Controlling fungal growth in sesame (Sesamum indicum L.) seeds with γ-irradiation: impacts on some properties of sesame oil


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**SUMMARY:** This study investigated the free fatty acids, fatty acid profile, total phenolics, and antioxidant activity of sesame seed oil extracted from γ-irradiated seeds and the decontamination effects of the treatment on fungal incidence in the seeds. Gamma irradiation reduced ($P \leq 0.05$) fungal growth and colony forming units of sesame seeds in a dose-dependent manner. The free fatty acid content of sesame oil decreased ($P \leq 0.05$) in irradiated samples compared to non-radiated controls, but there was no difference ($P \geq 0.05$) between samples treated at doses ≥ 1.0 kGy. A concomitant ($P \leq 0.05$) increase in total phenolic and scavenging activity was observed in the oil extracted from γ-irradiated sesame seeds in comparison with non-radiated samples, while free fatty acid (FFA) content decreased. The results obtained in the present study demonstrate that γ-irradiation at low doses can be used as an effective post-harvest preservation method for sesame seeds without a major effect on the quality of sesame oil.

**KEYWORDS:** Antioxidant activity; Free fatty acids; Gamma irradiation; Sesame seed oil; Total phenolics

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**RESUMEN:** Control del crecimiento de hongos en semillas de sésamo (Sesamum indicum L.) con irradiación γ: impactos en algunas propiedades del aceite de sésamo. Este estudio investigó los ácidos grasos libres, el perfil de los ácidos grasos, los fenoles totales, la actividad antioxidante del aceite de semillas de sésamo extraída de las semillas irradiadas con rayos gamma y los efectos de descontaminación del tratamiento sobre la incidencia de hongos en las semillas. La irradiación gamma redujo ($P \leq 0.05$) el crecimiento de hongos y las unidades formadoras de colonias en las semillas de sésamo de una manera dependiente de la dosis. El contenido de ácidos grasos libre del aceite de sésamo disminuyó ($P \leq 0.05$) en las muestras irradiadas en comparación con los controles no irradiados, pero no hubo diferencia ($P \geq 0.05$) entre las muestras tratadas a dosis ≥ 1,0 kGy. Se observó un aumento concomitante ($P \leq 0.05$) en la actividad fenólica total y de eliminación en el aceite extraído de semillas de sésamo irradiadas con rayos gamma en comparación con muestras no irradiadas, mientras que el contenido de ácidos grasos libres (FFA) disminuyó. Los resultados obtenidos en el presente estudio demostraron que la irradiación con rayos γ a dosis bajas se puede usar como un método efectivo de conservación del sésamo después de la cosecha sin un efecto importante en la calidad del aceite de sésamo.

**PALABRAS CLAVE:** Aceite de semilla de sésamo; Ácidos grasos libres; Actividad antioxidante; Fenoles totales; Irradiación gamma

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1. INTRODUCTION

Oil seeds are important food stuff all over the world, and they are considered as a major source of good quality edible proteins and oils due to their availability, great acceptability by religious and health-conscious consumers, and relatively low cost compared to animal-based foods (Hassan et al., 2018). The Sesame seed is one of the oldest seeds used for oil production and the current world production of this important crop is 6.1 million tons (FAO, 2018). In recent years, the world production of sesame seeds has increased and this can be attributed to its wide applications in industries, particularly in the production of healthy and nutritious products. Oil is an important component of seeds and its value varies due to the difference in seed type and the extraction method used. Sesame seeds have been reported to contain up to 63% oil (Hassan et al., 2018) with several health-promoting properties. Nutritionally, sesame oil is a very rich source of unsaturated fatty acids such as oleic and linoleic acids, which makes it a perfect raw material for the production of margarine and cooking oil. In addition, sesame oil contains a substantial amount of lignans (sesamin, sesamolin and sesaminol), tocopherols and phytosterols (Elleuch et al., 2011). These compounds have been proven to have many health benefits including blood pressure reduction, hypocholesterolemic effect, antioxidant and anticancer activities (Elleuch et al., 2011). Owing to these nutritional and health-promoting effects, sesame oil may be useful industrially for the production of functional foods and might also find applications in the pharmaceutical industry. However, in spite of the nutritional and health-promoting effects of sesame seeds, post-harvest attack by insects, fungi, bacteria and pests continue to pose a threat to the effective utilization of the seeds for various applications. It is therefore essential to apply an appropriate treatment to the seeds to limit post-harvest decomposition in quality without major damages to the nutritional and health-promoting properties.

Various methods have been developed to control food spoilage and improve the safety of foods. Chemical preservation, freezing, chilling, canning, and pasteurization have been successfully used to control food spoilage (Agrios, 2005). Irradiation, which involves the exposure of food materials to different doses, is an effective method for controlling food spoilage due to the effect of its primary and secondary radiolysis on the microorganism; although it could produce chemical reactions in food materials (Hassan et al., 2018). However, the effects of these chemical reactions are derived from various factors such as dose rate, quantity of dose absorbed, availability or unavailability of oxygen, and temperature (Brewer, 2009). Mahmoud et al., (2016) reported that irradiation treatment was effective in improving the quality and dietary attributes of stored agricultural products, while maintaining its shelf-life and hence, irradiation could be a better replacement to chemical preservation. In addition, irradiations leave no residue in food materials, unlike other methods such as chemical treatments. Interestingly, ionizing radiation applied at doses below 10 kGy had no effect on micro or macronutrients and it enhanced the shelf-life of food (Brewer, 2009). Recently, it has been reported that gamma irradiation of sesame seed flour at doses ≤ 2.0 kGy improved its functional properties (Hassan et al., 2018). Another report indicated that the total phenolic compounds in almond skin extract increased following irradiation at doses below 4 kGy (Harrison and Were, 2007). To date, studies on the impact of gamma rays on the quality of sesame oil are limited (Affify et al., 2013; Al-Bachir, 2017). However, information on the effect of gamma irradiation on the FFA, total phenolics, and antioxidant activity of oil extracted from sesame seeds is scarce. Therefore, this study was conducted to investigate the decontaminating effect of gamma irradiation on the fungal growth and colony formation of sesame seeds and the impact of this treatment on the FFA, fatty acid profile, total phenolics, and antioxidant activity of sesame oil extracted from γ-irradiated sesame seeds.

2. MATERIALS AND METHODS

2.1. Sample preparation

The sesame seeds used in the present study were procured from a sesame farm in Gadairf, Sudan. The seeds were transported to the laboratory immediately after harvest, cleaned, sorted and kept in plastic bags at 4 °C before use.

2.2. Gamma radiation treatments

Sesame seeds were sealed in plastic bags prior to and during the irradiation process. The samples were irradiated at the Kaila irradiation processing unit, Sudanese Atomic Energy Corporation (SAEC) using an experimental cobalt- 60 gamma source (Nordion gamma cell 220 - Excell) at the doses of 0.5, 1.0, 1.5, and 2.0 kGy and with a dose rate of 1.98 kGy/h. To ensure uniform dose delivery and minimize the variations in radiation received by the samples, both sides of the samples were exposed to irradiation (double-side irradiation). Three dosimeters (Gafchromic HD-810 film, International Specialty Products, NJ, USA; FAO/IAEA/USDA 2003) were included in each batch of seeds and read after irradiation with a Radiachromics reader (Far West Technology Inc., CA, USA) to measure the dose received by the batch. Triplicate samples of sesame seeds were irradiated, and all treatments
were repeated three times. Non-irradiated seeds were used as a control.

2.3. Fungal growth and colony formation

The fungal growth of the treated and untreated seeds was measured following the Agar method according to ISTA (2006). For the assessment of seed infection, the seeds from each treatment were surface disinfested to eliminate the possible growth of other microorganisms on the seed’s surface. About 25 seeds were placed on PDA (Potato Dextrose Agar) in each petri dish. The plates were incubated at 20-25 °C for 5 days. The total number of infected seeds with the pathogen were counted and calculated into a percentage of infection using the following formula:

\[
\text{Fungal growth} \, (\%) = \frac{\text{Number of seeds with fungal growth}}{\text{Total number of the seeds}} \times 100
\]

The fungal colony formation unit per gram (CFU/g) of treated and untreated samples was determined according to standard methods (AOAC, 1995). One ml of selected dilution (10⁻³) of each sample was poured and plated (in triplicate) onto petri dishes containing sterile PDA and then incubated at 25 ± 2 °C for 5 days. Colony formation was observed and identified according to spore morphology under a stereoscopic microscope, and the colonies on the reverse side of the petri-dishes, as well as the fruiting body, were observed under a compound microscope.

2.4. Oil extraction

Oil was extracted from the seeds using the cold extraction method. About 50 g of crushed sesame seeds were shaken with petroleum ether (1:10 w/v) for 24 h at room temperature. The solvent was then removed from the oil using a rotary evaporator. Finally, the oil was placed in a glass vessel at ambient temperature to completely remove any residual solvent. The oil was stored under refrigeration at approximately 4 °C until analysis.

2.5. Preparation of methanolic extract

Methanolic extract was prepared according to the method proposed by Szydlowska-Czerniak et al., (2008). Briefly, 500 mg oil were dissolved in 1.5 mL of n-hexane and then extracted with methanol (3 × 1 mL with stirring for 2 min). The mixture was incubated for 16 h in the dark. The methanolic extract was washed with 2.5 mL of n-hexane and dried under vacuum using a rotary evaporator and kept at 4 °C for further analysis.

2.6. Determination of total phenolic content of the oil

The total phenolics of the oil were determined by the Folin-Ciocalteu’s reagent method (Waterhouse, 2001). In this assay, a 20 µL aliquot of extract in methanol (1:10 w/v) was transferred to a test tube for reaction with the Folin-Ciocalteu reagent (0.5 mL). A standard curve was prepared using different concentrations of Gallic acid dissolved in methanol (R² = 0.9967). The TPC was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight.

2.7. Determination of antioxidant activity

The scavenging activity of diphenyl- 2-picryl-hydrazyl (DPPH) radicals of methanolic extracts was measured according to the method reported by Chang et al., (2001). Briefly, 100 µL of methanolic extract of sesame oil was added to the mixture of 1 mL of DPPH in methanol and 0.5 mL of Tris-HCl buffer (pH 7.5). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min and then the absorbance was measured spectrophotometrically at 517 nm. Methanol was used as a blank. The inhibitory effect of DPPH was calculated according to the following formula:

\[
\text{Inhibition} \, (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

2.8. Determination of free fatty acids

The analysis of the FFA content in sesame seed oil was made using the AOAC official method (AOAC, 1995). The acidity of sesame oil was evaluated as milligrams of potassium hydroxide required to neutralize one gram of oil.

2.9. Fatty acids composition

The fatty acid composition was determined according to Thies (1971) by using Gas Chromatography (GC) after derivatization to fatty acid methyl esters with 0.5 M Na-methyleate in methanol. The GC (TRACE GC Ultra, Thermo Fisher Scientific, Milan, Italy) used in this analysis was equipped with a Macherey-Nagel Permabond FFAP-0.25μm column (25 m × 0.25 mm and 0.25 μm film thickness), split injection at the rate of 1:70, and a hydrogen carrier gas at a pressure of 100 kPa. The injector/detector temperature was set to 230 °C and the oven temperature was 210 °C. Fatty acids were expressed as a percentage of the total.
fatty acids. The identification and quantification of fatty acid methyl ester was calculated by comparing retention times of the peaks with those of fatty acid standards (Mixture 463, Nu-Chek-Prep, Inc., Elysian, MN).

2.10. Statistical analysis

Analysis of variance (one-way ANOVA) was used to study the effect of gamma radiation on the fungal growth and colony formation of sesame seeds and the fatty acid content, free fatty acids, total phenolics, and antioxidant activity of sesame seed oil. Significant differences were accepted at the probability of $P \leq 0.05$ and least significant difference (LSD) was used to compare means (SAS 8.0 software, SAS Institute, Inc., Cary, NC, USA). The analyses of fungal growth, colony formation, fatty acid composition, free fatty acids, total phenolics, and antioxidant activity were performed in triplicate and values are presented as the means ± standard deviation.

3. RESULTS

3.1. Effect of irradiation on fungal incidence in sesame seed

The impact of gamma radiation on the percentage of fungal growth and colony formation (cfu/g) in sesame seeds is shown in Table 1. The treatment of sesame seeds with gamma rays at the doses used significantly ($P \leq 0.05$) reduced the fungal growth in a dose-dependent manner. It can be observed from the table that fungal growth was 98.7% before irradiation. The fungal growth reduced to 76.0% at 0.5 kGy radiation dose, reaching the lowest value (9.3%) in the sample treated at 2 kGy. Comparably, the efficacy of gamma radiation in lowering fungi counts was noticed. Before irradiation, the colony formation was impossible to count, as too many colony forming units were observed. Colony formation however decreased ($P \leq 0.05$) as the irradiation dose increased, reaching its minimum ($1.0 \times 10^2$ cfu/g) at 2 kGy.

3.2. Effect of gamma irradiation on total phenolics in sesame seed oil

The effect of gamma radiation on the total phenolic content of sesame oil is shown in Figure 1. Gamma radiation had a significant ($P \leq 0.05$) effect on the total phenolic contents in sesame seed oil. The non-radiated control had significantly ($P \leq 0.05$) lower total phenolic contents compared to the samples treated at doses ≥ 1.0 kGy. The total phenolic contents in the oil extracted from γ-irradiated seeds concomitantly ($P \leq 0.05$) increased as the radiation dose elevated. The highest phenolic content was observed in the oil extracted from the seeds irradiated at 2.0 kGy, while the lowest value was observed in the control. Accordingly, total phenolic contents in sesame oil, which have many health benefits, can be improved by treating the oil with gamma rays at the doses used in the present work.

3.3. Effect of gamma irradiation on antioxidant activity of sesame seed oil

The DPPH Scavenging activity of the oil extracted from sesame seeds treated with different doses of gamma rays is presented in Figure 2. The result showed that sesame oil was significantly ($P \leq 0.05$) affected by γ-irradiation. Generally, the antioxidant activity of the oil samples concomitantly ($P \leq 0.05$) increased as the irradiation doses increased from 0.5 to 2.0 kGy. Maximum scavenging activity was observed at the maximum radiation dose of 2.0 kGy. Interestingly, the non-radiated sample had the lowest ($P \leq 0.05$) scavenging activity of all samples. These findings revealed that gamma radiation of

<table>
<thead>
<tr>
<th>Radiation dose (kGy)</th>
<th>Fungal growth (%)</th>
<th>Colony formation (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>98.7 ± (2.309)</td>
<td>Uncountable</td>
</tr>
<tr>
<td>0.5</td>
<td>76.0 ± (0.000)</td>
<td>2.4 × 10^5 ± (1.002)</td>
</tr>
<tr>
<td>1.0</td>
<td>17.3 ± (2.309)</td>
<td>2.0 × 10^6 ± (0.985)</td>
</tr>
<tr>
<td>1.5</td>
<td>13.3 ± (2.309)</td>
<td>5.0 × 10^5 ± (3.020)</td>
</tr>
<tr>
<td>2.0</td>
<td>9.3 ± (2.309)</td>
<td>1.0 × 10^5 ± (1.400)</td>
</tr>
</tbody>
</table>

Data was analyzed using one-way ANOVA. Values are means ± standard deviation (n = 3). Values not sharing a common superscript are significantly ($P \leq 0.05$) different as assessed by LSD.
sesame seeds at doses of 0.5-2.0 kGy could be used to enhance the scavenging activity of sesame seed oil.

### 3.4. Effect of gamma radiation on the FFA composition of sesame seed oil

The FFA content of sesame oil as affected by the gamma radiation of sesame seeds is presented in Fig. 3. The results revealed that the FFA in sesame seed oil decreased with an increase in the radiation dose. The oil extracted from non-radiated samples had significantly (*P* ≤ 0.05) higher levels of FFA than those extracted from radiated samples. The oil extracted from sesame seeds irradiated at a radiation dose of 0.5 kGy had significantly (*P* ≤ 0.05) higher contents of FFA than those extracted from seeds irradiated at 1.0, 1.5 and 2.0 kGy. However, the oil samples from the seeds irradiated at 1.0, 1.5 and 2.0 kGy were not different (*P* ≥ 0.05) from one another in terms of their FFA contents.

### 3.5. Effect of gamma radiation on fatty acid profile of sesame seed oil

The effect of different doses of gamma rays on the fatty acid contents of sesame seed oil is presented in Figure 4. The main fatty acids identified in oils extracted from both radiated and non-radiated sesame seeds were palmitic acid (C16-0), stearic acid (C18-0), oleic acid (C18-1), linoleic acid (C18-2), α-linoleic acid (C18-3), and arachidic acid (C20-0). Oleic acid was the most abundant fatty acid in sesame seed oil, while α-linoleic acid was the least. The results showed that gamma irradiation of sesame seeds at different doses had an insignificant (*P* ≥ 0.05) effect on the individual fatty acids present in sesame seed oil. No significant (*P* ≥ 0.05) difference was observed between the oil extracted from the control and irradiated seeds for all the fatty acids. However, the presence of high amounts of oleic and linoleic acids in sesame seed oil is an indication that sesame seed oil is highly nutritious and could also serve as an index of oil stability since the stability of oil depends on the ratio of oleic to linoleic acid present in it.

### 4. DISCUSSION

The fungal incidence results obtained in this research showed that gamma irradiation significantly reduced the fungi growth and colony formation in sesame seeds. In addition, the fungal growth and colony formation were affected by irradiation in a dose-dependent fashion. The highest growth rate was observed in non-irradiated seeds and the growth decreased concurrently with an increase in the radiation dose. The inhibition of fungal growth and colony formation by gamma rays could be attributed to the damaging effects of gamma rays on the fungal membrane which might lead to losses in proteins,
nucleic acids, and osmotic balance, thereby causing the death of the fungal cells (McNamara et al., 2003). A similar observation on the reduction in fungal growth was reported for millet grains treated with low doses of gamma rays (Mahmoud et al., 2016). In addition, the irradiation of walnut kernels also caused a significant reduction in fungi growth and colony formation (Al-Bachir, 2004). Moreover, a significant reduction in the growth and survival of common seed-borne fungi (Alternaria sp., Aspergillus sp., Trichoderma sp., and Curvularia sp.) on different types of seeds following gamma irradiation at doses of 1-3 kGy has been reported (Maity et al., 2008). The decontamination of sesame seeds with gamma rays at the doses applied in this study could elongate the shelf-life and preserve the quality of sesame seeds during post-harvest processing.

The results obtained for total phenolics in this study showed that the gamma irradiation of sesame seeds increased the total phenolic contents of sesame oil in a dose-dependent manner. The increase in total phenolic compounds in sesame seed oil could be attributed to the release of phenolic compounds from glycosidic components of the seeds and degradation of larger phenolic compounds into smaller ones, thereby increasing their solubility and extractability in the oil. Information on the effect of gamma irradiation on the total phenolics of sesame oil is scarce in the literature, although the diverse effects of gamma irradiation on the total phenolics of other plant materials has been reported. Ghadi et al. (2015) reported that gamma radiation at different doses significantly increased the total phenolics of mazafati dates. In addition, Harrison and Were (2007) reported that gamma radiation increased the phenolic contents in soybean and spices. In contrast, Apaydin et al. (2017) reported a significant reduction in total phenolic compounds after the irradiation of grape seed oils. Differences between these studies could be attributed to the variations in the plant material used, applied gamma ray doses, treatment conditions, extraction solvents, and analysis methods. Interestingly, the enhancement of total phenolics in sesame seed oil following irradiation at the doses applied in the current study could increase the health potentials of the oil.

In the study to determine the antioxidant activity of oil extracted from sesame seeds treated with different doses of gamma rays, the DPPH scavenging activity was assessed. The results revealed that gamma radiation affects the scavenging activity of the sesame seed oil in a dose-dependent manner. The highest scavenging activity was observed in the oil irradiated at 2 kGy and this implies that irradiation could be employed to increase the antioxidant activity of sesame seed oil. An increase in antioxidant activity following gamma irradiation might be due to the formation of free phenolic compounds as a result of a radiation-induced breakdown of glycosides (Variyar et al., 2004) and subsequently the release of more phenolic compounds (Dixit et al., 2010) that positively contribute to the total antioxidant activity (Apaydin et al., 2017). Studies on the effect of gamma irradiation on the antioxidant activity of sesame oil are limited; however, there are various reports on the impact of such treatment on the antioxidant activity of other plant materials. Similar to these findings, gamma irradiation significantly increased the antioxidant activity of grape seed oil (Apaydin et al., 2017), almond skin extract (Harrison and Were, 2007), and mazafati dates (Ghadi et al., 2015). Strikingly, improving the antioxidant activity of sesame seed oil with gamma irradiation treatment could add more nutritional and health value to the oil.

The quantity of FFA in oil is an important quality factor and its determination is essential for industrial purposes since its presence in large quantity can change the organoleptic and physicochemical properties of oil. The decrease in FFA contents of sesame seed oil as gamma radiation doses increase is desirable. The decrease in FFA of sesame seed oil could result from the deactivation of lipases by gamma rays, thereby preventing the release of more FFA into the irradiated oil. In addition, a loss in volatile FFA could occur during the gamma irradiation treatment of sesame seeds leading to the reduction in FFA in the oil. The decrease in FFA as radiation doses increased as observed in this study is similar to those reported for irradiated pearl millet kernels (Mahmoud et al., 2016) and wheat bran (Pankaj et al., 2013). However, Zoumpoulakis et al. (2012) reported an opposite result for irradiated sesame seed oil. According to Zoumpoulakis et al., (2012), FFA concomitantly increased as the irradiation doses increased in sesame seed oil. Also, Apaydin et al., (2017), Bhatti et al., (2013) and Geigel et al., (2011) reported that the FFA contents in grape seed oils, almond oil, hazelnuts, walnuts, almonds and pine nuts, increased with increasing radiation doses. Guler et al., (2016) reported that gamma radiation had no significant effect on the FFA in hazelnuts and walnuts of both irradiated and non-radiated nuts. The differences observed for FFA in these studies might be due to the moderately higher doses (≥ 2.5 kGy) used compared to the one used in the current study (≤ 2.0 kGy) as well as the variation in the genetic makeup of the irradiated materials. The results of this study revealed that gamma irradiation at lower doses (≤ 2.0 kGy) could be employed as an effective method to stabilize and lengthen the storage life of sesame seed oil. This is because it can be observed from the result that the FFA present in oil extracted from radiated seeds is lower than that of non-irradiated ones and FFA is an indicator of rancidity that is instrumental to the development of off-flavor and off-odor in oil during storage.
The prominent fatty acids identified in both radiated and non-radiated sesame seed oil were palmitic acid, stearic acid, oleic acid, linoleic acid, α-linoleic acid and arachidic acid. Our findings revealed that gamma radiation had no significant effect on the fatty acid profile of sesame seed oil. Similar results were reported for oil extracted from walnuts irradiated at doses of 2-10 kGy (Iqbal et al., 2016) and oil extracted from peanuts irradiated at doses 1-3 kGy (Al-Bachir, 2015). In contrast, Afify et al., (2013) reported that gamma irradiation at a dose of 7.5 kGy significantly increased saturated fatty acids (palmitic and stearic acids) and decreased the unsaturated fatty acids (oleic and linoleic acids) of sesame oil. In addition, Al-Bachir (2017) reported increases in palmitic and stearic acids and a reduction in the linoleic acid of sesame oil extracted from seeds irradiated with 9 kGy. Moreover, Al-Bachir (2014) reported that oleic, linoleic and stearic acids increased when almond oil was irradiated at doses 1-2 kGy and the values decreased when irradiation doses increased to 3 kGy. The variations among these studies might be due to the variation in the seeds used and irradiation doses applied. The fatty acid content of sesame seed oil is similar to that of other oils extracted from radiated seeds such as peanut oil (Al-Bachir, 2015) and sesame, peanut and soybean oils (Afify et al., 2013).

CONCLUSIONS

The results obtained showed that gamma radiation could be used as an alternative to heat or chemical treatment in the preservation and value addition to sesame seeds. Gamma radiation caused a significant increase in the total phenolic composition and scavenging activity of sesame seed oil, while decreasing the fungal growth and colony formation in the seeds and free fatty acid formation in the oil. Irradiation of sesame seeds, however, had no significant effect on individual fatty acids in the oil extracted from treated seeds. In conclusion, gamma radiation could be an alternative to chemical treatment for disinfecting sesame seeds and extending the shelf-life of sesame seed oil without destroying its bioactive compounds.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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