

Changes in Volatile Compounds of γ -Irradiated Fresh Cilantro Leaves during Cold Storage

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Consumption of salsas and dishes containing cilantro has been linked to several recent outbreaks of food-borne illness due to contamination with human pathogens. Ionizing irradiation can effectively eliminate food-borne pathogens from various vegetables including cilantro. However, the effect of irradiation on aroma of fresh cilantro is unknown. This study was conducted to investigate the effect of irradiation on volatile compounds of fresh cilantro leaves. Fresh cilantro leaves (*Coriandrum sativum* L.) were irradiated with 0, 1, 2, or 3 kGy γ radiation and then stored at 3 °C up to 14 days. Volatile compounds were extracted using solid-phase microextraction (SPME), followed by gas chromatographic separation and mass spectra detection at 0, 3, 7, and 14 days after irradiation. Most of the volatile compounds identified were aldehydes. Decanal and (*E*)-2-decenal were the most abundant compounds, accounting for more than 80% of the total amount of identified compounds. The amounts of linalool, dodecanal, and (*E*)-2-dodecenal in irradiated samples were significantly lower than those in nonirradiated samples at day 14. However, the most abundant compounds [decanal and (*E*)-2-decenal] were not consistently affected by irradiation. During storage at 3 °C, the amount of most aldehydes peaked at 3 days and then decreased afterward. Our results suggest irradiation of fresh cilantro for safety enhancement at doses up to 3 kGy had minimal effect on volatile compounds compared with the losses that occurred during storage.

KEYWORDS: Irradiation; cilantro; volatile; storage

INTRODUCTION

The fresh leaves of cilantro are highly regarded in the cuisine of China, Mexico, South America, India, and Southeast Asia. However, soups and salsas have caused several recent outbreaks of food-borne illness (1). Fresh cilantro leaves, an ingredient in these foods, have been suggested as the source of the pathogens. In recent surveys of both domestic and imported produce, the U.S. Food and Drug Administration has found a high rate of pathogens (*Salmonella* and *Shigella*) recovered from cilantro (2, 3). A high rate of *Cryptosporidium* oocysts, a parasite, has also been found in cilantro (4); therefore, a means of eliminating food-borne pathogens from cilantro is needed. Irradiation is well documented to effectively inactivate food-borne pathogens and parasites (5, 6); however, the effect of irradiation on the quality of cilantro has not been reported.

One of the major attributes of cilantro, as an herb, is aroma quality (7). Aroma quality, paramount to the culinary value of the fresh herb, usually decreases before visual quality decreases (8). The aroma in fresh cilantro stems from volatile compounds; therefore, the impact of irradiation on volatile compounds of cilantro is a major concern. Fruit and essential oils have been used for medicinal herb and flavoring agents. Much information

is available on the identification of volatile components of cilantro fruit essential oils (9–13). Limited information on volatile compounds of fresh cilantro leaves exists in the literature. Schratz and Qadry (14) investigated the volatile oil composition of cilantro during plant development. They identified (*E*)-2-tridecenal, decanal, and borneol in vegetative organs. MacLeod and Islam (15) identified 17 volatile compounds from cilantro leaves, most of which were aliphatic aldehydes with 7-dodecenal, dodecanal, and decanal being the most abundant. Potter and Fagerson (16) identified 37 volatile constituents from cilantro leaves, including alkenals, alkanals, alkenols, and alkanols. Smallfield and others (17) found that the most abundant volatile compound of whole cilantro plants (including fruit) was (*E*)-2-decenal. In many of these studies, compounds were extracted using steam distillation or solvent extraction, and identifications were based on mass spectra. In addition, absolute quantification of the compounds in fresh cilantro leaves has not been reported. Also, information about changes in production of volatile compounds by fresh cilantro leaves during postharvest storage is not available. In the present study, we used the solid-phase microextraction (SPME) technique to extract volatile compounds from cilantro leaves at different storage times (days) after treatment with different levels of irradiation. The extracted compounds were identified and quantified by GC/MS comparison with authentic standards.

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The best postharvest conditions for preservation of fresh cilantro leaves include storage at low temperature with high humidity in air (7, 8). Under these conditions, a shelf life of 14 days can be expected if aroma is considered an important attribute (8). The objective of this study was to investigate the impact of irradiation on production of volatile compounds immediately after irradiation as well as during a 14-day postirradiation storage period at 3 °C.

MATERIALS AND METHODS

Standard compounds were obtained from Sigma Chemical Co. (St. Louis, MO). The purity of all reference compounds was greater than 90%.

'Slobolt' cilantro (*Coriandrum sativum* L) was harvested by uprooting the whole plants at a farm near Great Meadows, NJ. After harvest, the samples, placed into perforated plastic bags, were transported on ice in insulated chests and then stored at 3 °C overnight. Roots were cut off and discarded. The leaves were then washed with cold tap water and excess water was drained by laying the samples on tissue paper. Yellow and senescent leaves were discarded. The cilantro leaves were then packaged in zipper plastic bags (16.5 × 8.2 cm) perforated with two holes (3 mm in diameter each). Four packages containing cilantro were randomly assigned to each treatment. Each bag contained 50 g of cilantro. All preparation procedures were performed at 10 °C. After packaging, the cilantro leaves were stored at 3 °C overnight before irradiation at 5 ± 2 °C to doses of 0, 1, 2, or 3 kGy. The samples were then stored at 3 °C up to 14 days. Volatile compounds were measured initially (immediately after irradiation), and 3, 7, and 14 days after irradiation.

Irradiation and Dosimetry. The samples were irradiated using a self-contained cesium-137 γ radiation source (Lockheed Georgia Co., Marietta, GA) with a dose rate of 0.098 kGy/min. Variations in radiation dose absorption were minimized by placing the samples within a uniform area of the radiation field, by irradiating them within a polypropylene container (4-mm wall) to absorb Compton electrons, and by using the same geometry for sample irradiation during the entire study. During irradiation, temperature in the radiation chamber was controlled by flushing the gas phase of liquid nitrogen into the upper portion of chamber. To reduce possible effects of nitrogen during irradiation, all samples were placed in the chamber with nitrogen flushing for the same total period (33.72 min). Routine dosimetry was performed using 5-mm-diameter alanine pellets (Bruker Instruments Inc., Billerica, MA). The pellets were placed into 1.2-mL cryogenic vials (Nalgene, Rochester, NY), and the cryogenic vials were placed with the samples before irradiation. Absorbed doses were measured using a Bruker EMS 104 EPR analyzer and calculated in comparison with a standard curve. Actual doses were within 5% of the target doses.

Analysis of Headspace Volatile Compounds. Samples (5 g) taken from a single bag were homogenized at room temperature with 25 mL saturated CaCl₂ (18) using a homogenizer (Virtishear, Virtis, Gardiner, NY) at a speed setting of 70 for 1 min. Saturated CaCl₂ solution was prepared by adding excess CaCl₂ to deionized water and then boiling the solution for 1 h to remove volatile impurities from the CaCl₂ solution (18). The solution was then used for sample homogenization. Volatile compounds in the homogenates were extracted using a solid-phase microextraction (SPME) method (19) with modification (20). To reduce the variation caused by individual fibers, only two fibers that had similar responses were used in the entire experiment. A 5-mL aliquot of homogenate was transferred into a 40-mL vial (9.2 × 2.6 cm). The vial, sealed by a Teflon-lined septum and a screw cap, was incubated in a 55 °C water bath with stirring for 30 min before a SPME fiber [2 cm 50/30 μ m divinylbenzene/Carboxen/poly(dimethylsiloxane)] was inserted into the headspace of the sample bottle. The distance between the tip of fiber to the surface of homogenate was approximately 4 cm. 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 an HP-5 trace analysis column (30 m × 0.32 mm i.d., 0.25 μ m film thickness). The temperature of

Table 1. Volatile Compounds Detected in Nonirradiated Cilantro Measured on the Day of Irradiation

compound	retention index ^a	concentration (μ g/g FW)	% of total
(E)2-hexenal	(2.97)	0.44 ± 0.10	0.23
nonane	(3.59)	8.11 ± 1.84	4.25
octanal	800	1.24 ± 0.23	0.65
linalool	(6.92)	4.07 ± 0.54	2.13
nonanal	900	0.54 ± 0.09	0.29
decanal	1000	98.21 ± 3.45	51.51
(E)2-decenal	1058	60.27 ± 7.81	31.61
undecanal	1100	1.12 ± 0.07	0.59
(E)2-undecenal	1160	1.52 ± 0.14	0.80
dodecanal	1200	1.42 ± 0.27	0.74
(E)2-dodecenal	1262	9.42 ± 0.60	4.94
(E)2-tridecenal	1361	1.16 ± 0.17	0.61
tetradecanal	1400	3.15 ± 0.69	1.65
total		190.67 ± 11.76	100

^a Retention index values are Kovats indices based on the retention times of the saturated *n*-aldehydes (16). Numbers in parentheses are uncorrected retention times in minutes. ^b Values are means of four replicate samples ± standard errors.

the GC was held at 60 °C for 2 min, increased to 200 °C at 10 °C·min⁻¹, then increased to 250 °C at 25 °C·min⁻¹, and held for 2 min at the final temperature. Helium was the carrier gas at a linear flow rate of 20.7 cm·s⁻¹. The MSD was operated at SCAN mode. Compounds were identified by comparison of 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. A series of concentrations of standard compounds were prepared in saturated CaCl₂ solution. The concentrations of volatile compounds were 0, 0.1, 1, 10 PPM for nonane, octanal, linalool, decanal, (E)-2-decenal, undecanal, (E)-2-undecenal, dodecanal, (E)-2-dodecenal, (E)-2-tridecenal, and tetradecanal; and 0, 0.01, 0.1, and 1 PPM for (E)-2-hexenal and nonanal. The solutions were mixed well by stirring for 2 h at room temperature. Volatile compounds were then analyzed using the same procedures as described above. Compounds were quantified using selected ions, 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.

Statistical Analysis. Data were subjected to statistical analysis using SAS version 6.12 (SAS Institute, Raleigh, NC). The least significant difference (LSD) analysis was performed using the general linear model (GLM) procedure. Only significant ($P < 0.05$) results are discussed.

RESULTS AND DISCUSSION

Eleven aldehydes, one alcohol, and one hydrocarbon were identified by comparing the spectra and retention times of samples with those of standards (Table 1). The aldehydes were straight-chained alkanals and alkenals ranging from C₆ to C₁₄. Decanal and (E)-2-decenal were the most abundant compounds, accounting for more than 80% of total amounts of identified compounds.

(E)-2 Hexenal content of cilantro did not change during the first 7 days of storage, then increased from day 7 to 14 (Figure 1A). Irradiation had no significant ($P < 0.05$) effect on content of (E)-2-hexenal. Nonane content of cilantro did not change during storage and was not significantly ($P < 0.05$) affected by irradiation (data not shown). Octanal content of all cilantro samples decreased during storage (Figure 1B), and irradiation had no effect on octanal content except that samples irradiated at 2 kGy had higher content than nonirradiated samples at day 7.

Irradiation had no effect on linalool content of fresh cilantro measured immediately after irradiation (Figure 1C). However,

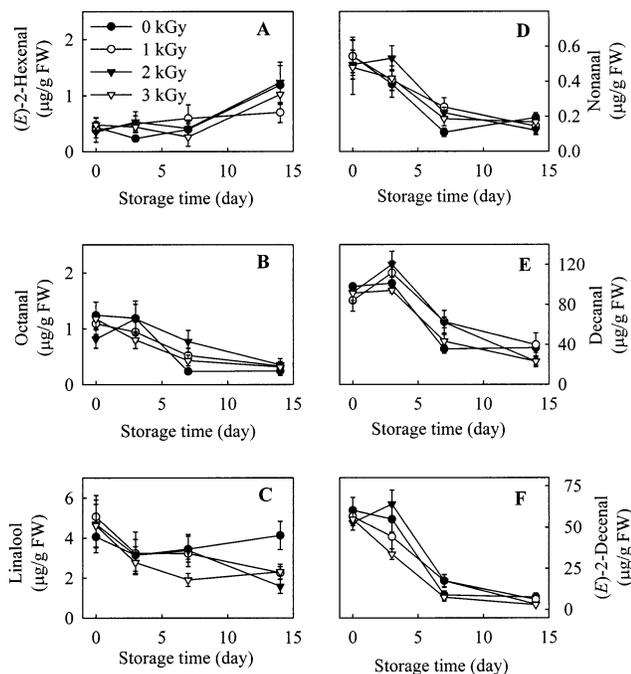


Figure 1. Changes in the contents of (*E*)-2-hexenal (A), octanal (B), linalool (C), nonanal (D), decanal (E), and (*E*)-2-decenal (F) in cilantro leaves during storage at 3 °C. Fresh cilantro leaves were irradiated with 0, 1, 2, and 3 kGy γ rays, and then stored at 3 °C up to 14 days. Volatile compounds were measured at days 0, 3, 7, and 14. Vertical bars represent standard errors of four replicates.

samples irradiated at 3 kGy had significantly ($P < 0.05$) lower linalool content at day 7. At day 14, samples irradiated at all doses had significantly lower linalool content than nonirradiated samples. Linalool is an acyclic monoterpene. We have found that acyclic terpenes are more sensitive to irradiation than cyclic terpenes in orange juice (20).

Linalool constitutes two-thirds of cilantro fruit oil volatiles and is regarded as one of the flavor-impact compounds for fruit essential oil (10, 11, 21). We found that linalool is a minor component of cilantro leaf volatiles. Potter and Fagerson (16) did not find any linalool in fresh cilantro leaves. Smallfield et al. (17), however, identified linalool as one of the cilantro leaf volatiles.

Nonanal content of all cilantro samples decreased during storage (Figure 1D) and irradiation had no effect on nonanal content except that cilantro irradiated at 1 kGy had more nonanal than nonirradiated samples at day 7.

Decanal content did not change much during the first 3 days of storage and then decreased rapidly (Figure 1E). Irradiation generally had no effect on decanal content except cilantro irradiated at 1 and 2 kGy had higher ($P < 0.05$) decanal content than nonirradiated samples at 7 days of storage.

(*E*)-2-Decenal content of all samples decreased rapidly during storage (Figure 1F). At the end of 14 days storage, (*E*)-2-decenal was less than 13% of original amounts. Irradiation had no effect on (*E*)-2-decenal content except samples irradiated at 3 kGy had lower (*E*)-2-decenal content than nonirradiated cilantro at day 14.

Undecanal content of both irradiated and nonirradiated samples had similar changes during storage, a maximum at day 3, decreased production thereafter (Figure 2A). Cilantro irradiated at 1 kGy had higher undecanal content than nonirradiated samples at day 7. Irradiation at other doses had no effect on undecanal content during the entire storage period.

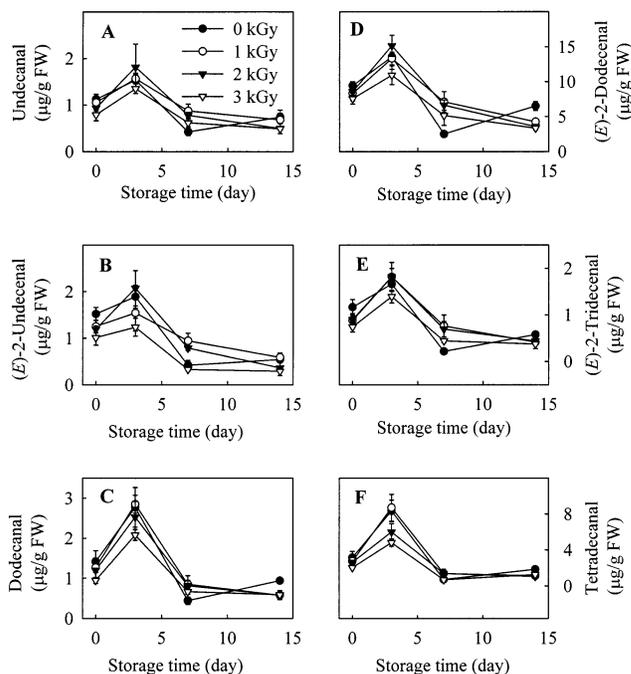


Figure 2. Changes in contents of undecanal (A), (*E*)-2-undecenal (B), dodecanal (C), (*E*)-2-dodecenal (D), (*E*)-2-tridecenal (E), and tetradecanal (F) in cilantro leaves during storage at 3 °C. Fresh cilantro leaves were irradiated with 0, 1, 2, and 3 kGy γ rays, and then stored at 3 °C up to 14 days. Volatile compounds were measured at days 0, 3, 7, and 14. Vertical bars represent standard errors of four replicates.

(*E*)-2-Undecenal content of all samples also had a maximum at day 3 followed by decreased production (Figure 2B). Cilantro irradiated at 3 kGy had lower (*E*)-2-undecenal content than controls measured immediately after irradiation. Samples irradiated at 1 and 2 kGy had higher content than controls at day 7.

Contents of dodecanal, (*E*)-2-dodecenal, (*E*)-2-tridecenal, and tetradecanal had similar changes during storage (Figure 2C–F). Maximum amounts were observed at day 3 and decreased afterward. Although control samples had lower (*E*)-2-dodecenal content on day 7 than samples irradiated at 1 kGy, all irradiated samples had lower dodecanal and (*E*)-2-dodecenal contents than controls at day 14. At day 0, samples irradiated at 3 kGy had lower (*E*)-2-tridecenal content than controls, whereas at day 7, samples irradiated at 1 and 2 kGy had higher (*E*)-2-tridecenal content than controls. At day 3, samples irradiated at 3 kGy had the lowest tetradecanal content, and at day 14, control samples had higher tetradecanal content than those irradiated at 2 kGy.

Neophytadiene was tentatively identified in cilantro by comparing spectra of sample compounds with those contained in the Wiley-NBS library. The identification could not be confirmed because of unavailability of the authentic compound. Neophytadiene is presumably a metabolite of chlorophyll, and has a grassy flavor (22). Although many other minor volatile compounds were not identified, most of the major peaks have been identified and quantified. We did not observe any significant effect by irradiation on these unidentified compounds.

We found that decanal and (*E*)-2-decenal were the most abundant compounds of fresh cilantro volatiles, which generally is in agreement with the finding of Smallfield et al. (17) and Potter and Fagerson (16). Many others have found very low or no (*E*)-2-decenal (14, 15). The difference in preparation and extraction of volatile compounds may account for different

profiles of volatile compounds in cilantro among our and previous studies. The difference between our results and others may also be due to variation between cilantro varieties. Substantial quantitative difference was observed between two commercial samples of cilantro (23).

Our results show that nonane is another major (4.25% of total) component of cilantro leaf volatile compounds. Potter and Fagerson (16) found a very low amount of nonane, whereas MacLeod and Islam (15) found no nonane in fresh cilantro leaves. Mookherjee and others (21) reported that the concentration of nonane in living leaves was 15% and decreased dramatically upon picking. The variation in our results and others on nonane may be due to differences in storage conditions, stage of growth, and storage/display time before analysis. Cilantro volatile compounds may exhibit significant variation in composition because of ontogenetic factors (21). In the present study, the cilantro at the vegetable stage was harvested directly from a farm and used in the experiment within 48 h. Nonane possesses a very characteristic fresh cilantro leaf odor (15). Our results showed that nonane content of cilantro did not change during storage at 3 °C, and irradiation at doses up to 3 kGy did not have an effect on nonane content.

The exact pathway(s) of volatile compound synthesis by fresh cilantro is unknown. The simplicity of volatile compounds of cilantro (most are straight-chained aldehydes) as well as the similar response to irradiation implies a common pathway. The presumed source of the aldehydes in the cilantro leaf volatile is peroxidation of unsaturated fatty acids (16, 24). Our results suggest, for several volatile compounds, there seems to be a peak at day 3. The initial increase in production of volatile compounds observed from day 0 to day 3 may reflect a stress response to the cold storage. Smallfield and others (17) found that relative levels of (*E*)-2-decenal and oil yield of chopped cilantro decreased during the 24-h storage at ambient temperature (19–38 °C). Our results show that amounts of most volatile compounds decreased from day 3 to day 14 during storage at 3 °C. Cold storage has been found to reduce production of volatile aldehydes by tomato fruit (18). Sensory evaluation also suggests a progressive decrease in aroma during cold storage of fresh cilantro (8, 25). The decrease in aldehydes during storage at 3 °C observed in the present study is likely due to reduced rates of enzymatic biogenesis of these compounds during the cold storage period.

Ionizing radiation generally decreased production of volatile compounds in fresh fruits and vegetables. A dose of 2.5 kGy reduced production of many volatile compounds by pear fruit (26). Our previous study on apple fruit suggested that irradiation inhibited production of ripening-related volatile compounds (27). An irradiation-induced decrease in volatile compounds has also been observed in orange fruit (28) and fresh mushroom (29). In general, irradiation only induced quantitative changes in some of the volatile compounds, and the effect of irradiation on production of volatile compounds depended on radiation dose. Production of volatile compounds recovered during postirradiation storage when a low radiation dose was applied. However, the quantities of some major volatile compounds were lower in ginger rhizomes irradiated at 0.05 kGy than in nonirradiated rhizomes after 3 months of storage, although irradiation did not have an impact on volatile compounds immediately after treatment (30).

We used saturated CaCl₂ to homogenize cilantro leaves because saturated CaCl₂ has been found to deactivate enzyme systems (18). Furthermore, addition of salts to samples also increases extraction efficiency by accelerating the release of

volatile compounds into headspace of vials, and therefore, into the SPME fiber (31).

Our quantification of volatile compounds in cilantro leaves is based on the standard curve established in saturated CaCl₂ because cilantro leaves were homogenized in saturated CaCl₂ before extraction. The quantification may be slightly different from actual amounts. Also, compounds may not be completely separated because of the short run time used in our GC temperature program. Nevertheless, our results did not reveal any consistent effect by irradiation on volatile compounds. Sensory evaluation conducted on the samples suggested irradiation at doses up to 3 kGy did not significantly affect the typical aroma of cilantro leaves (25), corresponding to our measurements of volatile compounds in the present study.

Irradiation at all doses reduced amounts of linalool, dodecanal, and (*E*)-2-dodecenal after 14 days of postirradiation storage. Major volatile compounds, such as decanal, were not affected by irradiation. Furthermore, amounts of total volatile compounds were not significantly ($P < 0.05$) affected by irradiation during the entire storage period, although the amount tended to be lower with increasing radiation dose. No new volatile compound was induced by irradiation.

In summary, fresh cilantro leaves can be irradiated at doses up to 3 kGy with minimal effect on the volatile composition. Production of volatiles was more affected by cold storage than by the irradiation treatments. Thus, irradiation can be used for pathogen inactivation without major loss of volatile compounds.

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