489	50.583	52.604	47.330	8.64	1042	limonene
0.206	0.094	0.189	0.102	0.206	0.231	0.184	0.128	9.35	1073	p-cresol
0.492	0.486	0.547	0.632	0.492	0.496	0.527	0.571	10.03	1103	linalool
0.036	0.060	0.058	0.155	0.036	0.037	0.034	0.152	10.54	1126	trans-rose oxide
0.224	0.244	0.287	0.365	0.224	0.253	0.269	0.284	10.64	1130	ethyl 3-hydroxyhexanoate
0.092	0.114	0.164	0.221	0.092	0.144	0.154	0.252	11.82	1183	
0.108	0.222	0.289	0.463	0.108	0.238	0.347	0.477	12.11	1197	a-terpineol
0.141	0.121	0.090	0.153	0.141	0.038	0.057	0.090	12.33	1207	
0.029	0.045	0.061	0.105	0.029	0.029	0.030		12.72	1225	nerol
0.023	0.027			0.023	0.031	0.031		12.86	1231	neral
0.048	0.094	0.167	0.241	0.048	0.040	0.056	0.066	13.25	1250	carvone
0.023	0.028	0.058	0.100	0.023	0.037	0.041		14.73	1321	eugenol
0.043	0.066	0.094	0.145	0.043	0.053	0.054	0.070	15.27	1347	E-2-undecenal
0.023	0.036	0.044	0.066	0.023	0.023	0.029		16.51	1410	b-demascenone
0.052	0.063	0.074	0.125	0.052	0.061	0.072	0.077	16.93	1432	
0.045	0.060	0.070	0.090	0.045	0.058	0.067	0.069	17.49	1461	wine lactone
0.064	0.074	0.091	0.122	0.064	0.074	0.095	0.091	17.99	1488	
0.229	0.267	0.321	0.402	0.229	0.251	0.297	0.294	18.15	1497	
2.727	3.196	3.834	4.751	2.727	3.058	3.537	3.642	18.37	1508	b-ionone
0.112	0.144	0.135	0.190	0.112	0.149	0.144	0.160	18.53	1517	
0.197	0.227	0.274	0.339	0.197	0.232	0.258	0.269	18.81	1533	
0.025	0.039	0.052	0.073	0.025	0.040	0.033		19.41	1565	dodecanoic acid
0.027	0.054	0.057	0.103	0.027	0.047	0.049	0.070	21.26	1672	
0.032	0.027		0.053	0.032	0.030	0.026		21.73	1699	b-sinensal
0.087	0.099	0.153	0.237	0.087	0.100	0.184	0.126	22.76	1761	a-sinensal
0.207	0.257	0.300	0.446	0.207	0.229	0.283	0.370	23.78	1825	nootkatone
0.015	0.023	0.082	0.078	0.015	0.055	0.067		24.33	1859	
1.196	1.400	0.671	2.300	1.196	1.182	0.673	1.899	24.65	1880	
0.691	1.358	1.213	3.544	0.691	2.768	3.851	2.216	24.88	1895	
0.123	0.160	0.212	0.354	0.123	0.150	0.181	0.270	29.97	2251	
0.151	0.383	0.272	0.176	0.151	0.236	0.321	0.139	30.44	2286	
0.027	0.123	0.063	0.315	0.027	0.113	0.108	0.195	30.74	2308	
0.081	0.193	0.141	0.246	0.081	0.188	0.270	0.192	31.01	2329	
0.035	0.054	0.037		0.035	0.049	0.076	0.190	31.24	2346	
0.126	0.185	0.110	0.273	0.126	0.177	0.261	0.246	31.36	2356	
0.083	0.093	0.053		0.083	0.078	0.118		31.69	2381	

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BIOGRAPHICAL SKETCH

Kristin Nelson graduated from Niceville High School in 1997. She received a Bachelor of Science degree in Chemical Engineering from the Georgia Institute of Technology in 2002, graduating with honors. Kristin completed her Master of Science in food science and human nutrition at the University of Florida in 2005. Her research was in the field of flavor chemistry and was conducted at the Citrus Research and Education Center in Lake Alfred, Florida. She is now part of Kerry's graduate student management training program working in Lakeland, Florida, in the flavor division. As part of the program, Kristin trains in various areas of the company such as research and development, beverage applications, pilot plant scale-up, production, quality control, and analytical analysis.