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Investigation of Radiation-Induced Free Radicals and Luminescence Properties in Fresh Pomegranate Fruits

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ABSTRACT: Radiation-induced free radicals and luminescence properties were investigated in γ-irradiated (0−3 kGy) pomegranate (*Punica granatum* L.) fruits. Photostimulated luminescence (PSL) analysis showed limited applicability, and only 3 kGy-irradiated pomegranates showed positive PSL values (>5000 PCs). Thermoluminescence (TL) glow curve features, such as intensity and the presence of maximum glow peak in radiation-specific temperature range (150−250 °C), provided definite proof of irradiation, and the TL ratios (TL_1/TL_2) also confirmed the reliability of TL results. Scanning electron microscopy energy dispersive X-ray (SEM-EDX) analysis of the separated minerals showed that feldspar and quartz minerals were responsible for the luminescence properties. Radiation-induced cellulose radicals were detected in the seeds and rinds by ESR analysis. The ESR results were better in freeze-dried samples than in alcohol-extracted ones. A positive correlation was found between the ESR and TL signal intensities and irradiation doses; however, the most promising detection of the irradiation status was possible through TL analysis.

KEYWORDS: *γ-irradiation, pomegranate, quarantine, identification, photostimulated luminescence, thermoluminescence, electron spin resonance*

■ INTRODUCTION

Pomegranate (*Punica granatum* L. Punicaceae) is a high-valued delicious fruit that is extensively consumed worldwide largely due to its unique sensory and nutritional properties.[1] The current world pomegranate production amounts to approximately 1,500,000 tons.[2] Local cultivation in Korea accounts for an area of about 161.4 ha, which is steadily increasing. The local cultivars are liked among consumers because of their stronger sour taste compared with imported pomegranates.[3]

In the past decade, the industry and agricultural production have adapted to meet higher market demands for pomegranate.[4] However; there has been an overall increase in foodborne illnesses due to enhanced production, consumption, and distribution of fresh produce over the last two decades. Pathogenic contamination of fresh produce may occur before or after harvest, but once contaminated, produce is difficult to sanitize.[5]

Food irradiation, along with several other nonthermal processing technologies, has been recognized as a significant tool to ensure food safety by the U.S. Food and Drug Administration (FDA) and the World Health Organization (WHO).[6] Irradiation is also a viable postharvest quarantine disinfestation treatment of fresh agricultural commodities majorly devised to prevent migration of potentially damaging organisms to new areas.[7] The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA), under the regulations in "Fruits and Vegetables Section" (7 CFR part 305), has enforced the mandatory requirement to irradiate fresh pomegranate fruits with a minimum dose of 0.4 kGy on import either outside or inside the United States. The designed phytosanitary treatment would be helpful to eliminate a mite (*Tenuipalpus granati*), the false spider mite (*Tenuipalpus punicae*), and a bacterium (*Xanthomonas axonopodis* pv. *punicae*) from fresh pomegranate fruits.[8] Alighourchi et al.[9] reported the effects of γ-irradiation (0−10 kGy) on the stability of anthocyanins and inhibition of microbial growth in the juice of different pomegranate cultivars during storage at 4 °C. γ-Irradiation at 0.5 and 2 kGy reduced the growth rate of bacteria and fungi of the selected pomegranate juices during storage with negligible effect on nutritional and sensory profiles. However, at higher doses (>2 kGy) a considerable decrease of total anthocyanin content was observed.

International trade stresses the implementation of various national and international regulations to ensure proper labeling demonstrating the confirmation of specified applied irradiation doses. Therefore, the need for authentic detection methods is amplified to boost the trade and acceptability of irradiated foods. It is unfortunate that none of the currently available identification techniques has the potential to recognize all irradiated foodstuffs.[10] Many studies demonstrate the identification of different irradiated fruits,[11,12] but there is no published data found on the identification of irradiated fresh pomegranate fruits.

The present investigation was conducted to characterize the radiation-induced free radicals and luminescence properties for the effective identification of irradiated pomegranate fruits. ESR analysis of fresh produce requires effective drying pretreatments, for which freeze-drying and alcohol extraction methods were compared. It is demonstrated that photostimulated luminescence (PSL) and thermoluminescence (TL) character-

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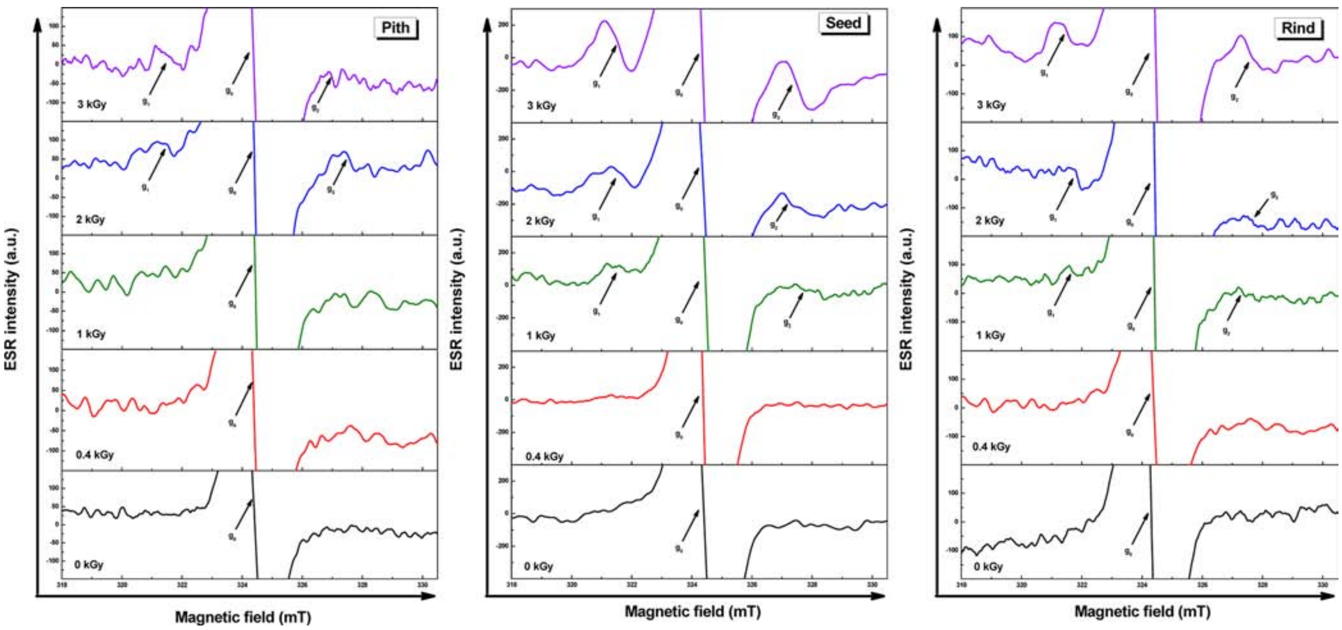

Figure 1. Effects of irradiation doses on ESR spectra of different parts of pomegranate fruits on freeze-drying.

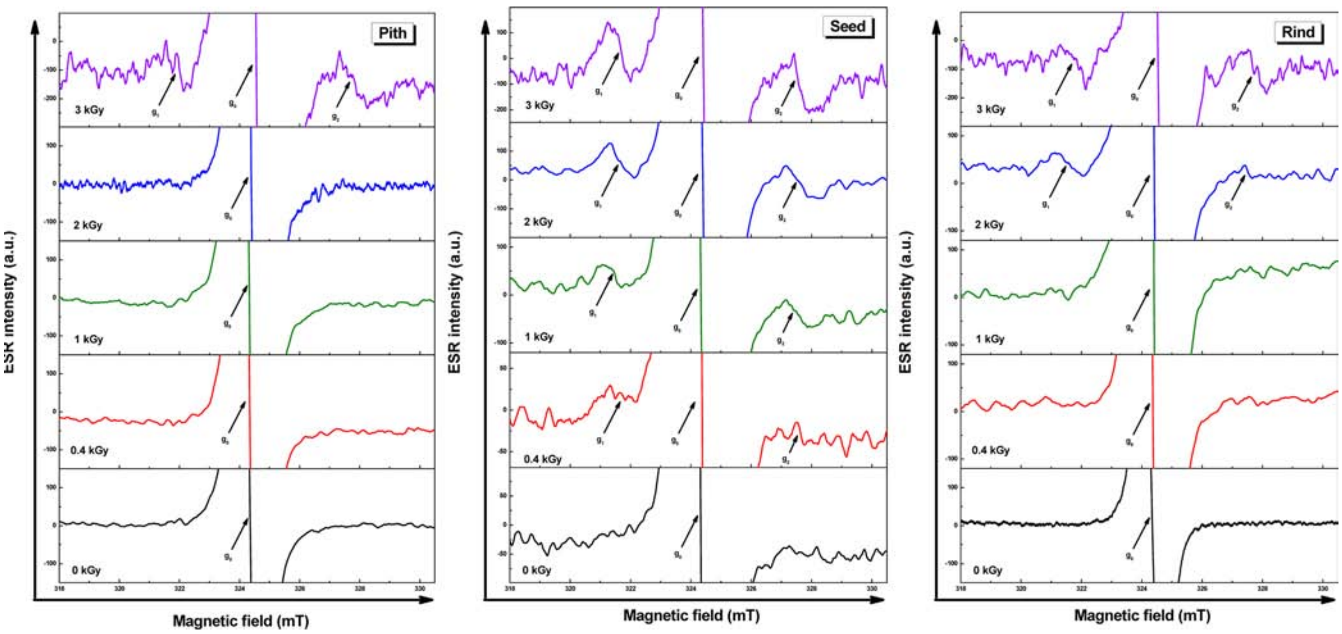

Figure 2. Effects of irradiation doses on ESR spectra of different parts of pomegranate fruits on alcohol extraction.

istics are largely dependent on the composition of contaminated inorganic minerals[13] that was determined by scanning electron microscopy energy dispersive X-ray (SEM-EDX) technique.

■ MATERIALS AND METHODS

Pomegranate Fruit Samples and γ-Irradiation. Pomegranate fruits were obtained from the Goheung County, which is a major pomegranate production zone in Korea. The fruits were properly packed in polyethylene bags and labeled with the specific dose. The packed fruits were irradiated (0, 0.4, 1, 2, and 3 kGy) at room temperature at the Korean Atomic Energy Research Institute (KAERI) in Jeongeup, Korea. A Co-60 γ-ray source (AECL, IR-79, Nordion International Co. Ltd., Ottawa, Canada) was used at a dose rate of 2.1 kGy/h. Dose calibration was conducted with alanine dosimeters of 5 mm diameter (Bruker Instruments, Rheinstetten, Germany). The fruit samples were kept at room temperature (23 ± 2 °C) in the laboratory before and after the irradiation treatment. The relevant sample preparation was performed, and the analyses of irradiated samples were carried out within 3 days after irradiation treatment. There were 16 fruits separated for the PSL measurements (4 fruits for each dose), and about 60 fruits (15 fruits for each dose) were selected for the TL analysis. The TL and ESR analyses were performed in triplicate, whereas 10 counts were recorded for the PSL measurements.

ESR Analysis. European standard EN 1787[14] was used to determine the free radicals by ESR spectroscopy. Pomegranate fruits ($n = 9$) were carefully separated into different parts (rind or peel, pith, white carpellary membranes, and seeds) with a sharp sterile blade.[15] The samples were freeze-dried or extracted with alcohol[16] to reduce the moisture content. ESR spectrometer (JES-TE 300, JEOL Co. Ltd, Tokyo, Japan) was used as described earlier by Akram et al.[16]

PSL Analysis. The pomegranate fruit rind was carefully separated and irradiated with the food screening system (Serial: 0021, Scottish

Universities Research and Reactor Center, Glasgow, UK) used for the PSL analyses according to European standard EN 13751.[17] The external surface of the detached rind was kept in an upward direction. The petri dishes (Bibby sterilin type 122) were protected with respective lids to eliminate the possibility of any outside contamination. Passive light conditions were adopted during the PSL analysis. The PSL signals emitted from the samples per second were automatically accumulated and presented as photon counts (PCs) per minute. The results obtained were interpreted by using threshold limits (negative <700 PCs/min and positive >5000 PCs/min). The intermediate results (700−5000 PCs/min) were further confirmed using other analytical techniques.

TL Analysis. European standard EN 1788[18] was used for the TL analysis of separated inorganic minerals (≥0.2 mg) through density separation technique. The whole fruits (n = 5) were used to collect the minerals amount for one TL measurement. A TLD (Harshaw 4500, Thermo-Fisher Scientific Inc. Waltham, MA, USA) was used as previously described by Akram et al.[16]

SEM-EDX Measurements. Polymineral composition of the separated minerals, from the pomegranate fruits, was analyzed with SEM-EDX. A field emission scanning electron microscope (S4300, Hitachi, Tokyo, Japan) equipped with an energy dispersive X-ray (EDX) spectrometer was used at an accelerating voltage of 15 kV.

RESULTS AND DISCUSSION

ESR-Based Characterization of Free Radicals To Identify Irradiated Pomegranate Fruits. In food materials of plant origins, irradiation can generate free radicals in cellulose and crystalline sugars, which could serve as irradiation detection markers on ESR analysis.[19] In fresh agricultural produce with higher moisture contents, the radiation-induced free radicals are not as stable as those from dried food materials. In addition, sugar does not exist in crystalline form in fresh fruits and vegetables, so production of crystalline sugar radicals upon irradiation is not possible. Generally, the hard parts (seeds, shell, skin, etc.) of fresh fruits and vegetables are analyzed for ESR-based identification of cellulose radicals. Effective drying techniques are also needed as pretreatments to reduce the moisture content with no or negligible effect on the radiation-induced free radicals (temperature sensitive), resulting in clear or improved ESR spectral features.[16]

The ESR spectra of the different parts of control and irradiated pomegranate fruits are shown in Figures 1 and 2. A central ESR signal (g = 2.0045) was observed in all parts of the pomegranate fruit, regardless of the irradiation history and the pretreatment technique used. Semiquinone radicals are generated by the oxidation of polyphenolic compounds within the plant matrix, which are considered to be responsible for this central ESR signal.[20] Different scientists have also reported a similar ESR signal due to organic radicals from different foods of plant origins.[19,21,22] A significant difference was observed in the intensities of the central ESR signals from different parts of the same fruit, whereas the effect of different sample pretreatments was also prominent (Figure 3). The seed part of the fruit generated high-intensity signals compared to any other fruit part. The signal intensities were more prominent from the freeze-dried samples compared to the alcohol-extracted samples. Relatively weak ESR signals were produced from the rind part of the fruit, particularly after alcohol extraction. Similarly, from the pomegranate fruit pith, a very low intensity signal was observed. No ESR signals were detected from the white carpellary membranes of the fruits (data not shown). The variation in ESR response of the different parts of the same fruit can be attributed to the differences in their chemical composition.[13] The results were similar to those described for the different parts of kiwifruits[12] and dried mushrooms.[13]

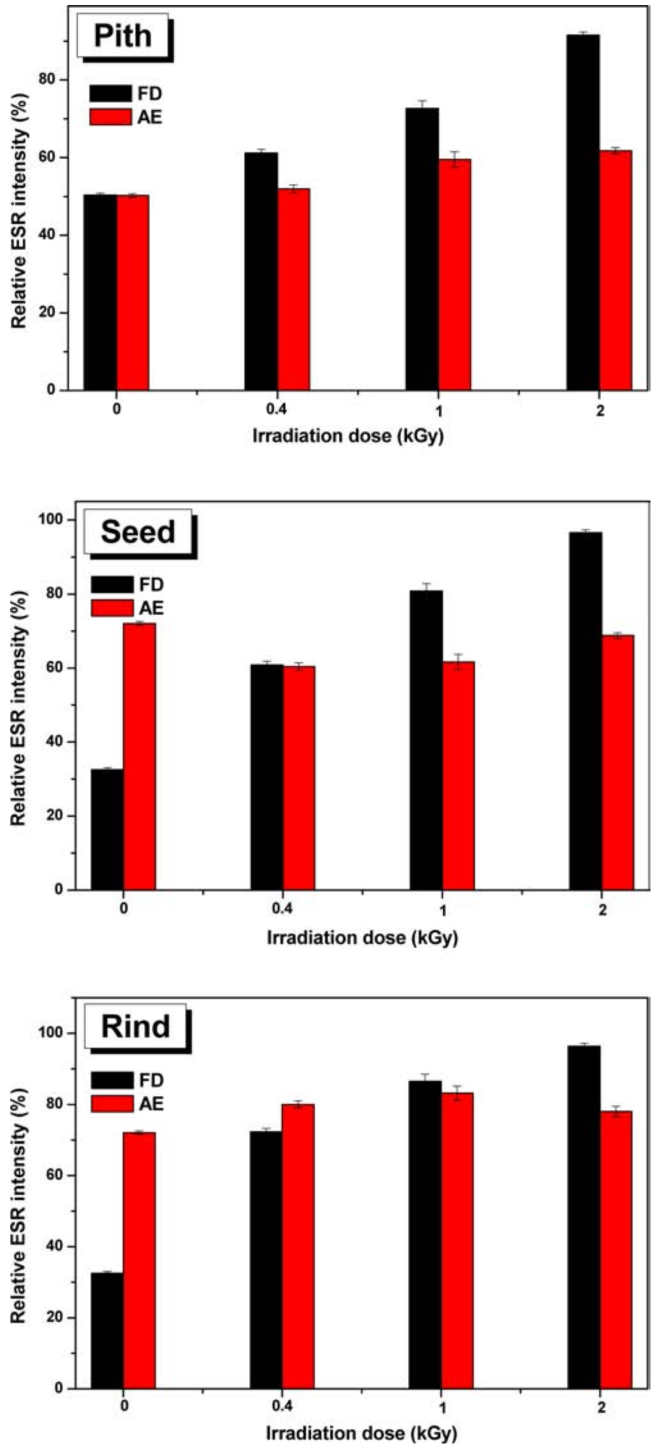

Figure 3. ESR intensity of different parts of irradiated pomegranate fruits at different pretreatments (freeze-drying, FD; alcohol extraction, AE).

Irradiation induced two side peaks with the central signal (also appearing in the nonirradiated samples), where the main signal also showed an increase in intensity upon irradiation in freeze-dried samples (Figure 3). These side peaks were detectable in samples irradiated at ≥1 kGy, when the results were clear in seeds followed by the rind samples. The radiation-specific side signals with a mutual distance of 6 mT were

Table 1. Accumulated PSL Photon Counts of Irradiated Pomegranate Fruits

	irradiation dose				
measurement	0 kGy	0.4 kGy	1 kGy	2 kGy	3 kGy
1	273	523	1637	2778	11047
2	305	473	1096	5966	5361
3	319	448	1719	3956	8539
4	311	415	1254	2009	14158
5	300	523	1813	6225	6573
6	266	508	1881	5846	4806
7	337	512	1451	5400	7532
8	300	481	1004	4441	7115
9	264	496	1850	2001	10483
10	310	435	1137	3710	7541
mean value[a]	299 ± 24	481 ± 38	1484 ± 339	4233 ± 1612	8316 ± 2850

[a]Mean ± SD (n = 10).

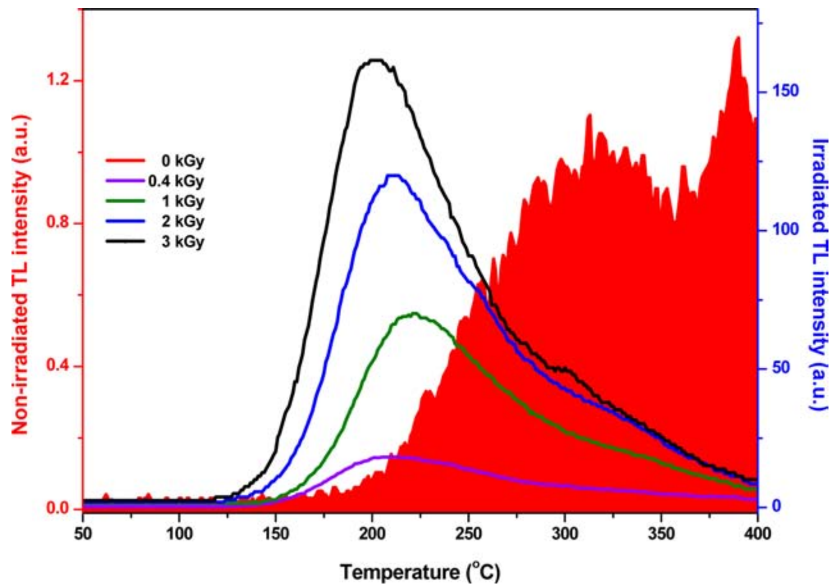

Figure 4. TL glow curves of the minerals separated from irradiated pomegranate fruits.

associated with radicals generated by irradiation in cellulose-containing foods.[23] Deighton et al.[24] identified the left side peak due to radiation-induced cellulose radicals, whereas the lignin radicals were responsible for the right side peak. The right peak signal was found to be sensitive to irradiation as well as heat processing.[25] Figures 1 and 2 show that the distance ($g_1 - g_2$ = 5.544−6.379) and g values (g_1 = 2.014−2.027 and g_2 = 1.984−1.987) of the two side peaks did not vary considerably for the samples (different parts) and pretreatments. These results are in close agreement with those stated by de Jesus et al.[26] on irradiated fruit pulp samples.

The freeze-drying treatment provided overall better results when the signals from the same part of the fruit were compared with those of the alcohol-extracted sample (Figure 1). The results were contradictory to those provided by Delincée and Soika[19] showing improved ESR results for alcohol-extracted plant food samples. The intensities of the side peaks in comparison to the main signal (signal ratio) showed significant differences depending upon the part of fruit used for the ESR analysis and sample pretreatments, in which the freeze-dried seed samples had the best results. The intensities of radiation-specific side signals with respect to the main signal are important for easy detection of irradiation treatment and were reported to be approximately 5% in irradiated *Foeniculi fructus*[27] and 50% in irradiated citrus fruits[21] compared to the total intensity of the central signal.

Luminescence (PSL and TL) Properties for the Identification of Irradiated Pomegranate Fruits. The contaminating inorganic minerals, especially silicate materials, present on food can absorb energy upon irradiation in charge carriers trapped at structural or interstitial sites. External light or heat stimulation of these minerals can discharge the stored energy, yielding a measurable luminescence. On the basis of this radiation-specific luminescence behavior of inorganic minerals on foods, the PSL and TL techniques have been devised to analyze the irradiation history of food samples.[10,17]

PSL has proved its potential as an effective and rapid screening technique for the identification of irradiated samples.[17] This method has been successfully tested as an initial screening tool for numerous products including shrimp and prawn, herbs and spices, and fruits and vegetables. The results reported as "intermediate" or "positive" by PSL analysis were further confirmed with more valid TL measurements.[28] PCs for the control and irradiated pomegranate fruits are

Table 2. TL Ratios (TL_1/TL_2) of Minerals Separated from Irradiated Pomegranate Fruits

TL parameter	irradiation dose 0 kGy	0.4 kGy	1 kGy	2 kGy	3 kGy
TL_1	0.2 ± 0.0[a]	232.3 ± 24.0	660.9 ± 32.2	1250.3 ± 64.3	1555.2 ± 61.1
TL_2	381.4 ± 79.9	716.0 ± 18.6	835.8 ± 35.5	817.7 ± 66.3	742.0 ± 53.2
TL ratio	0.001 ± 0.000	0.324 ± 0.025	0.793 ± 0.070	1.532 ± 0.065	2.107 ± 0.229

[a]Mean ± SD (n = 3).

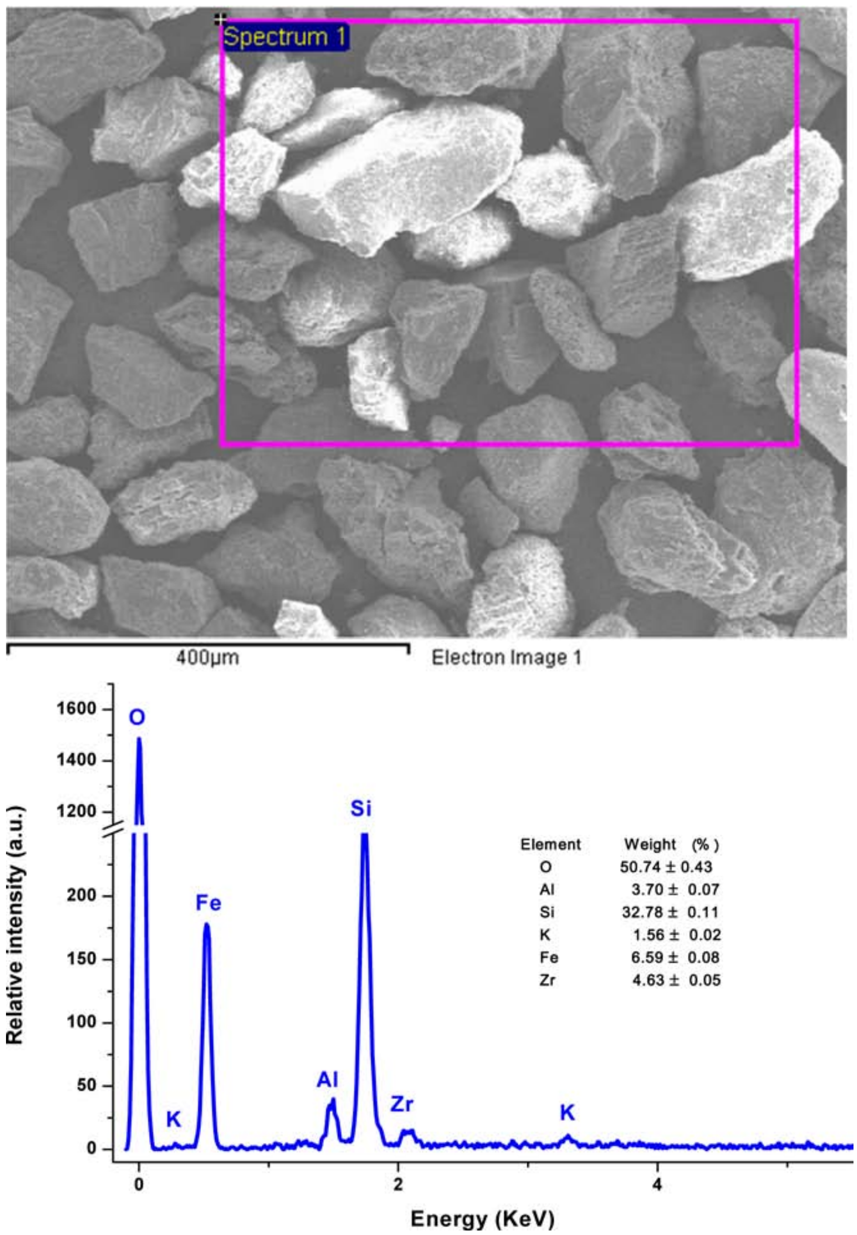

Figure 5. SEM-EDX analysis of the minerals separated from pomegranate fruits.

presented in Table 1. Nonirradiated and 0.4 kGy irradiated fruits had negative counts (<700 PCs). Intermediate counts (700−5000 PCs) were produced by 1 and 2 kGy irradiated pomegranates. Only the 3 kGy treated fruits were in the positive PSL range (>5000 PCs). However, there was a direct increasing relationship between PCs and irradiation dose. Irregularity observed in the PSL values can be ascribed to the differences in the amount and composition of the inorganic minerals on the fruit surfaces.[29] Poor results due to low PSL sensitivity and a lack of contaminating minerals in irradiated fresh mushrooms were also observed by Akram et al.[16]

The TL technique needs sufficient amounts of silicate minerals to be separated from the foods. TL measurements were performed to confirm the irradiation status of pomegranate fruits after the silicate enriched mineral fraction was obtained through a density separation method.[18] Irradiated foodstuffs usually show a glow curve peak in the temperature range of 150−250 °C, whereas low natural radioactivity produces TL signals above 300 °C in nonirradiated samples.

In the present investigation, all nonirradiated fruits produced a weak TL glow curve with the highest intensity at about 300 °C (Figure 4), confirming the absence of any ionizing radiation application. The TL ratio (<0.1) was obtained to confirm the authenticity of the minerals used to achieve reliable results. All irradiated pomegranates exhibited typical TL glow curves with maximum peaks in the temperature range of 150−200 °C (Figure 4). In addition, all of the irradiated pomegranates provided TL ratios >0.1 (Table 2), approving the characteristics of the minerals on the TL disks to achieve effective results. The TL results proved to be the most promising because clear identification was possible even at the lowest applied dose of 0.4 kGy (Figure 4). Different scientists have shown the improved potential of the TL technique over others, such as PSL and ESR analyses, to characterize the irradiation history of spices,[29] kiwifruits,[12] sesame seeds,[30] sauce,[31] potatoes,[32] and fresh mushrooms.[16]

Radiation-induced luminescence properties are primarily dependent upon the amount and composition of inorganic minerals present on the surfaces of samples.[17,18] The food materials become contaminated with these minerals from the environment during cultivation, harvesting, transportation, or processing. The fruits were washed to obtain the inorganic minerals for SEM-EDX analysis. Figure 5 exhibits the composition of inorganic minerals separated from the pomegranate fruits. Major mineral components observed on the pomegranate fruits were quartz and sodium feldspar, which accounted for the key radiation-specific luminescence properties of the samples. In addition, iron-containing minerals were also observed. Recently, Shin et al.[33] have successfully used iron-containing minerals for TL analysis to characterize the irradiation status of dried spices. The influence of the quartz to construct a TL glow curve is usually veiled due to its low sensitivity upon irradiation, in which the feldspar minerals usually define the overall TL glow curve properties in irradiated food samples.[32] Comparable findings were also reported for contaminating inorganic minerals separated from irradiated fresh mushrooms[10] and animal feed.[34]

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Notes

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