Journal of Advancements in Food Technology
Volume 8 | Issue 1

Research Article Open Access

Gamma Radiation Effects on Physicochemical Properties of Organic Fruits

Jessica Scifo[1*], Rocco Carcione[1], Ilaria Di Sarcina[1], Beatrice D'Orsi[1], Leonardo Lanzetta[2], Silvia Massa[3], Valentina Mastrobuono[3,4], Elisabetta Bennici[3] and Alessia Cemmi1

[1]ENEA Nuclear Department (NUC), Casaccia Research Center, Via Anguillarese, 301, 00123 Rome, Italy

[2]Sapienza University, Dipartimento di Ingegneria Astronautica, Elettrica ed Energetica (DIAEE), Rome, Italy

[3]ENEA, Department for Sustainability (SSPT) Casaccia Research Center, Via Anguillarese, 301, 00123 Rome, Italy

[4]University of Tuscia, DAFNE – Department of Agriculture and Forest Sciences, Viterbo, Italy

***Corresponding Author**: Jessica Scifo, ENEA Nuclear Department (NUC), Casaccia Research Center, Via Anguillarese, 301, 00123 Rome, Italy, E-mail: jessica.scifo@enea.it

Citation: Jessica Scifo, Rocco Carcione, Ilaria Di Sarcina, Beatrice D'Orsi, Leonardo Lanzetta, et al. (2026) Gamma Radiation Effects on Physicochemical Properties of Organic Fruits. J Adv Food Technol 8(1): 101

Received Date: March 06, 2026 **Accepted Date:** March 24, 2026 **Published Date:** March 27, 2026

Abstract

Different methods are nowadays employed for the preservation of postharvest fruits quality, such as chemical treatments, heating and freezing. However, the application of these methodologies often causes heavy losses during storage, due to pathogenic microorganisms' and insects' activity. Gamma irradiation exposure, being a non-thermal process with high penetration depth, constitutes an effective way for postharvest food preservation. This work investigates the effects of gamma irradiation on organic pistachios and blueberries using non- or minimally destructive spectroscopic techniques, namely Electron Spin Resonance (ESR), micro-Raman, and Fourier-Transform Infrared (FTIR) spectroscopy. Organic pistachio kernels were subjected to several gamma radiation absorbed doses (0 Gy, 500 Gy, 1000 Gy, 2000 Gy and 4000 Gy), whereas organic blueberries underwent gamma irradiation at absorbed doses of 0 Gy, 250 Gy and 1000 Gy. In both cases the dose rate value was 1 kGy/h in air and at room temperature. The spectroscopic analysis of irradiated organic pistachio kernels highlights the preservation of functional groups and unsaturations at lower doses showing stability of C=O and C–H bands in FTIR and the consistent C=C/CH ratio in Raman. Only at 4000 Gy marked changes are detected, showing a decrease in unsaturations and an increase in radical formation, without evidence of oxidative degradation. The UV-visible spectrum of organic blueberries after irradiation test reveals the level of its anthocyanins was not affected by irradiation, indicating that these compounds are stable also after irradiation at 1000 Gy. The FTIR-ATR spectral analysis suggests that no substantial oxidation takes place even at the highest irradiation dose tested (1000 Gy). These results confirm the potential of gamma irradiation as a safe and effective method to extend organic fruits shelf life without compromising molecular integrity.

Keywords: Gamma irradiation, Food Preservation, Organic Fruits, Agri-Food Sector, Spectroscopic Non- Or Minimally Destructive Techniques

Introduction

The preservation of postharvest fruit quality is a key challenge in the agri-food sector, with significant implications for food safety, nutritional value, marketability, and waste reduction [1,2]. Fresh fruits are inclined to microbial spoilage, enzymatic degradation, and loss of sensory and nutritional properties during storage and distribution [3]. In this context, identifying and validating effective postharvest preservation strategies is therefore essential to extend shelf life while maintaining the chemical integrity and organoleptic quality of the product. Different methods are nowadays used for the preservation of postharvest fruits quality, such as chemical treatments, heating and freezing. However, the application of these techniques often causes important heavy losses during storage, due to pathogenic microorganisms' and insects' activity. In addition, physical and chemical techniques can leave dangerous residues on treated fruits, resulting in risks to human health [4-6]. Gamma radiation, which is characterized by high penetration power, is a safe technology used for postharvest food preservation [7-9]. Despite the use of ionizing radiation on food widespread in many countries, the potential physicochemical changes induced by radiation still represent topical issues for the agri-food supply chain. The present study focuses on the characterization of food matrices, such as organic pistachio and blueberries, by using spectroscopic techniques not conventionally used in this sector [10,11] before and after gamma irradiation tests. The samples were irradiated at different absorbed doses, in air and at room temperature at the Calliope Co-60 irradiation facility at ENEA Casaccia Research Center (Rome, Italy) [12]. Simple and effective protocols based on non- or minimally destructive techniques, such as Electron Paramagnetic Resonance (EPR) spectroscopy, micro-Raman spectroscopy and Fourier-Transform Infrared spectroscopy (FTIR), were developed.

Pistachios and blueberries were selected as model food matrices for this work due to their high nutritional value, economic relevance, and sensitivity to postharvest deterioration. Pistachios are rich in unsaturated lipids and bioactive compounds, making them valuable from both a dietary and commercial perspective, but potentially susceptible to oxidative processes [13,14]. Blueberries, on the other hand, are soft fruits with high water content and phenolic compounds, known for their antioxidant properties but also for their perishability and vulnerability to microbial spoilage [15]. Among the key properties of blueberries, anthocyanins, a class of water-soluble pigments belonging to the flavonoid family, are particularly abundant in the fruit matrix, where they contribute to the fruit's characteristic blue-purple coloration. In addition to their protective role in plants, these compounds have attracted increasing scientific interest because of their wide range of beneficial properties for human health. Indeed, numerous studies have demonstrated the antioxidant, anti-inflammatory, neuroprotective, and cardioprotective effects of anthocyanins, as well as their potential involvement in the mitigation of chronic diseases such as obesity and diabetes [16]. The stability/conservation of anthocyanins is of considerable interest, as their degradation can have a substantial impact on the color, sensory acceptance, and nutritional value of blueberry fruits. The present study aimed to assess the effect of different doses of gamma radiation (250 Gy and 1000 Gy) on the anthocyanin profile, evaluated using UV-VIS spectrophotometry. Variation in spectral characteristics, such as band shifts and changes in absorbance intensity, were monitored as indicators of potential irradiation-induced modification in anthocyanin molecules. UV-VIS spectrophotometric techniques provide a cheap, rapid and accessible alternative to more complex and resource-intensive analytical methods, for estimating total phenolic content in plant extracts. Although this method does not enable the identification of individual molecules, it allows a qualitative estimation of different classes of specialized metabolites such as anthocyanins, phenolic acids, stilbenes, flavanols, and tannins, based on known absorbance peaks [17]. Moreover, it is considered a "consumer-friendly" technique due to its ease to use, minimal sample preparation and suitability for routine and preliminary analysis in both research and industrial setting.

Given such considerations, investigating the effects of gamma irradiation on these two distinct matrices by coupling a series of spectroscopic techniques offers a comprehensive understanding of how irradiation impacts food with different biochemical compositions. This comparative approach aims to assess the suitability of gamma irradiation as a non-thermal preservation method for diverse fruit-based food products.

Materials and Methods

Samples

This study involved the analysis of two distinct biological sample types: organic pistachios harvested in the southern region of Italy and organic blueberries harvested in the central Italy. Organic pistachio kernels were subjected to different gamma radiation absorbed doses (0, 500, 1000, 2000 and 4000 Gy) at a dose rate value of 1 kGy/h in air at room temperature to determine the effects of exposure on fruit properties. Only the green part of the kernel has been irradiated.

Organic blueberries were subjected to gamma irradiation at low absorbed doses (0, 250 and 1000 Gy) at a dose rate value of 1kGy/h in air at room temperature to determine the effects of irradiation on fruit quality. In particular, the stability/conservation of anthocyanins is of considerable interest, as their degradation can have a substantial impact on the color, sensory acceptance, and nutritional value of these fruits.

Samples Preparation

A total of five organic pistachio kernels were selected for the study. To ensure methodological consistency and meet the geometric requirements for specific analyses, the kernels were first peeled and then cut into sections of approximately 100 mg each. In this way, each individual kernel was divided into five slices, and each slice was randomly assigned to one of the five target absorbed doses: 0 (unirradiated reference sample), 500, 1000, 2000, and 4000 Gy. This approach allowed each kernel to serve as its own reference, allowing to obtain five biological replicates for each dose level.

Analogously to pistachio preparation, a group of fifteen blueberries was selected based on strict visual criteria to ensure a reproducible sample set. Only fruits free from visible mold and other macroscopic defects were included. Both the skin and pulp were processed together to represent the whole fruit properties. The selected blueberries were divided into standardized aliquots of around 30g each. Two aliquots of 26.42 g 27.48 g were irradiated at doses of 250 Gy and 1000 Gy, while a 28.25 g aliquot was kept unirradiated as a control group (0 Gy). The obtained samples were lyophilized in a freeze dryer (Modulyo Freeze Dryer, Freeze Dryer Model, Edwards) at −60 °C and 0.2 mbar for five days.

Phenolic compounds of lyophilized blueberry were extracted by using a modified protocol described by Wang et al 2013. Briefly, 30 mg of freeze-dried powdered was mixed with 600 μL of MeOH: H2O (70:30 v/v) and incubated on a rotator for 1 hour at room temperature. After centrifugation (Centrifuge 5415R, Eppendorf) at 9300g for 5 min at 10 °C, the supernatant was collected. Spectrophotometric analysis (BioSpectometer, Eppendorf) was performed. Extraction was done in duplicate.

Gamma Irradiation Test

The irradiation tests described in this study were conducted at the Calliope gamma irradiation facility, located at the ENEA Casaccia Research Center in Rome, Italy. The facility is equipped with a 60Co radioisotope source array emitting gamma rays with a mean energy of 1.25 MeV. The samples were irradiated in air under ambient temperature and humidity conditions, at different absorbed doses as previously mentioned, by using a dose rate of about 1 kGy/h that is commonly available in gamma irradiation facilities. All dose and dose rate values are referred to water. The dose rates were experimentally determined at the Calliope facility's dosimetry laboratory using Fricke and alanine-ESR dosimetric systems. The facility also features a characterization laboratory, where various analyses were performed before and after irradiation. [12]. The irradiation tests are conducted in air at room temperature.

Characterization Techniques

EPR spectra were acquired using a Bruker e-scan EPR spectrometer operating in the X-band at a frequency of 9.4 GHz and a magnetic field range of 3390–3580 G. The samples were placed in standard quartz tubes. All spectra were normalized to the sample mass to analyse the EPR dose-response curves. The measurements are performed for the not irradiated sample and right after the end of the irradiation tests for samples irradiated at the 3 absorbed doses. For each sample, three independent spectra were recorded. The EPR spectra reported as representative for each dose group correspond to samples with the following specific masses: 92.20 mg (0 Gy), 72.42 mg (500 Gy), 133.54 mg (1000 Gy), 74.27 mg (2000 Gy), and 75.40 mg (4000 Gy). These values were used to normalize the signal intensity, ensuring that the observed variations in radical concentration are strictly related to the absorbed dose and not to mass fluctuations.

FTIR spectra of both pistachios and blueberries were recorded using a Perkin-Elmer Spectrum 100 FT-IR spectrometer in ATR mode, over the range of 650–4000 cm^{-1}, before and after irradiation. Background (air) was subtracted from each spectrum prior to analysis. For each sample, three independent spectra were acquired. For each biological replicate, at least two independent spectra were acquired to confirm consistency.

Raman spectra were acquired using a Horiba XploRA Plus spectrometer with a 785 nm excitation laser, a 20-second acquisition time, 50 mW laser power, and a 1200 gr/mm diffraction grating at 10X objective magnification. Prior to analysis, baseline subtraction was applied to the Raman spectra. For each pistachio biological replicate, at least two independent spectra were acquired.

UV-VIS spectra were acquired by using a BioSpectometer by Eppendorfin the wavelength range of 250-800nm, employing standard plastic cuvette with a 1 cm path length. The instrument is a single beam absorption spectrophotometer with a reference beam, equipped with a xenon lamp as light source and a CMOS diode array detector. The blank was represented by the extraction solvent MeOH: H2O (70:30 v/v). For each condition, two spectra were recorded.

It is to be noted that, while a broad dataset was obtained for each analysis, the figures presented in this study illustrate the most representative spectra for each dose group to clearly highlight the radiation-induced modifications.

Results And Discussion

Organic pistachio kernels were subjected to gamma irradiation at absorbed doses of 0, 500, 1000, 2000 and 4000 Gy at a dose rate value of 1 kGy/h to determine the effects of irradiation on fruit properties. Only the green part of the kernel has been irradiated. To investigate the behavior of the radical species induced by gamma irradiation that are responsible for the post-irradiation processes, the EPR spectra (represented by the first derivative of the physical signal) were recorded for the samples before and after irradiation. The measurements performed right after the end of the irradiation tests before and after irradiation at 500, 1000, 2000 and 4000 Gy absorbed are shown in Figure 1.

As shown in Figure 1, an increase in EPR signal intensity is observed for all samples after gamma irradiation. This trend is caused by the formation of free radicals within the pistachio kernel matrices as a direct consequence of their interaction with gamma rays.

The EPR signals were processed and analysed. By integrating twice, the curves reported in Figure 1, the area of the signals, proportional to the number of radicals present in the samples, were obtained. The trend of the signal area, normalized to the sample mass, is reported in Figure 2 as a function of the total absorbed dose.

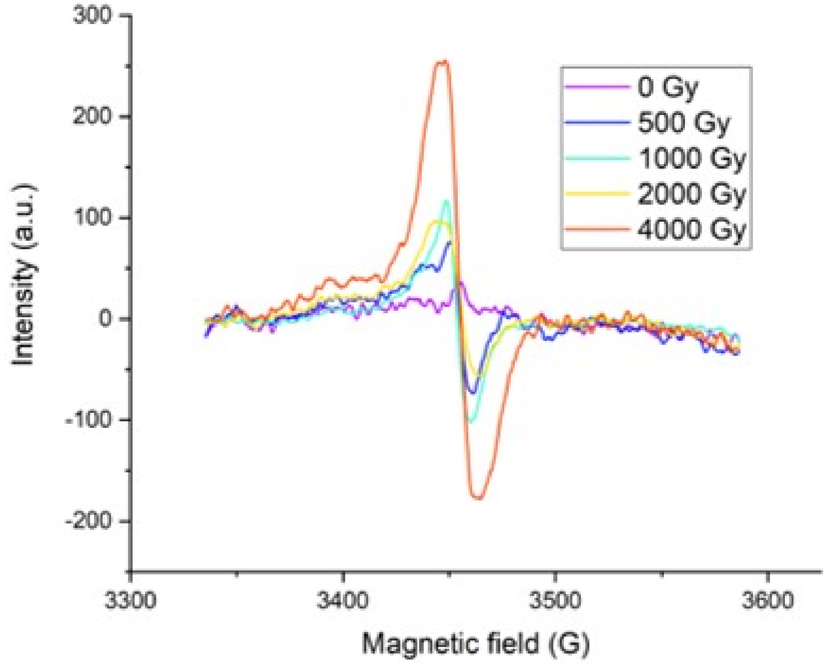

Figure 1: EPR spectra of pistachio kernel before (violet line) and after (blu line for 500 Gy, light blu line for 1000 Gy, yellow line for 2000 Gy and light red line for 4000 Gy) irradiation

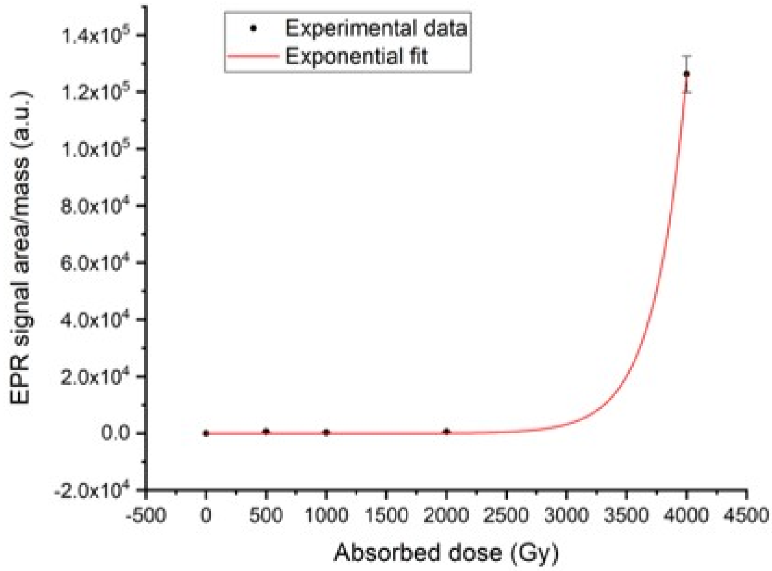

Figure 2: Area of The EPR Signals, Normalized to The Samples Mass, As A Function of The Absorbed Dose at The Total Absorbed Dose Values: 0 Gy, 500 Gy, 1000 Gy, 2000 Gy And 4000 Gy (Black Points). The Continuous Line Represents the Best Fit (Exponential Curve)

The EPR Analysis Indicates a Drastic Increase in Radicals' Formation At 4000 Gy.

This finding suggests that beyond a certain threshold, the interaction between gamma rays and the organic pistachio matrix leads to a nonlinear amplification of radiolytic events. In particular, the exponential increase may be indicative of cumulative damage mechanisms, such as chain scission and the formation of new reactive sites, which in turn generate more radicals [18]. In complex matrices like pistachio, this could reflect a transition from a relatively stable structural regime to a state in which radicals accumulate faster than they are neutralized. Given such considerations, this exponential trend seeks the importance of carefully optimizing irradiation doses to avoid crossing a threshold beyond which molecular degradation becomes significant to food quality and safety.

To evaluate the modifications induced by gamma irradiation on the chemical composition and molecular structure of pistachio fruits, FTIR-ATR measurements and Raman spectroscopy analyses were respectively performed. In Figure 3, the FTIR-ATR spectra of pistachio fruits before and after irradiation at 500, 1000, 2000 and 4000 Gy are reported.

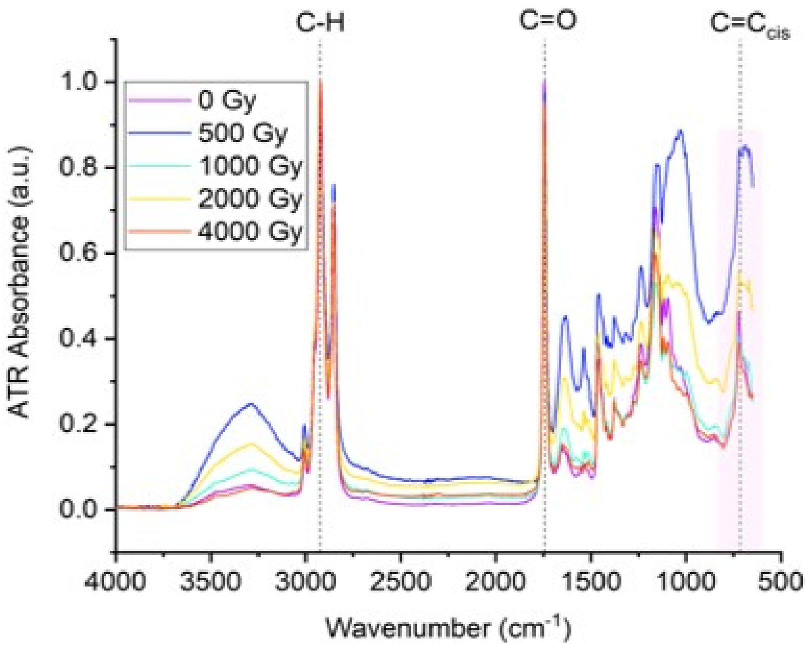

Figure 3: FTIR-ATR spectra of pistachio fruits before irradiation (0 kGy) and after irradiation at 500, 1000, 2000 and 4000 Gy along with main signals' attribution

As shown in Figure 3, the FTIR-ATR spectra of pistachio fruits exhibit distinct absorption bands that correspond to major functional groups in their molecular composition [19,20]. A broad band around 2900 cm^{-1} is attributed to the stretching vibrations of C–H bonds, which are commonly found in aliphatic chains of lipids and other organic compounds [21]. Another prominent band appears near 1730 cm^{-1}, corresponding to the stretching vibration of carbonyl (C=O) groups, typically associated with esters or carboxylic acids, likely originating from lipid or pectin components [21]. Additionally, a signal observed around 750 cm^{-1} is characteristic of out-of-plane bending vibrations of cis-configured carbon–carbon double bonds (C=Ccis), which are often present in unsaturated fatty acids [22]. Notably, when pistachio fruits are subjected to gamma irradiation, the FTIR-ATR spectra show no significant changes. This indicates that the primary chemical structure of the fruit components remains largely stable under irradiation, and the functional groups detected by infrared spectroscopy are not appreciably altered by the treatment.

In Figure 4 the Raman spectra of pistachio fruits are shown before and after irradiation at 500, 1000, 2000 and 4000 Gy.

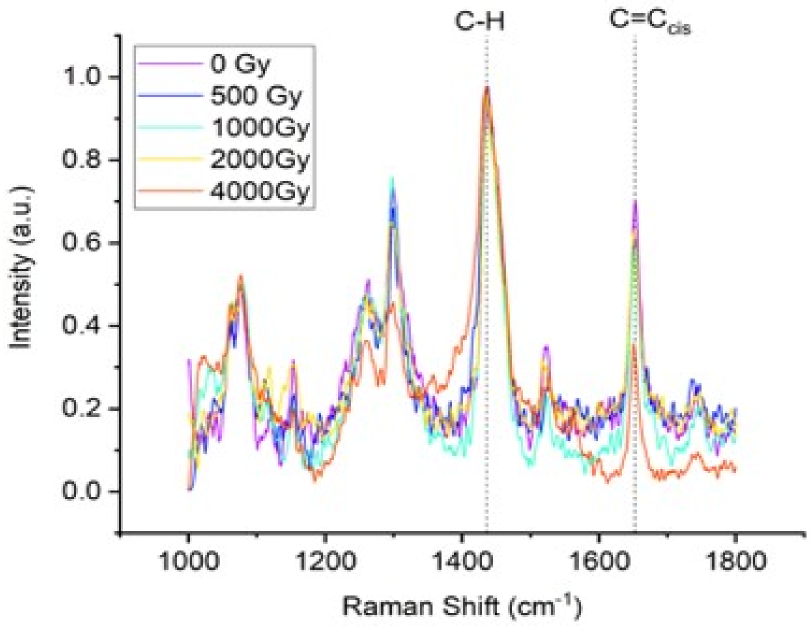

Figure 4: Raman spectra of pistachio fruits before irradiation (0 kGy) and after irradiation at 500, 1000, 2000 and 4000 Gy along with main signals' attribution

The Raman spectra of pistachio fruits before and after gamma irradiation provide significant insights into the structural changes induced by high-energy treatment. Two prominent bands are observed at approximately 1430 cm^{-1} and 1620 cm^{-1}, which are respectively attributed to the vibrational modes of C–H bonds and C=C bonds in the cis-configuration [23,24]. These signals are indicative of the molecular features commonly associated with lipids and unsaturated compounds present in the pistachio matrix.

In non-irradiated samples (0 Gy) and those irradiated up to 2000 Gy, the ratio between the integrated areas of the C=C and C–H bands is kept stable at around 0.8, suggesting no appreciable change in the relative abundance of unsaturated groups compared to aliphatic chains. However, for samples irradiated at 4000 Gy, this ratio is lower than 0.5. This decrease implies a reduction in the number of C=C double bonds, pointing to structural degradation processes that preferentially affect unsaturated moieties. The breakdown or transformation of these unsaturations is consistent with molecular damage or rearrangements initiated by the interaction with gamma photons.

This spectroscopic evidence complements the FTIR-ATR results, which show no major changes in the overall chemical composition of the fruit across the same irradiation range. While FTIR indicates that functional groups such as carbonyls and C–H bonds remain stable, Raman spectroscopy reveals more subtle, yet critical, modifications at the molecular level. In particular, the modification of the unsaturation degree can produce potential changes in the chain entanglement of packing order.

These observations are in agreement with EPR measurements, which demonstrate a significant increase in the number of radical sites only at doses of 4000 Gy and above. Taken together, the combined spectroscopic analyses support the conclusion that up to 2000 Gy, pistachio fruits do not undergo substantial alterations in radical content, compositional chemistry, or molecular structure. In contrast, irradiation at 4000 Gy leads to a marked increase in radical formation and a reduction in unsaturation, without evidence of oxidative degradation as suggested by the stable FTIR profile.

The most plausible mechanism for these changes involves the formation of radicals at the site of double bonds, which then undergo recombination reactions. Rather than reacting with atmospheric oxygen or water-derived radicals, these sites may engage in crosslinking processes, thereby decreasing unsaturation without producing oxidized products. This interpretation points out the importance of combining different spectroscopic techniques to fully understand the molecular-level effects of gamma irradiation on complex biological matrices like pistachio fruits.

Given such considerations, the combination of EPR, Raman and FTIR-ATR spectroscopy analyses indicate that gamma irradiation at the investigated dose range does not cause dramatic or disruptive effects on pistachio kernel. The absence of significant degradation suggests that the structural integrity of the pistachio is largely preserved under these conditions. Organic blueberries were subjected to gamma irradiation at low absorbed doses (0, 250 and 1000 Gy) at a dose rate value of 1kGy/h to evaluate the effects of irradiation on fruit physico-chemical properties. In particular, the stability/conservation of anthocyanins is of considerable interest, as their degradation can have a substantial impact on the color, sensory acceptance, and nutritional value of these fruits. From the UV-visible spectrum (Figure 5) of the polar extract, it was possible to identify characteristic absorption wavelengths corresponding to specific anthocyanins, as reported in literature.

The peak at 282 nm is possibly attributable to delphinidin, cyanidin 3-glucoside, petunidin, malvidin 3-glucoside [25]; peak at 322 nm to pelargonidin-3,5-glucoside, malvidin 3-glucoside [26, 27] and peak at 530 nm to delphynidin-glycoside, cyanidin 3-glucoside and malvidin 3-glucoside [25,26,28]. To better compare the different conditions, spectra were represented in arbitrary units (a.u.) (i.e., units normalized to one, used to represent the ratio of a measured value to the maximum or reference value), set equal to 1.

The real absorbance values, diluted at 1:10, are reported in Table 1. Although the UV-visible spectra of polar extracts showed a decrease in absorbance at 1000 Gy and a slight increase at 250 Gy compared to the untreated sample, these variations were not statistically significant. As highlighted by the histograms (Figure 6), the optical density (O.D.) values at the three analyzed peaks (282 nm, 322 nm and 530 nm) do not show statistically significant differences, suggesting an overall stability in the qualitative profile of polar compounds across intensities. The statistical analysis was performed using GraphPad Prism (version 9.5.0). Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. The level of statistical significance was set at $p < 0.05$. The analysis was conducted on two independent biological replicates.

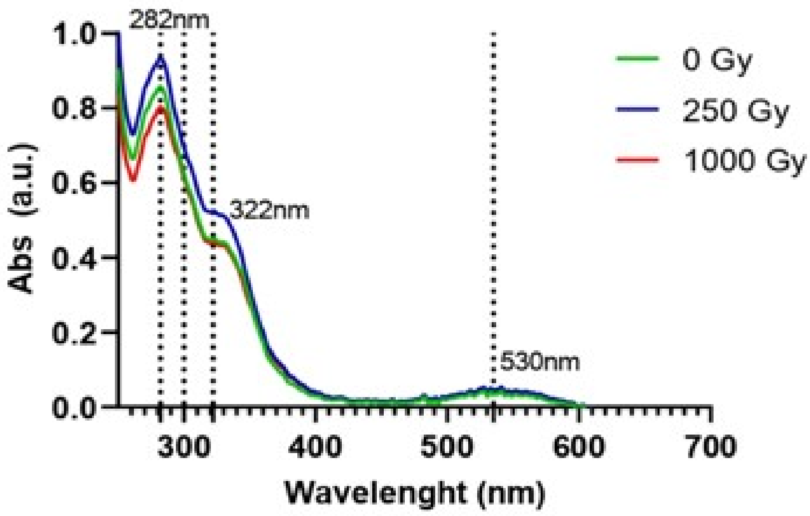

Figure 5: UV-VIS spectra of the hydroalcoholic extracts (MeOH: H_2O 70:30) obtained from lyophilized blueberry samples under different gamma radiation conditions

Table 1: Main Absorption Peaks in the UV-VIS Spectra of The Blueberry Hydroalcoholic Extracts, With the Corresponding Compounds (According to Literature) And the Relative Absorbance Values At 0, 250 And 1000 Gy

Compounds	Literature	λ (nm)	0 Gy	250 Gy	1000 Gy	Δmax 250-0
Delphinidin, Cyanidin 3-glucoside, petunidin, malvidin 3-glucoside	Ryu et al., 2017, Lohachoompol et al., 2004	282 nm	21.251 ± 0.948	23.123 ± 0.608	19.902± 0.296	1.872
Pelargonidin-3,5-glucoside, malvidin 3-glucoside	Jordheim et al., 2007, Lohachoompol et al., 2004	322 nm	11.173 ± 0.58	13.01± 0.324	10.950 ±0.144	1.837
Delphynidin-glycoside, Cyanidin 3-glucoside, malvidin 3-glucoside	Miljković et al., 2018, Jordheim et al., 2007, Lohachoompol et al., 2004	530 nm	0.715 ± 0.115	0.991 ± 0.032	0.818 ± 0.024	0.276

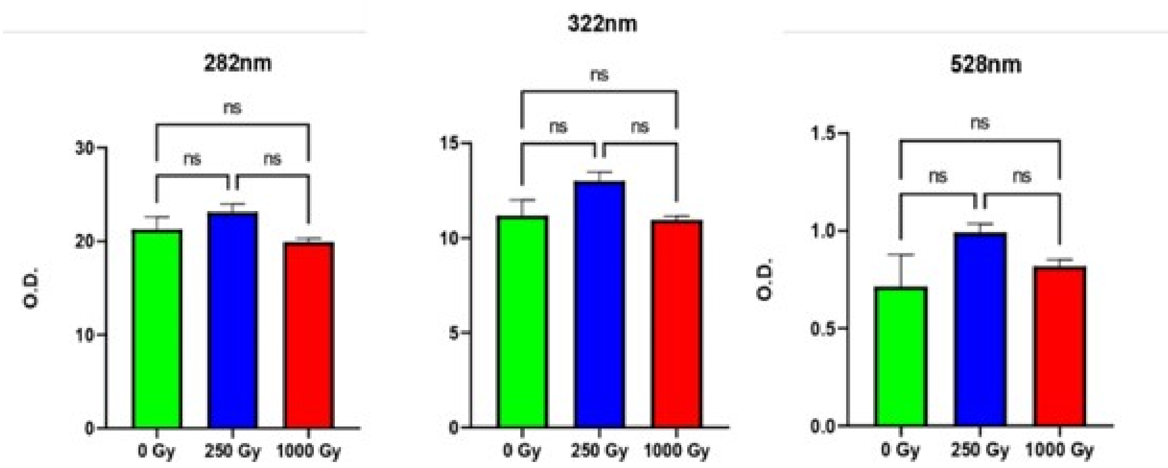

Figure 6: Histogram displays the optical density (O.D.) at characteristic peaks (282nm, 322nm and 528nm) for samples exposed to different gamma radiation doses (0 Gy-green, 250Gy-blue,1000Gy-red). The analysis of variance was performed by one-way ANOVA with multiple comparisons and Tukey's post-hoc test

To evaluate the blueberries modifications in chemical composition, FTIR-ATR measurements were performed on the same lyophilized samples. In Figure 7, the FTIR-ATR spectra are reported.

As shown in Figure 7, the FTIR-ATR signals are typical of blueberries [28,29]. In particular, each spectrum exhibits five absorption signals corresponding to O–H (3000–3700 cm−1) and C–H (2800–3000 cm−1) moieties stretching vibrations, modes of C=O bond (around 1730 cm−1), C=C/H2O bands (around 1620 cm−1) and the carbohydrate fingerprint area (pink box between 800 and 1200 cm−1) [30,31]. Further signals below 800 cm−1 are ascribable to the vibrations of pyranose rings in glycosidic units. To qualitatively evaluate the oxidation effects produced by the gamma irradiation on the chemical composition of blueberries, the carbonyl index (CI) parameter was derived from an absorption band assigned to oxygen-containing moieties, such as the C=O band. The data are referred to the absorbance intensity of the CH peak, that was chosen as a reference signal by considering that the effective breaking of C–H bonds and detachment of radiolytic hydrogen atoms from oat macromolecules occurred at the beginning at doses to 100 kGy. On these bases, the CI values, expressed as the ratio between the absorbance of C=O and CH signals, are around 0.5 for all the samples, indicating no significant increase in oxidation degree after irradiation. Analogously, the Carbohydrate fingerprint region shows no significant modification in absorbance values among the samples before and after irradiation. This suggests that irradiation up to 1000 Gy does not produce relevant changes on glycosidic units, indicating structural preservation of polysaccharides.

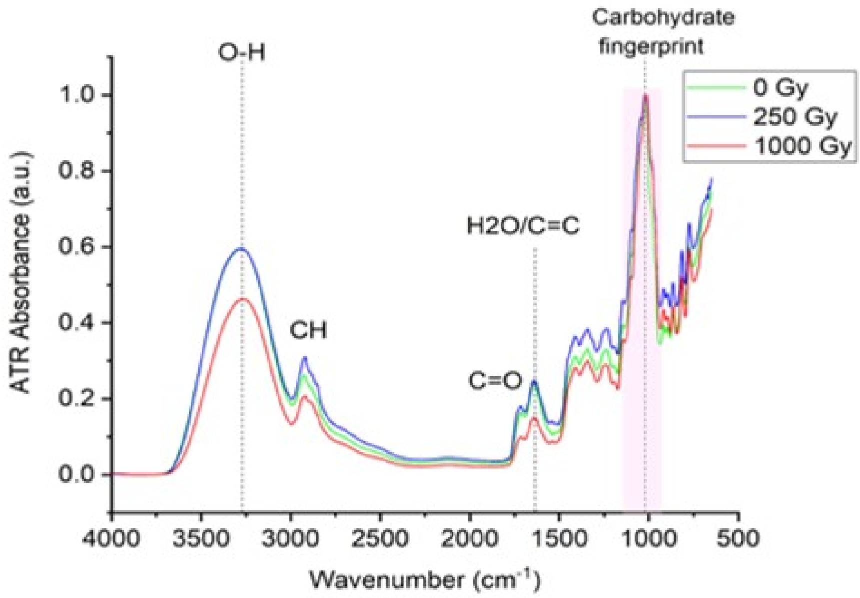

Figure 7: FTIR-ATR Spectra of Lyophilized Blueberries Fruits Before Irradiation (0 Kgy) And After Irradiation At 250, 500 And 1000 Gy Along with Main Signals' Attribution.

Taken together, these observations support the conclusion that gamma irradiation up to 1 kGy does not compromise the molecular integrity of key blueberry components, making it a viable and safe technique for post-harvest preservation.

Conclusions

This work aims to investigate the effects of gamma irradiation on organic pistachios and blueberries using non- or minimally destructive spectroscopic techniques. The combined FTIR-ATR, Raman, and EPR analyses indicate that gamma irradiation up to 2 kGy produces no significant alterations in the molecular structure or chemical composition of pistachio fruits. The stability of C=O and C–H bands in FTIR and the consistent C=C/CH ratio in Raman spectra suggest preservation of functional groups and unsaturations at lower doses. Only at 4 kGy notable changes are detected, including a decrease in unsaturations and an increase in radical formation, without evidence of oxidative degradation. These findings support the potential of gamma irradiation as a safe and effective method to extend pistachio shelf life without compromising molecular integrity.

Analogously, gamma irradiation of blueberries up to a dose of 1 kGy appears promising as a method to extend their shelf life without significantly altering their chemical composition. The UV-visible spectrum shows that the level of anthocyanins in blueberries was not affected by irradiation, indicating that these compounds are stable after irradiation at 250 and 1000 Gy. The FTIR-ATR spectral analysis reveals consistent absorption patterns across all samples, both irradiated and non-irradiated, particularly in regions associated with key functional groups such as O–H, C–H, C=O, and the carbohydrate fingerprint region. The calculated carbonyl index (CI) remains stable at approximately 0.5 across all treatments. This suggests that no substantial oxidation occurs even at the highest irradiation dose tested. Furthermore, the carbohydrate fingerprint region (800–1200 cm^{-1}) and signals attributable to glycosidic units below 800 cm^{-1} show no significant differences in absorbance, indicating structural preservation of polysaccharides.

Author Contributions

Conceptualization: Rocco Carcione, Alessia Cemmi, Silvia Massa, Valentina Mastrobuono, Jessica Scifo.

Methodology: Rocco Carcione, Silvia Massa, Valentina Mastrobuono, Jessica Scifo.

Formal analysis and investigation: Elisabetta Bennici, Beatrice D'Orsi, Rocco Carcione, Silvia Massa, Valentina Mastrobuono, Jessica Scifo.

Writing - original draft preparation: Rocco Carcione, Silvia Massa, Valentina Mastrobuono, Jessica Scifo.

Writing - review and editing: Elisabetta Bennici, Rocco Carcione, Alessia Cemmi, Ilaria Di Sarcina, Beatrice D'Orsi, Leonardo Lanzetta, Silvia Massa, Valentina Mastrobuono, Jessica Scifo.

Resources: Alessia Cemmi.

Supervision: Jessica Scifo.

All authors have read and agreed to the published version of the manuscript.

Declarations

Funding

The authors declare that no funding was received for this work.

Conflict Of Interest

The authors declare no conflicts of interest.

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