E-sensing, calibrated PSL, and improved ESR techniques discriminate irradiated fresh grapefruits and lemons

Yunhee Jo^{1,2} · Kashif Ameer³ · Namhyeok Chung¹ · Yoon-Han Kang⁴ · Dong U. Ahn⁵ · Joong-Ho Kwon¹

Revised: 24 July 2019 / Accepted: 29 August 2019 / Published online: 5 September 2019
© Association of Food Scientists & Technologists (India) 2019

Abstract Grapefruit and lemon were exposed to phytosanitary irradiation doses of 0, 0.4, and 1 kGy, and then electronic-sensing screening of irradiation status and identification of radiation-induced ESR markers were conducted during 20 days storage. Codex standard photo-stimulated luminescence measurement (PSL₁) was not a reliable indicator of irradiation status. Electronic tongue and electronic nose, however, showed potential as screening tools for discriminating irradiated fruits from non-irradiated counterparts based on principal component analysis of taste attributes and flavor patterns. Calibrated PSL approach clearly distinguished irradiated from non-irradiated samples based on the PSL ratio (PSL₂/PSL₁). Verification of irradiation status by electron spin resonance spectroscopy revealed clear paramagnetic centers from both irradiated fruits samples and ethanol-vacuum drying pretreatment improved radiation-induced ESR signal detection; not prominent enough in 0.4 kGy-irradiated commodities during 20 days of storage. Peel parts of both

fruits showed high limonene which proportionally increased with irradiation doses ($p < 0.05$ kGy).

Keywords Citrus · Phytosanitary irradiation · Detection · Electronic sensing · Volatiles

Introduction

Citrus, also described as *agrumes* because of its sour attribute, includes major fruit crops that are widely popular because of their worldwide availability and contribution to diets intended for human consumption. Fruits in the citrus family are grown worldwide and include numerous natural species; however, the most common citrus fruits are oranges, lemons, limes, grapefruit, and tangerines. Massive growth has been recorded with respect to the annual global production of citrus fruits over the past several decades, with nearly 30 million metric tons produced in the 1960s and over 105 million metric tons in the years 2000–2004 (Liu et al. 2012). The nutritional significance of citrus fruits is well-documented, as they contain many health-beneficial nutrients such as dietary fiber, pectin, vitamin C, flavonoids, coumarins, and carotenoids. Citrus fruits are consumed as fresh commodities or are processed for juice extraction. Among citrus fruits, grapefruit and lemon are considered very important in the food, cosmetics, and flavor industries (Yoo et al. 2004). Thus, consumption of citrus fruits on a daily basis may be advantageous in terms of their nutraceutical and medicinal benefits.

Food irradiation is an important postharvest technology that is used commercially to enhance the shelf-life of agricultural products, particularly fruits and vegetables with high marketability (Farkas and Mohácsi-Farkas 2011). Moreover, the nutritional adequacy of irradiated

✉ Joong-Ho Kwon
jhkwon@knu.ac.kr

¹ School of Food Science and Biotechnology, Kyungpook National University, Daegu 41566, South Korea

² Health Research Department, Health and Environment Institute of Daegu, Daegu 42183, South Korea

³ Institute of Food and Nutritional Sciences, PMAS-Arid Agriculture University, Rawalpindi 46300, Pakistan

⁴ Department of Food Processing and Distribution, Gangneung-Wonju National University, Wonju-si, Gangwon-do 26403, South Korea

⁵ Department of Animal Science, Iowa State University, Ames, IA 50011-3150, USA

foods is well-documented and major health authorities have endorsed the safety of this technology (WHO 1981). To regulate the global trade of irradiated foodstuffs, it is necessary to ensure valid detection so that consumers may be protected from misinformation because of technological misuse. Standard detection methods have been developed by the European Committee of Standardization (CEN). Irradiated foodstuffs are detected according to guidelines of European Standards, which include physical methods, such as photostimulated luminescence (PSL) and thermoluminescence (TL) (EN 1788 2001; EN 13751 2009). Moreover, electron spin resonance (ESR) spectroscopy is used widely to validate the irradiation status of various foodstuffs by measuring cellulose, crystalline sugar, and bone tissue hydroxyapatite matrix (Ahn et al. 2012; EN 13708 2001). Exposure to ionizing radiation may affect the odor and taste profiles of foodstuffs depending upon the applied dose, and electronic sensing (E-sensing) techniques (electronic tongue: E-tongue and electronic nose: E-nose) show potentials as rapid screening tools by comparing odor and taste attributes at different applied doses. Hence, inexpensive, simple, reliable, and rapid detection methods are needed for differentiating irradiated foods from their non-irradiated counterparts (Khan et al. 2002).

This study was conducted to evaluate the potential of calibrated PSL with electron beam (E-beam) to screen irradiated grapefruit and lemon (0, 0.4, and 1 kGy) during storage (20 days). A new E-sensing approach involving E-tongue and E-nose was employed to differentiate between irradiated and non-irradiated samples by principal component analysis (PCA). ESR spectroscopic analysis was utilized to ensure the reliability of the calibrated PSL and E-sensing screening. Moreover, volatile flavor profiles of irradiated grapefruit and lemon were evaluated soon after irradiation treatment.

Materials and methods

Sample preparation and irradiation

Two types of citrus fruits, grapefruits (*Citrus paradise*) and lemons (*Citrus limon*) imported from the USA, were purchased from a local market in Daegu, Korea. Three cardboard box packages (36 × 24 × 12 cm, 5 kg) for each citrus fruit were procured and stored at a temperature of 4 °C. The fruits were subjected to e-beam irradiation treatment at doses of 0, 0.4, and 1 kGy with an applied dose rate of 0.6 kGy/h using a high-energy linear accelerator (ELV-4, 10 MeV, 10 kW, EB Tech., Daejeon, Korea). Dosimetry for absorbed doses was performed using alanine dosimeter pellets with 5 mm diameters (Bruker Instruments, Billerica, MA, USA) placed on the top and

bottom of the boxes. A Bruker EMS 104 EPR analyzer (Bruker Instruments) was employed to analyze free radicals. After irradiation, the citrus fruits were stored at 4 ± 2 °C for 20 days.

PSL measurement

The fruit peels were separated and cut into small pieces (0.5 × 0.5 cm) using a sterile peeling knife. PSL measurement were performed following the standard method of EN 13751 by employing the SURCC PPSL screening system (serial 0021, SURRC, Scottish Universities Research and Reactor Centre, Glasgow, UK). Disposable petri dishes (50 mm in diameter, Bibby sterlin type 122, Glasgow, UK) were utilized for the placement of each sample (≤ 5 g). During screening, the signals emitted from the samples were automatically accumulated and measurements were recorded as photon counts (PCs)/min. The guidelines of the EN 13751 protocol were used for interpretation of the results using a lower threshold level (T_1 : 700 PCs/min) and upper threshold level (T_2 : 5000 PCs/min) (EN 13751 2009). A normalization step was followed according to the European standard to decrease the scattering of luminescence results, and then re-irradiation at a normalization dose of 1 kGy was performed for both irradiated and non-irradiated samples. The scattering of signals between these two levels were classified as intermediate, which necessitated further investigation of the samples under consideration. All experiments were carried out under subdued light conditions.

E-tongue analysis

Ten fruits were peeled and juice was extracted by blending in a blender. The homogenate was filtered through a filter paper (Whatman filter paper No. 4) for E-tongue and E-nose analyses. The samples were analyzed with an ASTREE Electronic Tongue system (Alpha M.O.S., Toulouse, France). Six electrochemical sensors, which are part of the E-tongue configuration, were utilized for screening and an Ag/AgCl electrode was used as the reference. Each sensor was associated with the following specific taste attributes: SRS (sourness, astringency, bitterness), GPS (sourness, saltiness, metallic), STS (saltiness, spiciness, metallic), SPS (metallic, spiciness, umami), SWS (sweetness, sourness), and BRS (bitterness, astringency). For analytical purposes, 25 mL of each sample was used to measure signal intensity for 120 s, and the raw data were expressed as a function of voltage versus time. All sample were analyzed in five replications and cross-contamination was avoided by rinsing the electrochemical sensors after each analytical run. Principal component analysis (PCA)

was used to identify trends in the numerical data and individual patterns of both grapefruit and lemon samples.

E-nose analysis

Volatile compounds were analyzed with a HERACLES E-nose system (Alpha M.O.S.). The E-nose system was equipped with a sampling system, GC instrument that is consisted of two short columns of different polarities (DB5 and DB1701), and two flame ionization detectors. Each sample (2 mL) was injected into the headspace vial followed by capping with polytetrafluoroethylene/silicone septum (Supelco, Bellefonte, PA, USA). Vials were incubated for 10 min at 40 °C with agitation at 500 rpm. Accumulated gas was pumped into the headspace of GC functionality. As a carrier gas, H₂ was circulated in constant flow mode with a 1 mL/min of circulation rate and injector temperature of 200 °C. The oven temperature was varied as follows: 40 °C for 5 s; 4 °C/s ramp to 270 °C; and a holding period for 30 s. Five replicates of each sample were evaluated and trends in the numerical data for odor profiles were analyzed by PCA.

ESR spectroscopy

Prior to ESR measurements, the peel of each fruit was subjected to two different ESR pretreatments: (1) VD: vacuum drying (equipment) of all samples (Sholom and McKeever 2016), (2) EVD: ethanolic extraction of samples by mixing 30 g of each sample with 100 mL of ethanol followed by VD to reduce moisture content and improve ESR signals as described by Delincée and Soika (2002). Samples were pulverized and approximately 0.1 g of each pulverized sample was added to a quartz ESR tube with a 5-mm diameter. Plastic film was used to seal the open end of the tube and then the tube was stored in the dark in a desiccator at 40 ± 5% relative humidity. ESR signal measurement was performed according to the guidelines of the European standard of EN 1787. The ESR signals were measured with an X-band ESR spectrometer (JES-TE 300; Jeol Co., Tokyo, Japan) at ambient temperature. Measurements were recorded in triplicate (n = 3) under the following conditions: microwave power, 0.4 mW; magnetic center field, 324 ± 2 mT; modulation width, 0.79 mT; and amplitude of 790.

Analysis of volatile compounds

The peel and pulp parts were separated from each fruit and then subjected to freeze-drying in a freeze-drier (MFCD8515, IIShinBioBase, Gyeonggi-do, Korea). The dried samples were ground to a fine powder using a mortar and pestle followed by packing in polyethylene bags.

Volatile compounds of each sample were analyzed using a dynamic headspace-GC/MS unit (Model 6890/5973, Hewlett Packard Co., Wilmington, DE, USA) to quantify volatile compounds as described by Ahn et al. (2000). Approximately 3 g of samples was added to 40-mL vials followed by flushing of the headspace with helium (He) gas (99.99% purity). Flushing was performed for 5 s to minimize oxidative changes during the waiting time prior to analysis. Sample purging with He gas (40 mL/min) was carried out at 40 °C for 14 min. A silica/charcoal column (Tekmar–Dohrmann, Mason, OH, USA) was used to trap volatile compounds for desorption into the GC column at 220 °C for 1 min. Separation of volatile compounds was improved by using a modified column. Volatile compounds were injected into the column by employing a split inlet (175 °C inlet temperature with split ratio of 49:1). During ramping, the oven temperatures were varied as follows: 7 °C for 2.5 min, increased to 25 °C at a rate of 3 °C/min, increased to 120 °C at a rate of 10 °C/min, and finally increased to 200 °C at a rate of 20 °C/min. Liquid nitrogen was used to cool the oven to ambient temperature. Selective mass detectors were set to an ionization potential of 70 eV; carbon dioxide peaks were eliminated by scanning the mass range from 46.1 to 550 at a scan velocity of 2.94 scans/s. Volatile compounds were identified by comparing the mass spectra with those of the library. The amount of each volatile compound released is reported in terms of peak area.

Statistical analysis

Statistical analysis system (SAS) software (ver. 8.1) (SAS Institute Inc., Cary, NC, USA) was employed for data analysis. The results were expressed as the mean ± standard deviation. Differences between means were analyzed by one-way analysis of variance and their significance was evaluated by Duncan's multiple range test. Differences were considered significant at $p < 0.05$.

Results and discussion

Screening characteristics of PSL analysis

Food stuffs contain several intrinsic inorganic particles, also known as contaminating dust, which absorb energy; this absorbed energy is optically stimulated and can be used to determine the luminescence characteristics of irradiated materials. These radiation-induced luminescence characteristics may be used to screen and classify irradiated foodstuffs (Jo et al. 2015). In accordance with the European Standard of CEN, the PSL method can be employed for screening of irradiated foodstuffs. Generally, no prior

sample pretreatment is required for the PSL technique and this method may be used for large-scale screening (Akram et al. 2012a). The results of PSL screening for both grapefruit and lemon are shown in Table 1. The PCs of the first PSL measurement (PSL₁) showed increasing tendency in gradual and dose-dependent manner, whereas all irradiated samples showed negative results (< 700 PCs) according to EN 13751 (2009) guidelines. The sensitivity of the PSL technique for specific samples relies on the quantity and types of minerals present in foodstuffs. Generally, signals below the lower threshold level (< 700 PCs) indicate unirradiated samples or may be associated with the low-sensitivity of the irradiated food materials. Therefore, PSL₁ measurement of an unknown sample is not a valid indicator of irradiation status.

Screening characteristics of E-tongue and E-nose analyses

E-tongue taste attributes were determined for both grapefruit and lemon samples from 0 to 20 days of storage; the results are shown in Fig. 1a, b. Numerical data trends were determined by PCA for both for irradiated and non-irradiated samples of grapefruit (Fig. 1a) and lemon (Fig. 1b). For grapefruits, both principal components of PC1 and PC2 contributed to the variance at 0 day of the storage period with individual contribution rates of 95.54% and 3.77%, respectively, indicating that the scattering of distinct groups (0, 0.4, and 1 kGy) was accounted for by the major

proportion by PC1 alone. On day 20 of the storage period, irradiated grapefruit samples were clearly discriminated from the control (0 kGy), although the 0.4 kGy sample showed similar results as the control (0 kGy) in the PCA score plot (Fig. 1a). A similar trend was observed in lemon, and clear discrimination of irradiated samples (0.4 kGy) from the control was possible on day 0 and even after 20 days of storage, although PC1 showed a decreasing trend in the variance contribution on day 20 (86.71%) as compared to that on day 0 (93.95%) (Fig. 1b). The discrimination ability of E-tongue based on the objective analysis of taste profiles accompanied by PCA has been reported for rice (Chung et al. 2017) and aged vinegars (Jo et al. 2016).

The results of E-nose analysis on days 0 and 20 are presented for both grapefruit and lemon in Fig. 1c, d. For grapefruit samples on day 0, all groups (0, 0.4, and 1 kGy) were distinctly recognized in the PCA score plot. However, the control (0 kGy) and 0.4 kGy were very close to each other, indicating that the low dose irradiation (0.4 kGy) was not sufficient to distinguish irradiated samples from the control, while 1 kGy was located further away and could be clearly recognized from the control and 0.4 kGy. On day 20 of storage, all distinct groups were at different positions on the PCA score plot and irradiated samples were clearly discriminated from the control (Fig. 1c). For lemon, the contribution of PC1 and PC2 to total variance showed a decreasing trend with an increasing storage period from 98.73% (day 0) to 95.82% (day 20). However,

Table 1 Changes in PSL intensities (PCs/min) and PSL ratios (PSL₂/PSL₁) of E-beam irradiated grapefruits and lemons during storage at 4 °C

Sample	Irradiation dose (kGy)	Storage period (day)		
		0	10	20
<i>PSL intensities¹</i>				
Grapefruit	0	190 ± 108 ^{Ba} (N)	271 ± 59 ^{Ba} (N)	228 ± 50 ^{Ba} (N)
	0.4	325 ± 77 ^{ABa} (N)	352 ± 13 ^{Aa} (N)	376 ± 74 ^{Aa} (N)
	1	397 ± 105 ^{Aa} (N)	322 ± 66 ^{ABa} (N)	378 ± 108 ^{Aa} (N)
Lemon	0	346 ± 38 ^{Aa} (N)	296 ± 87 ^{Ba} (N)	304 ± 54 ^{Ba} (N)
	0.4	347 ± 88 ^{Aa} (N)	328 ± 44 ^{Ba} (N)	318 ± 46 ^{Ba} (N)
	1	397 ± 157 ^{Aa} (N)	479 ± 87 ^{Aa} (N)	417 ± 88 ^{Aa} (N)
<i>PSL ratios²</i>				
Grapefruit	0	24.54 ± 8.24 (N)	18.04 ± 6.87 (N)	17.88 ± 2.98 (N)
	0.4	9.53 ± 4.20 (P)	8.85 ± 2.04 (P)	7.32 ± 1.72 (P)
	1	6.68 ± 2.59 (P)	6.65 ± 1.30 (P)	8.58 ± 2.52 (P)
Lemon	0	11.35 ± 2.07 (N)	23.47 ± 10.75 (N)	13.22 ± 1.40 (N)
	0.4	6.57 ± 1.44 (P)	12.61 ± 3.42 (N)	17.21 ± 3.41 (N)
	1	8.82 ± 0.45 (P)	7.33 ± 2.21 (P)	18.56 ± 6.69 (N)

Values followed by different uppercase letters within a column and by different lowercase letters within a row are significantly different ($p < 0.05$) based on Duncan's multiple range test

¹PCs < 700: non-irradiated (N); 700 < PCs < 5000: intermediate (M); PCs > 5000: irradiated (P)

²Ratio > 10: non-irradiated (N); ratio < 10: irradiated (P)

(A) E-tongue / Grapefruit **(B) E-tongue / Lemon** **(C) E-nose / Grapefruit** **(D) E-nose / Lemon**

Fig. 1 Changes in E-tongue taste attributes from PCA of E-beam irradiated grapefruits (a) and lemons (b) and E-tongue volatile flavor patterns from PCA of irradiated grapefruits (c) and lemons (d) using E-nose during storage at 4 °C

all irradiated samples could be clearly distinguished from non-irradiated samples at day 0 and 20. The low-dose sample (0.4 kGy) was positioned close to the control on day 0, but was clearly distinguished from the control on day 20. It was possible to screen irradiated lemon samples from non-irradiated counterparts even after prolonged storage of 20 days (Fig. 1d). The potential application of E-nose as a screening tool for discriminating irradiated samples such as, anchovy sauce (Kim et al. 2004), spices (Sanyal et al. 2014), tomatoes (Winqvist et al. 1995) and mushrooms (Akram et al. 2012b) has also been reported based on the changes in volatile profiles.

Calibrated PSL screening characteristics

The PSL technique involves an initial intensity measurement for screening purposes and a calibration method for classifying irradiation status. The calibrated PSL ratio (PSL_2/PSL_1) was used to enhance the reliability of the screening results because of insufficient PSL sensitivity. Samples exhibiting PSL ratios of more than 10 were identified as non-irradiated, whereas samples with PSL ratios < 10 were categorized as irradiated. Calibrated PSL ratios of citrus fruits are shown in Table 1. Regardless of the storage period, control samples (0 kGy) of both grapefruit and lemon showed PSL ratios of more than 10, and thus their non-irradiated status was confirmed. In contrast, 0.4 and 1 kGy irradiated samples of grapefruit were identified as irradiated by their PSL ratios of < 10. Similarly, calibrated PSL characteristics were observed during storage for irradiated oranges. The samples irradiated at 2 kGy showed calibrated PSL ratios of < 10 until a storage period of 3 weeks under dark conditions and hence the calibrated PSL approach confirmed the irradiation status of e-beam-irradiated oranges (Jo et al. 2015). However, e-beam irradiated lemon samples after 10 and 20 days of storage showed PSL_1 less than 700 PCs and PSL ratios of more than 10, necessitating further confirmation of the irradiation status by detecting radiation-induced markers. These results agree with the findings reported by Kim et al. (2012) who reported intermediate PSL results for irradiated wheat samples and further confirmed the irradiation status by the TL technique. The (EN 13751 2009) protocol specifies the necessity to confirm the positive PSL screening result using another validated or standardized method, such as TL and ESR spectroscopy.

Verification of screening results by ESR spectroscopy

ESR spectroscopy is an effective method for characterizing irradiated foodstuffs by detecting radiation-induced markers for reliable identification. Plant materials containing cellulose may result in better findings regarding ESR analysis based on their irradiation history (Ahn et al. 2014). The results of ESR spectral features for both grapefruit and lemon are shown in Figs. 2 and 3, respectively. On day 0, non-irradiated grapefruit and lemon samples showed nonspecific ESR central signals, as shown in Figs. 2a and 3a. Side peak spacing of 6.00 ± 0.05 mT appeared in irradiated samples, demonstrating that cellulose radicals were radiation-specific markers in irradiated grapefruit and lemon samples (Figs. 2a, 3a). On day 0 in grapefruit, ethanol-vacuum drying (EVD) improved the signal intensity as compared to samples treated with vacuum drying (VD) only. Clear side peaks of cellulose radicals were visible in 0.4- and 1 kGy-irradiated EVD samples and EVD showed better results in terms of signal quality than the VD (Fig. 2a). After 20 days of storage, no radiation-specific marker was observed in either 0.4 kGy VD or EVD samples, while EVD samples showed clear cellulose signals from 1 kGy irradiated samples (Fig. 2b). Similarly, on 0 day of storage, the samples with low-dose irradiation (0.4 kGy) gave rise to only one side peak along with the central signal in VD lemon samples, whereas EVD samples showed two side peaks along with the central signal (Fig. 3a). On day 20, no specific radiation-induced cellulose signal was observed in 0.4 kGy-irradiated VD and EVD samples compared to the control (0 kGy), while EVD samples treated with 1 kGy showed clear side peaks on both sides of the central signals. VD samples showed only one clear signal along with an ambiguous peak. These results indicated that EVD pretreatment improved signal intensity and visibility compared to VD (Fig. 3b). EVD pretreatment led to more peak broadening of central signal in comparison with VD. Overall the ESR signals (Figs. 2, 3) in 0.4 kGy-irradiated stored commodities were not seemed to be prominent enough. Since, the legal dose limit for quarantine treatment is 0.4 kGy, the validity of this technique is also questionable and necessitates further inquiry about irradiation status. In a similar study, Ahn et al. (2012) reported that alcoholic extraction led to improved and clear visibility of a radiation-induced marker (cellulose) in fresh red ginseng samples with clear side peaks along with the central signal. In another study, ESR signals showed improvement with enhanced signal intensity for distinct identification of irradiated spice samples based on the emergence of clear cellulose signals. Alcoholic extraction pretreatment enhanced the paramagnetic radicals with unambiguous identification of irradiation status of spice samples (Ahn et al. 2014).

Fig. 2 Changes in ESR signals of E-beam irradiated grapefruits during storage at 4 °C (VD vacuum-drying, EVD ethanol extraction followed by vacuum drying, **Clear cellulose signal, *Ambiguous signal)

Changes in volatile compounds of irradiated grapefruit and lemon

Citrus fruits contain a wide range of volatile and aromatic compounds; particularly, the peel of lemon has been reported to consist of 62 unique aromatic compounds. In both grapefruit and lemon, limonene, myrcene, β -pinene, and γ -terpinene have been reported as the major volatile compounds (Lota et al. 2002). Both the peel and pulp of grapefruits and lemon were analyzed to assess changes in volatile compounds on day 0, as shown in Tables 2 and 3, respectively. Approximately 20 volatile compounds were

detected from the peel and pulp of grapefruit. Among all compounds, grapefruit peel exhibited ethanol, beta-pinene, myrcene, alpha-terpinene, limonene, and beta-terpinene as the major volatiles. Limonene was detected in significantly higher proportions in grapefruit peel from control samples (0 kGy), and an increasing tendency was observed in dose-dependent manner in irradiated samples. In contrast, a decreasing tendency was observed in limonene content from grapefruit pulp in irradiated samples compared to the control. Myrcene and 2,3,5-trimethyl-decane also showed increasing trends as the irradiation dose increased or compared with the control. While myrcene was not

Fig. 3 Changes in ESR signals of E-beam irradiated lemons during storage at 4 °C (VD vacuum-drying, EVD ethanol extraction followed by vacuum drying, **Clear cellulose signal, *Ambiguous signal)

detected in the pulp of irradiated grapefruit, 2,3,5-trimethyl-decane decreased in a dose-dependent manner. In the case of minor compounds, gamma-terpinene and alpha-terpinolene increased in irradiated peels, but remained undetected in pulp samples. Although E-nose results revealed that 0.4 kGy-irradiated samples on day 0 were distinctly different from the control samples, both groups (0 and 0.4 kGy) were located close to each other on the PCA score plot, suggesting similar volatile patterns for both samples. This tendency was confirmed by volatile compounds analysis of grapefruit pulp on day 0. Hexanal, 1-phellandrene, and camphene were not detected in either 0 or 0.4 kGy samples. Similarly, beta-pinene 2,2,8-trimethyl-

decane, 2,3,5-trimethyl-decane, limonene, 6-ethyl-2-methyl-octane, and gamma-terpinene showed no significant difference between the samples irradiated at 0.4 kGy and the control (0 kGy).

In lemon, 23 volatile compounds were detected, with acetaldehyde, methanol, ethanol, alpha-pinene, camphene, beta-pinene, myrcene, limonene, beta-terpinene, 1-methyl-2-(1-methylethyl) benzene, gamma-terpinene, and alpha-terpinolene as the major ones among all volatile compounds. Acetaldehyde was not detected in irradiated lemon peel, but was increased in the pulp compared to the control. Increasing trends were also observed for ethanol in both irradiated peel and pulp. Irradiation treatment increased the

Table 2 Changes in volatile compounds (unit: total ion counts $\times 10^4$) of E-beam irradiated grapefruits

Volatile compounds	Grapefruit peel			Grapefruit pulp		
	0 kGy	0.4 kGy	1 kGy	0 kGy	0.4 kGy	1 kGy
Methanol	2885 $\pm$ 336 ^c	3799 $\pm$ 228 ^b	5258 $\pm$ 296 ^a	ND	ND	ND
Ethanol	45,808 $\pm$ 2701 ^c	26,866 $\pm$ 3336 ^d	28,246 $\pm$ 1417 ^d	64,572 $\pm$ 5053 ^b	80,768 $\pm$ 4869 ^a	84,499 $\pm$ 2151 ^a
Hexanal	395 $\pm$ 41	460 $\pm$ 79	353 $\pm$ 74	ND	ND	ND
1-Phellandrene	191 $\pm$ 6	155 $\pm$ 18	138 $\pm$ 37	ND	ND	ND
alpha-Pinene	559 $\pm$ 122	539 $\pm$ 86	492 $\pm$ 106	179 $\pm$ 17	ND	ND
Camphene	163 $\pm$ 26	186 $\pm$ 37	133 $\pm$ 35	ND	ND	ND
beta-Pinene	903 $\pm$ 117	1144 $\pm$ 269	1159 $\pm$ 258	473 $\pm$ 50	265 $\pm$ 19	117 $\pm$ 8
Myrcene	1755 $\pm$ 237	2283 $\pm$ 44	2000 $\pm$ 309	134 $\pm$ 20	ND	ND
2,2,8-Trimethyl-decane	244 $\pm$ 16	280 $\pm$ 40	312 $\pm$ 14	297 $\pm$ 33	174 $\pm$ 40	ND
2,2,4,6,6-Pentamethyl-heptane	94 $\pm$ 7	88 $\pm$ 13	128 $\pm$ 16	113 $\pm$ 29	ND	ND
1-Phellandrene	321 $\pm$ 104	353 $\pm$ 103	309 $\pm$ 54	ND	ND	ND
2,3,5-Trimethyl-decane	802 $\pm$ 62	961 $\pm$ 124	924 $\pm$ 29	876 $\pm$ 61	473 $\pm$ 92	160 $\pm$ 31
alpha-Terpinene	701 $\pm$ 220	770 $\pm$ 141	673 $\pm$ 86	106 $\pm$ 9	ND	ND
Limonene	110,382 $\pm$ 12,605 ^a	116,380 $\pm$ 5373 ^a	118,206 $\pm$ 11,848 ^a	16,479 $\pm$ 1570 ^b	12,423 $\pm$ 1821 ^b	6142 $\pm$ 378 ^b
beta-Terpinene	1665 $\pm$ 197	2086 $\pm$ 85	1787 $\pm$ 205	1623 $\pm$ 64	631 $\pm$ 88	ND
1-Methyl-2-(1-methylethyl) benzene	745 $\pm$ 244	791 $\pm$ 55	719 $\pm$ 34	ND	ND	ND
trans-beta-Ocimene	202 $\pm$ 63	256 $\pm$ 35	237 $\pm$ 31	ND	ND	ND
6-Ethyl-2-methyl-octane	269 $\pm$ 9	314 $\pm$ 88	334 $\pm$ 8	412 $\pm$ 68	215 $\pm$ 11	ND
gamma-Terpinene	1582 $\pm$ 324	1613 $\pm$ 245	1601 $\pm$ 123	465 $\pm$ 75	310 $\pm$ 84	ND
alpha-Terpinolene	509 $\pm$ 96	610 $\pm$ 71	552 $\pm$ 45	ND	ND	ND

Values followed by different letters within a row are significantly different ($p < 0.05$) based on Duncan's multiple range test

content of alpha-pinene in lemon peel, while opposite effects were observed in pulp. Camphene remained stable in irradiated lemon peel and decreased in a dose-dependent manner in the pulp. Irradiated lemon peel showed enhanced beta-pinene content compared to the control, while a decreasing tendency was observed in lemon pulp. Slightly increasing trends were observed in myrcene content in case of both peel and pulp upon exposure to irradiation. Limonene as a major volatile compound significantly increased in irradiated peel and pulp compared to the control (0 kGy). A similar tendency was observed for beta-terpinene, while 1-methyl-2-(1-methylethyl) benzene in lemon pulp increased significantly upon exposure to irradiation. Significantly increased concentrations of gamma-terpinene were detected in irradiated lemon peel samples compared to the control and pulp also showed a similar tendency. Fan and Mattheis (2001) treated apple fruits (*Malus $\times$ domestica* Borkh., cv. Gala) and analyzed the effects of gamma irradiation on volatile compounds and found that gamma-irradiation reduced the production of volatile compounds in apple fruits in a dose-

dependent manner. Methyl or propyl esters were the most significantly inhibited volatile compounds, whereas a gamma irradiation at 0.44 kGy resulted in enhanced production of most esters during 21 days of storage at 20 °C. Fruits exposed to irradiation doses > 0.44 kGy lost their ability to synthesize volatile flavor compounds, and thus high-dose gamma irradiation may lead to very low production of aromatic volatile compounds.

Conclusion

Electronic sensing techniques, such as electronic tongue and electronic nose, clearly screened the irradiated samples from the control based on the PCA of taste attributes and volatile patterns. The calibrated PSL approach clearly distinguished irradiated grapefruit and lemon samples from non-irradiated samples based on the PSL ratio (PSL₂/PSL₁); irradiated samples showed PSL ratios of less than 10, while samples with PSL ratios of more than 10 were identified as non-irradiated. Overall, the PSL screening

Table 3 Changes in volatile compounds (unit: total ion counts $\times 10^4$) of E-beam irradiated lemons

Volatile compounds	Lemon peel			Lemon pulp		
	0 kGy	0.4 kGy	1 kGy	0 kGy	0.4 kGy	1 kGy
Acetaldehyde	863 $\pm$ 387	ND	ND	659 $\pm$ 13	649 $\pm$ 61	1451 $\pm$ 397
Methanol	2359 $\pm$ 90	5044 $\pm$ 581	5167 $\pm$ 464	ND	ND	ND
Ethanol	55,538 $\pm$ 3724 ^d	98,152 $\pm$ 3580 ^b	96,837 $\pm$ 6529 ^b	60,235 $\pm$ 1959 ^d	73,532 $\pm$ 1390 ^c	125,246 $\pm$ 4127 ^a
Hexanal	702 $\pm$ 88	598 $\pm$ 83	609 $\pm$ 38	809 $\pm$ 161	776 $\pm$ 40	647 $\pm$ 120
1-Phellandrene	1629 $\pm$ 241	2557 $\pm$ 169	2670 $\pm$ 367	90 $\pm$ 8	91 $\pm$ 8	54 $\pm$ 2
alpha-Pinene	4858 $\pm$ 478	6761 $\pm$ 367	7939 $\pm$ 661	568 $\pm$ 49	478 $\pm$ 68	431 $\pm$ 12
2,2,5,5-Tetramethyl hexane	199 $\pm$ 59	247 $\pm$ 67	271 $\pm$ 10	ND	ND	ND
(E)-2-hexanal	366 $\pm$ 34	328 $\pm$ 21	285 $\pm$ 53	ND	ND	ND
alpha-Fenchene	495 $\pm$ 90	592 $\pm$ 66	570 $\pm$ 61	82 $\pm$ 8	81 $\pm$ 0	51 $\pm$ 12
Camphene	3808 $\pm$ 641	3847 $\pm$ 903	3704 $\pm$ 617	385 $\pm$ 74	314 $\pm$ 98	211 $\pm$ 46
beta-Pinene	40,014 $\pm$ 1702 ^b	41,342 $\pm$ 1512 ^b	54,105 $\pm$ 2462 ^a	1382 $\pm$ 90 ^c	1498 $\pm$ 120 ^c	1285 $\pm$ 113 ^c
Myrcene	6416 $\pm$ 800	7200 $\pm$ 603	6969 $\pm$ 127	336 $\pm$ 71	339 $\pm$ 47	500 $\pm$ 13
2,2,8-Trimethyl-decane	287 $\pm$ 82	272 $\pm$ 41	290 $\pm$ 21	ND	ND	ND
1-Phellandrene	2057 $\pm$ 237	2371 $\pm$ 278	2172 $\pm$ 216	470 $\pm$ 50	461 $\pm$ 86	555 $\pm$ 39
2,3,5-Trimethyl-decane	1029 $\pm$ 82	986 $\pm$ 69	955 $\pm$ 67	1361 $\pm$ 164	1315 $\pm$ 82	2398 $\pm$ 225
alpha-Terpinene	8630 $\pm$ 388	8428 $\pm$ 240	7916 $\pm$ 801	811 $\pm$ 127	821 $\pm$ 105	1446 $\pm$ 203
Limonene	150,328 $\pm$ 5532 ^b	178,605 $\pm$ 6821 ^a	172,037 $\pm$ 9289 ^a	15,112 $\pm$ 1584 ^c	15,713 $\pm$ 1288 ^c	20,932 $\pm$ 1092 ^c
beta-Terpinene	5373 $\pm$ 328	5278 $\pm$ 294	5918 $\pm$ 226	3276 $\pm$ 801	3292 $\pm$ 570	5396 $\pm$ 510
1-Methyl-2-(1-methylethyl) benzene	7659 $\pm$ 809	7482 $\pm$ 407	6959 $\pm$ 909	2993 $\pm$ 190	2474 $\pm$ 214	4836 $\pm$ 819
trans-beta-Ocimene	3149 $\pm$ 525	3159 $\pm$ 255	2421 $\pm$ 379	ND	ND	180 $\pm$ 15
6-Ethyl-2-methyl-octane	475 $\pm$ 98	479 $\pm$ 72	442 $\pm$ 34	ND	ND	178 $\pm$ 35
gamma-Terpinene	35,539 $\pm$ 1705 ^b	45,668 $\pm$ 3714 ^a	45,119 $\pm$ 3088 ^a	2593 $\pm$ 557 ^c	2973 $\pm$ 599 ^c	4860 $\pm$ 665 ^c
alpha-Terpinolene	6431 $\pm$ 336	7471 $\pm$ 629	7056 $\pm$ 824	350 $\pm$ 102	360 $\pm$ 112	702 $\pm$ 104

Values followed by different letters within a row are significantly different ($p < 0.05$) based on Duncan's multiple range test

results were not a reliable indicator of detection. Further verification of irradiation status by electron spin resonance spectroscopy using ethanol-vacuum drying pretreatment resulted in reliable and improved detection of irradiated grapefruit and lemon samples with clear paramagnetic centers of radiation-induced markers compared to the conventional vacuum drying protocol. Overall the ESR signals in 0.4 kGy-irradiated stored commodities were not seemed to be prominent enough. Since, the legal dose limit for quarantine treatment is 0.4 kGy, the validity of this technique is also questionable and necessitates further inquiry about irradiation status Through quantitative analysis of volatile profiles of irradiated grapefruit and lemon, limonene was detected as the most prominent compound, which showed proportional increases as the irradiation dose increased.

Acknowledgements This research was supported by Kyungpook National University Research Fund, 2016.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Ahn DU, Jo C, Du M, Olson DG, Nam KC (2000) Quality characteristics of pork patties irradiated and stored in different packaging and storage conditions. *Meat Sci* 56:203–209. [https://doi.org/10.1016/S0309-1740\(00\)00044-9](https://doi.org/10.1016/S0309-1740(00)00044-9)
- Ahn JJ, Akram K, Jo DJ, Kwon JH (2012) Investigation of different factors affecting the electron spin resonance-based characterization of gamma-irradiated fresh, white, and red ginseng. *J Ginseng Res* 36:308–313. <https://doi.org/10.5142/jgr.2012.36.3.308>
- Ahn JJ, Sanyal B, Akram K, Kwon JH (2014) Alcoholic extraction enables epr analysis to characterize radiation-induced cellulosic signals in spices. *J Agric Food Chem* 62:11089–11098. <https://doi.org/10.1021/jf502258r>

- Akram K, Ahn JJ, Kwon JH (2012a) Analytical methods for the identification of irradiated foods. In: Belotserkovsky E, Ostaltsov Z (eds). Nova Science Publishers, Inc., New York, pp 1–48
- Akram K, Ahn JJ, Yoon SR, Kim GR, Kwon JH (2012b) Quality attributes of *Pleurotus eryngii* following gamma irradiation. *Postharvest Biol Technol* 66:42–47. <https://doi.org/10.1016/J.POSTHARVBIO.2011.12.001>
- Chung N, Jo Y, Joe MH, Jeong MH, Jeong YJ, Kwon JH (2017) Rice vinegars of different origins: discriminative characteristics based on solid-phase microextraction and gas chromatography with mass spectrometry, an electronic nose, electronic tongue and sensory evaluation. *J Inst Brew* 123:159–166. <https://doi.org/10.1002/jib.406>
- Delincée H, Soika C (2002) Improvement of the ESR detection of irradiated food containing cellulose employing a simple extraction method. *Radiat Phys Chem* 63:437–441. [https://doi.org/10.1016/S0969-806X\(01\)00533-3](https://doi.org/10.1016/S0969-806X(01)00533-3)
- EN 1787 (2000) Foodstuffs-detection of irradiated food containing cellulose by ESR spectroscopy. European Committee of Standardization (CEN), Brussels
- EN 13708 (2001) Foodstuffs-detection of irradiated food containing crystalline sugar by ESR spectroscopy. European Committee of Standardization (CEN), Brussels
- EN 1788 (2001) Foodstuffs-thermoluminescence detection of irradiated food from which silicate minerals can be isolated. European Committee of Standardization (CEN), Brussels
- EN 13751 (2009) Foodstuffs-detection of irradiated food using photostimulated luminescence. European Committee of Standardization (CEN), Brussels
- Fan X, Mattheis JP (2001) 1-Methylcyclopropene and storage temperature influence responses of ‘Gala’ apple fruit to gamma irradiation. *Postharvest Biol Technol* 23:143–151. [https://doi.org/10.1016/S0925-5214\(01\)00119-3](https://doi.org/10.1016/S0925-5214(01)00119-3)
- Farkas J, Mohácsi-Farkas C (2011) History and future of food irradiation. *Trends Food Sci Technol* 22:121–126. <https://doi.org/10.1016/J.TIFS.2010.04.002>
- Jo Y, Sanyal B, Chung N, Lee HG, Park Y, Park HJ, Kwon JH (2015) Calibrated photostimulated luminescence is an effective approach to identify irradiated orange during storage. *Radiat Phys Chem* 111:81–86. <https://doi.org/10.1016/J.RADPHYSCH.2015.02.024>
- Jo Y, Chung N, Park SW, Noh BS, Jeong YJ, Kwon JH (2016) Application of E-tongue, E-nose, and MS-E-nose for discriminating aged vinegars based on taste and aroma profiles. *Food Sci Biotechnol* 25:1313–1318. <https://doi.org/10.1007/s10068-016-0206-4>
- Khan AA, Khan HM, Delincee H (2002) Identification of irradiated spices using the novel technique of DNA comet assay. *J Food Sci* 67:493–496. <https://doi.org/10.1111/j.1365-2621.2002.tb10625.x>
- Kim JH, Ahn HJ, Yook HS, Kim KS, Rhee MS, Ryu GH, Byun MW (2004) Color, flavor, and sensory characteristics of gamma-irradiated salted and fermented anchovy sauce. *Radiat Phys Chem* 69:179–187. [https://doi.org/10.1016/S0969-806X\(03\)00400-6](https://doi.org/10.1016/S0969-806X(03)00400-6)
- Kim GR, Akram K, Ahn JJ, Kwon JH (2012) Identification of gamma ray and electron-beam irradiated wheat after different processing treatments. *J Cereal Sci* 56:347–351. <https://doi.org/10.1016/J.JCS.2012.02.013>
- Liu Y, Heying E, Tanumihardjo SA (2012) History, global distribution, and nutritional importance of citrus fruits. *Compr Rev Food Sci Food Saf* 11:530–545. <https://doi.org/10.1111/j.1541-4337.2012.00201.x>
- Lota ML, de Rocca SD, Tomi F, Jacquemond C, Casanova J (2002) Volatile components of peel and leaf oils of lemon and lime species. *J Agric Food Chem* 50:796–805. <https://doi.org/10.1021/JF010924L>
- Sanyal B, Ahn JJ, Maeng JH, Kyung HK, Lim HK, Sharma A, Kwon JH (2014) An improved approach to identify irradiated spices using electronic nose, FTIR, and EPR spectroscopy. *J Food Sci* 79:C1656–C1664. <https://doi.org/10.1111/1750-3841.12571>
- Sholom S, McKeever SWS (2016) Emergency EPR dosimetry technique using vacuum-stored dry nails. *Radiat Meas* 88:41–47. <https://doi.org/10.1016/J.RADMEAS.2016.02.014>
- WHO (1981) Wholesomeness of irradiated food. Report of a Joint FAO/IAEA/WHO Expert Committee Technical Report Series 659, World Health Organization, Geneva, Switzerland
- Winquist F, Arwin H, Lund E, Forster R, Day C, Lundstrom I (1995) Screening of irradiated tomatoes by means of an electronic nose. In: 8th International conference on solid-state sensors and actuators, and eurosensors IX. *Transducers’ 95*, vol 1. IEEE, pp 691–694. <https://doi.org/10.1109/sensor.1995.717325>
- Yoo ZW, Kim NS, Lee DS (2004) Comparative analyses of the flavors from hallabong (*Citrus sphaerocarpa*) with lemon, orange and grapefruit by spme and hs-spme combined with GC-MS. *Bull Korean Chem Soc* 25:271–279. <https://doi.org/10.5012/bkcs.2004.25.2.271>

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.