Degradation of Monoterpenes in Orange Juice by Gamma Radiation

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Single-strength orange juice was irradiated with 0, 0.89, 2.24, 4.23, and 8.71 gGy of gamma radiation at 5 °C and then stored at 7 °C for 21 days. Volatile compounds, isolated by solid-phase microextraction, were separated and identified using a gas chromatograph equipped with a mass selective detector. The majority of the volatile compounds were terpenes, and the most abundant volatile compounds were ethanol and limonene. Most volatile compounds were stable during the 21-day storage period except geranial and neral which decreased over time. Irradiation reduced the concentration of acyclic monoterpenes, such as geranial, neral, myrcene, and linalool 1 and 7 days after irradiation, but did not affect other monoterpenes, sesquiterpenes, or other volatile compounds. The reduction of acyclic monterpenes increased linearly with radiation dose, and correlated with an increase in thiobarbituric acid reactive substrates (TBARS) content. Reduction in the concentration of monoterpenes induced by irradiation was not significant 21 days after irradiation. Our results indicate that acyclic monoterpenes are sensitive to irradiation whereas most other volatile compounds are resistant.

Keywords: *irradiation; orange juice; SPME; terpenes; volatile compounds*

INTRODUCTION

Ionizing irradiation is a nonthermal technique that effectively inactivates human pathogens and reduces spoilage in juice (*1*−*3*) and other foods (*4*). However, irradiated juice may develop an off-flavor (*2*, *3*, *5*) described as "medicinal" and "cooked" (*5*). Irradiated orange juice has been found to be less acceptable than nonirradiated juice because of the development of this off-flavor (*5*, *6*). Much of the flavor in orange juice stems from volatile compounds, including volatile alcohols, aldehydes, esters, ketones, and hydrocarbons (*7*). Among the volatile compounds important to orange juice flavor, esters and aldehydes are the primary contributors (*8*). Marin et al. (*9*) reported that linalool, neral, geranial, vanillin, and two ethyl esters were the major aroma-impacting components. Using a GC−olfactometry technique, Bazemore et al. (*10*) showed the strongest aroma-active compounds were ethyl butyrate, (E)-2-nonenal, myrcene, decanal, and octanal. Limonene is also required in orange juice for acceptable flavor and mouthfeel (*11*).

Although no qualitative difference has been observed between volatiles isolated from control and irradiated citrus fruit, irradiation induces some quantitative differences in volatile production (*12*−*14*). A reduction in *β*-pinene production was observed from grapefruit irradiated at 0.5 kGy (*13*). Volatile terpene compounds of orange fruit increased with dose and post-irradiation holding time (*15*). However, the effect of irradiation on the aroma volatile compounds of orange juice is unknown.

Oxidation of unsaturated fatty acids released from lipids during processing and storage can cause significant alteration of flavor due to the formation of unpleasant aroma compounds, such as aldehydes and ketones (*16*, *17*). In high fat foods, such as meats, many irradiation-induced off-odor compounds originate from the oxidation of unsaturated fatty acids (*18*), and the lipid oxidation is correlated with the production of volatile compounds degraded from unsaturated fatty acids (*19*). The effect of irradiation on the lipid oxidation of orange juice and its relation to irradiation-induced off-odor is unknown. The objective of this study is to evaluate the impact of irradiation on volatile compounds and lipid oxidation of orange juice.

MATERIALS AND METHODS

Materials. Pasteurized single-strength orange juice was purchased from a local supermarket. Approximately 40 mL of juice was placed into 42-mL glass tubes sealed with Teflon-lined septa and screw caps. The tubes containing juice were stored at 7 °C overnight before being irradiated. There were four tubes per dose, and each tube was considered as a replicate. Ethanol, ethyl butyrate, ethyl hexanoate, α-pinene, *β*-pinene, myrcene, α-terpineol, octanal, limonene, linalool, geranial, and neral, decanal, and valencene standards were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Irradiation. Juice was irradiated with gamma radiation at doses of 0, 0.89, 2.24, 4.23, and 8.71 kGy. Irradiation was conducted using a self-contained, Lockheed Corporation ^{137}Cs gamma radiation source with a dose rate of approximately 0.1 kGy·min^{-1}. The dose rate was established using alanine transfer dosimeters from the National Institute of Standards and Technology (Gaithersburg, MD). The temperature was maintained at 5 ± 2 °C by injecting the gas phase from a liquid nitrogen tank into the radiation chamber. Routine dosimetry was performed using alanine dosimeters (Bruker Instruments Inc., Billerica, MA). The dosimeters were placed into 2-mL cryogenic vials (Nalgene, Rochester, NY), and the vials were taped onto the tubes containing the juice samples prior to irradiation. The free radical signal induced in response to radiation was quantified by inserting the alanine dosimeters into a 104 Electron Paramagnetic Resonance instrument (Bruker Instruments Inc., Billerica, MA). This instrument was

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10.1021/jf0013813
Published on Web 04/19/2001

calibrated in accordance with ASTM E1607-96 (*20*). After irradiation, volatile compounds and lipid oxidation in the juice sample were quantified after 1, 7, and 21 days at 7 °C.

Analysis of Headspace Volatile Compounds. Volatile compounds were extracted as described by Jia et al. (*21*) using a solid-phase microextraction (SPME) method (*22*). A 1-mL aliquot of orange juice was transferred into a 6-mL serum vial. The vial, sealed by a Teflon-lined septum and a screw cap, was incubated at 40 °C for 10 min before an SPME fiber, coated with 100 μm of poly(dimethylsiloxane), was inserted into the headspace of the sample bottle. After 30 min incubation, the SPME fiber with adsorbed volatile compounds was inserted into the GC injection port at 250 °C and held there for 2 min to desorb volatile compounds. Volatile compounds were separated by a Hewlett-Packard 5890/5971 GC−MSD (Agilent Technologies, Palo Alto, CA) equipped with a HP-5 trace analysis column (30 m H 0.32 mm i.d., 0.25 μm film thickness). The temperature of the GC was programmed from 60 to 160 °C at 8 °C·min^{-1}, then increased to 220 °C at 25 °C·min^{-1} and held for 3 min at the final temperature. Helium was the carrier gas at a linear flow rate of 20.7 cm·sec^{-1}. Compounds were identified by comparing spectra of the sample compounds with those of standards and with those contained in the Wiley-NBS library, as well as by comparing retention times of sample compounds with those of the standards.

Quantification of Volatile Compounds. Deodorized single-strength orange juice was prepared (*21*, *23*) by concentrating single-strength orange juice from 12.0 to 46 Brix using a vacuum rotary evaporator (Brinkmann Instrumental, Inc., Westbury, NY). The residual volatile compounds in the concentrated juice were then extracted twice with hexane (2:1). The separation of the juice phase from hexane was accelerated by centrifuging the mixture at 1000*g* for 10 min at 4 °C using a Sorvall RT6000B refrigerated centrifuge (DuPont, Wilmington, DE). The juice layer was collected, and the trace hexane residue in the juice was removed using the vacuum rotary evaporator. The concentrated, deodorized juice was diluted back to 12.0 Brix with distilled water. Standard mixtures with a series of concentrations of the 14 volatile compounds were prepared using the deodorized juice. The concentrations of volatile compounds were 0, 0.01, 0.1, and 1 ppm for ethyl hexanoate; 0, 0.1, 1, and 10 ppm for ethyl butyrate, α-pinene, β-pinene, α-terpineol, octanal, geranial, and neral; 0, 1, 10, and 100 ppm for myrcene, limonene, linalool, decanal, and valencene; and 0, 100, and 1000 ppm for ethanol. The deodorized orange juice containing the standard compounds was mixed well and stored at 7 °C overnight to equilibrate. The volatile compounds were then analyzed using the same procedures as described above. Compounds were quantified using selected ion monitoring for base peaks, and quantitative values were calculated using response factors generated with standard curves. Calibration factors were generated by plotting the selective ion counts against the concentrations of each volatile compound.

Lipid Oxidation. Lipid oxidation was measured by the thiobarbituric acid (TBA) reactive substrates (TBARS) assay (*24*). A 1.6-mL sample of diluted (4×) juice was added to a test tube containing 1.6 mL of either (i) −TBA solution: 20% (w/v) trichloroacetic acid and 0.01% butylated hydroxytoluene, or (ii) +TBA solution: containing the above plus 0.65% TBA. Samples were then mixed vigorously, heated at 95 °C in a water bath for 25 min, cooled, and centrifuged at 1300*g* for 10 min at 5 °C. Absorbance at 440, 532, and 600 nm was monitored using a Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). TBARS were calculated using the formulas developed by Hodges et al. (*24*).

$$[(Abs532_{+\mathrm{TBA}} - Abs600_{+\mathrm{TBA}}) - (Abs532_{-\mathrm{TBA}} - Abs600_{-\mathrm{TBA}})] = A \quad (1)$$

$$[Abs440_{+\mathrm{TBA}} - Abs600_{+\mathrm{TBA}})0.0571] = B \quad (2)$$

$$\mathrm{TBARS\ (nmol{\cdot}mL^{-1})} = [(A - B)/157000] \times 10^6 \quad (3)$$

Table 1. Volatile Compounds Detected in Non-Irradiated Orange Juice Measured One Day after Irradiation[a]

compound	retention time (min)	concentration (ppm)
ethanol	1.32	513.33
ethyl butyrate	2.23	0.73
α-pinene	3.67	1.46
β-pinene	4.15	0.05
ethyl hexanoate	4.42	0.06
myrcene*	4.48	5.09
octanal	4.53	0.52
limonene	5.03	131.94
linalool*	6.07	3.26
α-terpineol	7.72	1.04
decanal	7.86	1.88
neral*	8.43	0.10
geranial*	8.99	0.14
valencene	12.59	3.92

[a] Values are means of 4 replicate samples. Compounds reduced by irradiation are designated with an "*".

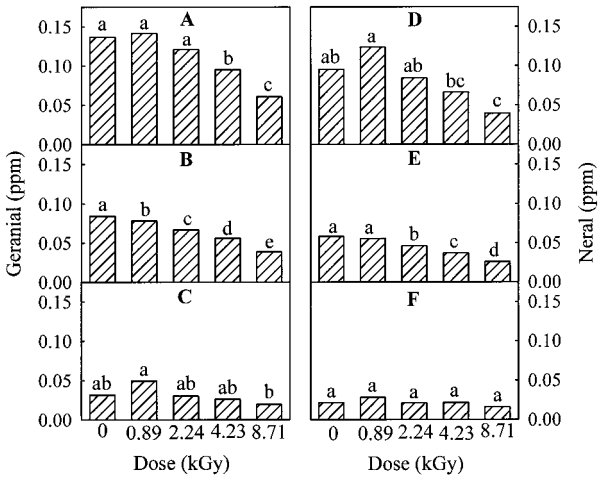

Figure 1. Concentrations of geranial (A, B, and C) and neral (D, E, and F) in irradiated and nonirradiated orange juice. Juices irradiated with 0, 0.89, 2.24, 4.23, or 8.71 kGy at 5 °C were stored at 7 °C for 21 days. Volatile compounds were measured after 1 (A and D), 7 (B and E), or 21 (C and F) days storage. Bars with same letter are not significantly different (LSD, $P < 0.05$). Comparisons were made within the same storage duration.

Statistical Analysis. Data were subjected to statistical analysis using SAS version 6.12 (SAS Institute, Raleigh, NC). LSD analysis was performed using the GLM procedure. The linearity effect of radiation dose was analyzed using orthogonal comparisons. Significance of polynomials was calculated using the Contrast statement of the GLM procedure.

RESULTS AND DISCUSSION

Fourteen volatile compounds identified by comparing the spectra and retention times of samples and standards included 3 alcohols, 2 esters, 4 aldehydes, and 5 hydrocarbons (Table 1). Most of the compounds were terpenes including terpene alcohols and terpene aldehydes. Ethanol was the most abundant compound followed by limonene. Most of the volatile compounds did not change during the 3 weeks storage at 7 °C except geranial and neral which decreased rapidly over time during storage in both irradiated and nonirradiated samples (Figure 1). The majority of the volatile compounds were not affected by irradiation. Irradiation, however, reduced the concentrations of myrcene, linalool, geranial, and neral in the samples stored for 1 and 7 days at 7 °C after irradiation (Figures 1 and 2). After 3 weeks storage, however, the differences among the

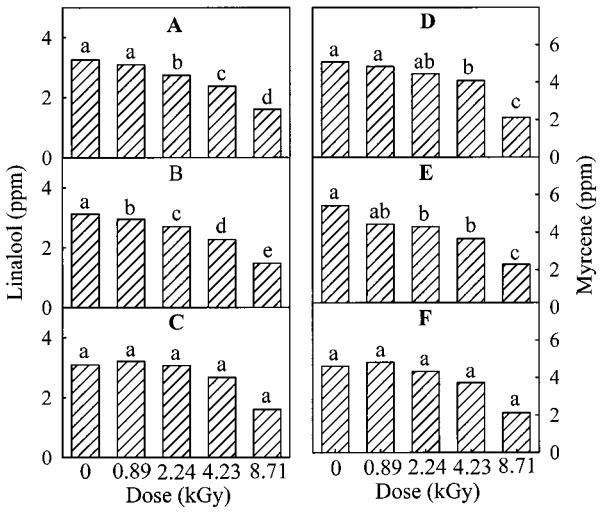

Figure 2. Concentrations of linalool (A, B, and C) and myrcene (D, E, and F) in irradiated and nonirradiated orange juice. Juices irradiated with 0, 0.89, 2.24, 4.23, or 8.71 kGy at 5 °C were stored at 7 °C for 21 days. Volatile compounds were measured after 1 (A and D), 7 (B and E), and 21 (C and F) days storage. Bars with same letter are not significantly different (LSD, $P < 0.05$). Comparisons were made within the same storage duration.

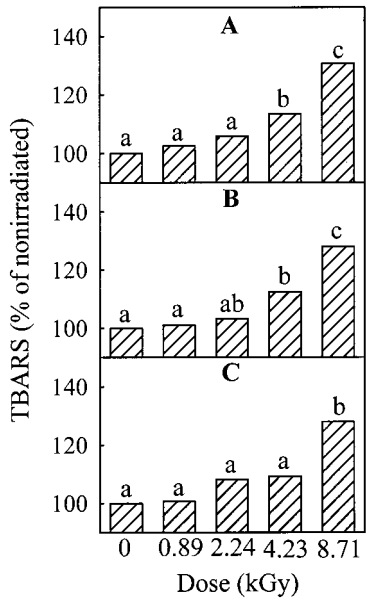

Figure 3. Lipids oxidation of irradiated and nonirradiated orange juice. Juices irradiated with 0, 0.89, 2.24, 4.23, or 8.71 kGy gamma rays at 5 °C were stored at 7 °C for 21 days. Lipid oxidation was assayed using the 2-thiobarbituric acid reactive substance (TBARS) assay after 1 (A), 7 (B), and 21 (C) days storage. Bars with same letter are not significantly different (LSD, $P < 0.05$). Comparisons were made within the same storage duration.

irradiation treatments were not significant, partially due to high variation among the replicates. Among the four compounds affected by irradiation, myrcene was the most abundant. Geranial and neral, the two isomers of citral, had similar changes during storage and responded similarly to irradiation. The decrease in geranial and neral during storage has been observed in aseptically packaged orange juice (*25*). Irradiation also reduces production of geranial and neral by ginger rhizomes (*26*).

All compounds reduced in quantity following irradiation were monoterpenes. Monoterpenes can be classified into acyclic, monocyclic, and bicyclic according to the number of rings in their structure. Only the concentrations of acyclic terpenes derivable by head to tail condensation of isoprene units without the ring structure, such as neral, geranial, linalool and myrcene, decreased after irradiation. The concentrations of monocyclic monoterpenes such as α-terpineol and limonene, and bicyclic monoterpenes such as α-pinene, were not influenced by irradiation. Valencene, a bicyclic sesquiterpene, was also resistant to irradiation. It appears that the ring structure of terpenes protects these compounds from degradation induced by irradiation. It is unclear what the radiolytic products of these acyclic terpenes are.

The concentrations of myrcene, linalool, geranial, and neral decreased linearly ($P<0.05$) with increased radiation dose. The irradiation-induced degradation rate for all four acyclic terpenes was similar, with 5.89, 5.66, 5.83, and 6.54% degraded per kGy for myrcene, linalool, geranial, and neral, respectively, indicating that all these compounds had a similar sensitivity to irradiation.

Consumption of orange juice has been linked to several recent outbreaks of illness associated with *Salmonella* and *E. coli* O157:H7 (*27*). Irradiation effectively eliminates these pathogens in juice (*1*, *28*). Variation in radiation resistance exists among different strains of *E. coli* O157:H7 and *Salmonella*, and *Salmonella* strains are more resistant to irradiation than *E. coli* O157:H7. The *D* value for the most resistant strain of *Salmonella* is 0.71 kGy. To achieve a 5-log reduction recommended by the National Advisory Committee on Microbiological Criteria for Food (*29*), a 3.55 kGy dose would be necessary for elimination of *Salmonella* and *E. coli*. O157:H7 (*1*, *28*). At the 5-*D* dose, myrcene, linalool, geranial, and neral would be reduced by 20.9, 20.1, 20.7, and 23.2%, respectively. Linalool, myrcene, neral, and geranial are important contributors to orange juice aroma (*9*, *10*). It is unclear whether the decrease in the concentrations of these compounds would impact the flavor of orange juice. Many other volatile compounds that contribute to juice aroma, including ethyl esters, decanal, octanal, and limonene, were not affected by irradiation at doses of 8.71 kGy or less. Besides the concentrations of individual compounds, other factors that influence the flavor of orange juice are the correct proportions of different compounds, taste threshold values of compounds, synergistic effects between volatiles, and interaction of nonvolatile with volatile compounds (*30*).

A number of other volatile compounds were tentatively identified in the orange juice by comparing spectra of sample compounds with those contained in the Wiley-NBS library. These compounds were α-phellandrene, β-terpinene, γ-terpinene, α-terpinolene, I-4-terpineol, nonanal, β-citronellol, β-elemene, α-selinene, β-selinene, δ-cadinene, γ-cardinene, and nootkatone. Among those compounds, only β-citronellol, an acyclic monoterpene, was significantly ($P<0.05$) reduced by 8.71 kGy radiation. Many other minor volatile compounds were not identified, but it appears that none of them were affected by irradiation. The analytical techniques used in this study did not detect any new compounds induced by irradiation.

TBARS has long been regarded as an index of lipid oxidation (*31*). Similar to the results in high fat foods (*19*), the TBARS content in orange juice increased following irradiation (Figure 3). The TBARS content

increased linearly with radiation dose when measured 1 day after irradiation and this increase was evident throughout the 3 week storage period (Figure 3). The increase in TBARS content was correlated with the reduction in myrcene, linalool, geranial, and neral ($R^2 = 0.99$, 0.98, 0.96, and 0.79, respectively). Terpenes or their radiolytic products did not contribute to the TBARS formation (data not shown). Although we did not detect any fatty acid-derived hydrocarbon volatile compounds, such as pentanal or hexanal, perhaps because of low concentrations and coelution with terpenes, the increase in TBARS content indicates that irradiation promoted lipid oxidation in orange juice. The amount of lipids in orange juice is low (<0.1%), but important to flavor. During storage of orange juice, the amount of free fatty acid increases while phospholipid concentration decreases (*32*). Flavor deterioration in orange juice may be associated with the oxidation of unsaturated fatty acids (*33*, *34*). The suspended matter in citrus juice contains most of the lipid fraction and is the principal contributor to off-flavor in aged orange juice (*33*, *34*). It is unclear whether irradiation-induced off-flavor is influenced by the amount of suspended matter.

The SPME fiber used in this study was more selective for nonpolar components such as terpenes (*35*). It is less effective for extracting polar, low-molecular-weight components such as ethanol. There was a large amount of variation among replicates for ethanol (data not shown), and it is possible that the compounds responsible for the off-flavor of irradiated juice could not be detected using this method.

α-Terpineol, along with 4-vinyl guaiacol and 2,5-dimethyl-3(2H)-furanone (furaneol) are known to contribute to off-flavor in aged orange juice (*36*). α-Terpineol is a degradation product of essential oil components such as limonene and linalool (*11*, *35*), and the rate of α-terpineol formation from linalool is faster than that from limonene (*35*). We observed a decrease in linalool but no corresponding increase in α-terpineol, possibly because α-terpineol degrades faster than its formation in orange juice (*35*) or the degradation of linalool caused by irradiation has a different pathway.

In summary, our results indicated that most volatile compounds in orange juice were stable during storage and during irradiation. However, acyclic monoterpene compounds such as linalool, myrcene, neral, and geranial were sensitive to irradiation, and the concentrations of these compounds decreased with increasing irradiation dose.

ACKNOWLEDGMENT

The technical assistance of Robert Richardson is gratefully acknowledged.

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Received for review November 17, 2000. Revised manuscript received March 7, 2001. Accepted March 20, 2001. Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature mentioned.

JF0013813