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RT/2025/10/ENEA

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Abstract
This study explores the effects of gamma irradiation on wheat samples, with a focus on improving our understanding of its influence on food safety, quality, and preservation. Particular attention is given to food matrices rich in polysaccharides, such as starch, which are key components of wheat. The experimental work was conducted at the Calliope ^{60}Co gamma irradiation facility at the ENEA Casaccia Research Centre (NUC-IRAD-GAM Laboratory, Rome). Employing non-destructive or minimally destructive techniques such as ATR-FTIR, Raman and EPR spectroscopies, we analysed compositional, structural, and radical content changes induced by gamma doses ranging from 0.1 kGy to 10 kGy. As a general trend, gamma irradiation up to 10 kGy does not produce remarkable degradation or depolymerization of wheat components. In more detail, variations in spectral data highlight dose-dependent molecular changes, particularly in carbohydrate and starch structures, with implications for food safety and consumer perception. The findings contribute to advancing gamma irradiation as a non-thermal, sustainable preservation method for diverse food matrices, aligning with global food security and safety goals. These approaches requiring minimal samples preparation can be extended in the development of screening methods for a wide range of polysaccharides in a variety of crops.

Keywords: *gamma irradiation; starch; food irradiation; spectroscopic methods; non-destructive techniques*

STUDIO DEGLI EFFETTI DELL'IRRAGGIAMENTO GAMMA SU CAMPIONI DI FRUMENTO E ALTRE MATRICI ALIMENTARI

Sommario
Questo studio esplora gli effetti dell'irraggiamento gamma su campioni di grano, con l'obiettivo di approfondire la comprensione della sua influenza sulla sicurezza alimentare, sulla qualità e sulla conservazione. Particolare attenzione è rivolta alle matrici alimentari ricche in polisaccaridi, come l'amido, che rappresentano componenti principali del grano. Il lavoro sperimentale è stato condotto presso la facility di irraggiamento gamma Calliope situata nel Centro Ricerche ENEA Casaccia (Roma), utilizzando una sorgente di cobalto-60. Impiegando tecniche non distruttive o in minima parte distruttive come le spettroscopie ATR-FTIR, Raman ed EPR, abbiamo analizzato i cambiamenti composizione, strutturali e del contenuto di radicali indotti da dosi gamma che vanno da 0.1 kGy a 10 kGy. Come tendenza generale, l'irraggiamento gamma fino a 10 kGy non produce una notevole degradazione o depolimerizzazione dei componenti del grano. Più nel dettaglio, le variazioni spettrali evidenziano cambiamenti molecolari dose-dipendenti, in particolare nelle strutture dei carboidrati e dell'amido, con implicazioni per la sicurezza alimentare e la percezione del consumatore. I risultati contribuiscono a promuovere l'irraggiamento gamma come metodo di conservazione non termico e sostenibile per diverse matrici alimentari, in linea con gli obiettivi globali di sicurezza e approvvigionamento alimentare. Questi approcci, che richiedono una preparazione minima dei campioni, possono essere estesi allo sviluppo di metodi di screening per una vasta gamma di polisaccaridi in diverse colture.

Parole chiave: irraggiamento gamma; amido; irraggiamento alimentare; metodi spettroscopici; tecniche non distruttive.

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Introduction

Food safety is a fundamental component of global health and sustainability. As the global population grows and supply chains become more complex, ensuring the safety and quality of food products from production through distribution has become increasingly challenging [1]. Contaminated food can carry a wide range of biological, chemical, and physical hazards, leading to foodborne illnesses that range from mild discomfort to life-threatening conditions. In this context, robust food treatment strategies are essential for protecting public health, reducing economic burdens, and supporting resilient food systems [1].

Food preservation techniques are broadly categorized into thermal and non-thermal methods. Traditional thermal treatments, such as pasteurization or sterilization, rely on heat to eliminate microbial contaminants. However, these methods often degrade heat-sensitive nutrients and alter sensory qualities. In contrast, non-thermal methods such as ionizing radiation offer effective microbial control while preserving nutritional and organoleptic properties. Among these, gamma irradiation has emerged as a particularly promising technology [2][3][4].

Gamma irradiation involves exposing food to high-energy photons, typically from a cobalt-60 (^{60}Co) source, to sterilize or decontaminate it. This process inactivates microorganisms, inhibits sprouting, and slows down enzymatic activity, all without inducing radioactivity or significantly affecting food composition. Other types of ionizing radiation used in the food industry include X-rays (up to 5–7.5 MeV) and electron beams (up to 10 MeV). The key to ensuring food quality lies in precisely controlling the absorbed radiation dose, which directly influences the extent of chemical and structural changes in organic food matrices [4].

Despite its proven efficacy and endorsement by international organizations such as the Food and Agriculture Organization (FAO), International Atomic Energy Agency (IAEA), and World Health Organization (WHO), gamma irradiation remains underutilized in many regions due to varying regulations and persistent consumer misconceptions. In particular, one of the main concerns is whether gamma irradiation may induce side effects on the macromolecular structures of the wheat products, compromising the nutritional qualities.

In this context, this report investigates the secondary effects produced by gamma irradiation on wheat matrices, with particular attention to structural and compositional changes resulting from different radiation doses. The experimental work was conducted at the Calliope Facility at the ENEA Casaccia Research Center, by using a cobalt-60 gamma source.

To characterize potential changes induced by irradiation, the study employed three non-destructive or minimally destructive spectroscopic techniques, such as:

- Attenuated Total Reflection–Fourier Transform InfraRed spectroscopy (*ATR-FTIR*) to detect molecular vibrations and functional group variations;
- *Micro-Raman* spectroscopy to assess structural integrity and detect specific molecular interactions;
- Electron Paramagnetic Resonance (*EPR*) to evaluate the presence and decay of free radicals generated by irradiation process.

Through comparative analysis of irradiated and non-irradiated samples, this report aims to confirm whether gamma irradiation is a safe, effective, and sustainable preservation method that enhances

shelf life and food safety without compromising the samples' integrity [5]. The integrated use of the above-described spectroscopic techniques provides a practical and efficient analytical approach for characterizing complex food matrices rich in polysaccharides[6]. These methods are non-destructive and involve minimal sample preparation, making them highly suitable for routine quality control and safety evaluation in the agrifood industry. Ultimately, the findings contribute to the broader understanding and potential implementation of gamma irradiation as a modern solution to food preservation challenges in the global agri-food industry [7].

1 Irradiation theory on food matrices

Gamma rays, characterized by their high frequency and energy, can penetrate through air at significant distances greater than those of both UV and X-ray photons [5]. As a result, gamma rays can penetrate food products to various depths, making them effective for both surface and bulk sterilization.

This feature of gamma radiation allows for the treatment of complex-shaped food items without the need to remove packaging.

Gamma irradiation has been widely recognized as a powerful tool for sterilization and decontamination by disrupting the covalent bonds within the DNA of viruses and bacteria, leading to their destruction. The most common sources of gamma radiation for food irradiation are radioactive isotopes, such as Cobalt-60 and Cesium-137. While both isotopes can be used, Cobalt-60 is preferred due to its water insolubility, reducing the risk of environmental contamination. In contrast, Cesium-137 is water-soluble, posing a higher risk of environmental contamination [8]. It is important to remember that although it is a radiation, gamma irradiation (from Cobalt-60 or Cesium-137) does not induce radioactivity within any kind of sample.

Gamma rays are particularly well-suited for the processing of bulk food items, as they can penetrate deeply. In contrast, electron beams are typically used for surface irradiation. Both gamma rays and electron beams are authorized for food irradiation, with specific regulations governing their use and the permissible radiation doses.

1.1 Gamma Irradiation: effects on food

Gamma radiation is widely used in the food industry to preserve food and ensure its hygienic safety. This process, known as food irradiation, involves exposing food to gamma radiation to sterilize, reduce contaminants, or increase shelf life. Gamma rays, like other radiations such as X-rays or electron beams (E-beams), employed in food irradiation are capable of breaking chemical and molecular bonds.

This process can influence various food properties such as sensory attributes, nutritional value, and digestion and absorption characteristics. The main effects of gamma radiation on food can be divided into microbiological effects, chemical effects, and impacts on nutritional and sensory properties [9].

1.1.1 Microbiological Effects

One of the primary benefits of gamma irradiation is the ability to significantly reduce microbial loads in food. Pathogenic bacteria such as Salmonella, Listeria monocytogenes, and Escherichia coli, along with molds, yeasts, and parasites, are effectively inactivated by the DNA and RNA-damaging effects of gamma rays. This prevents their replication and growth, ensuring safer food for consumers. Additionally, irradiation is highly effective in controlling pests, including worms and larvae that infest produce and meat. While spore-forming bacteria are more resistant to gamma radiation than vegetative bacteria, irradiation can still reduce spore loads, thereby mitigating spoilage and contamination risks. At high doses (from 25 kGy upwards), gamma radiation achieves complete sterilization, enabling the long-term storage of foods such as spices and herbs without refrigeration [10].

1.1.2 Chemical Effects

Gamma irradiation induces radiolysis reactions in food by breaking chemical bonds in organic molecules. This process generates free radicals, highly reactive species that typically recombine quickly without causing considerable damage. The chemical changes caused by irradiation are minimal and comparable to those that occur during cooking or drying.

While some degradation products may form, they are present in negligible amounts and are non-toxic, as confirmed by extensive toxicological studies. Fats in foods can undergo oxidation during irradiation, leading to rancidity in high-fat products like meat and dairy. However, these effects can be mitigated by using low radiation doses or packaging methods that limit oxygen exposure [11].

1.1.3 Nutritional Effects

The effects of food irradiation on nutrients have been the subject of extensive research, yielding significant insights and advancements.

- *proteins*: gamma irradiation affects proteins by altering their structure through reactions like oxidation, deamination, or cross-linking. These changes can improve the digestibility and bioavailability of proteins, making them easier for the body to absorb. However, doses greater than 10 kGy can cause significant structural damage, reducing functional properties like solubility or emulsification in certain proteins;
- *carbohydrates*: carbohydrates, particularly complex ones like starch, show structural changes under irradiation. Doses lower than 10 kGy can slightly increase digestibility, while higher doses can degrade molecular structures, such as the double helices in starch granules, or generate by-products from crystal radiolysis. These effects are typically dose-dependent but do not significantly alter the nutritional value of most carbohydrate-rich foods;
- *lipids*: lipids are more susceptible to irradiation-induced oxidation, particularly unsaturated fatty acids. This process can produce volatile compounds that lead to off-flavors in lipid-rich

foods. However, essential fatty acids like omega-3 and omega-6 remain stable under low-dose irradiation. Packaging that minimizes oxygen exposure can reduce oxidation;

- *vitamins*: among nutrients, vitamins are the most sensitive to gamma irradiation. Water-soluble vitamins, like vitamin C and certain B vitamins, can experience slight losses, as can fat-soluble vitamins such as vitamin E. Despite this, the reductions are comparable to those seen in other food processing methods like cooking or drying. Proper dose management can preserve most of the vitamin content;
- *bioactive compounds*: gamma irradiation can positively impact bioactive compounds like phenols and flavonoids by enhancing their extractability or antioxidant activity. In honey and strawberries, for instance, phenolic content and antioxidant capacity increased post-irradiation. The impact varies across food types and depends on the dose used.

Overall, gamma irradiation preserves the nutritional quality of food effectively while offering significant benefits for food safety and shelf life. Its impacts are mild and manageable through careful control of doses and environmental conditions during treatment [12], [13].

1.1.4 Sensory Effects

Gamma irradiation may slightly alter the sensory attributes of food, depending on the dose and the type of food. Changes in colour can occur, especially in foods with sensitive pigments. For instance, meats may darken or appear redder, while fruits and vegetables typically undergo minimal colour changes. Taste and odour can also be affected at higher doses, particularly in fatty foods, due to fat oxidation, which can impart an "irradiated" flavour or smell.

These effects are often minimized by using moderate doses or vacuum packaging to limit oxygen exposure. Textural changes are another potential effect, particularly in fruits and vegetables, which may become softer after irradiation. However, the doses typically used for preservation and microbial safety rarely cause significant textural alterations [14].

In summary, gamma irradiation is a versatile and effective technology for enhancing food safety, reducing spoilage, and extending shelf life. Its effects on food are well-researched, with minimal impacts on nutritional and sensory properties when applied at appropriate doses. By carefully balancing dose levels and packaging techniques, gamma irradiation ensures safe, high-quality food for consumers [15].

Food irradiation has been approved by various international agencies, including the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the Food and Drug Administration (FDA) in the United States. Numerous studies have confirmed that irradiated food does not become radioactive and that the chemical changes induced by irradiation do not pose a risk to human health. The use of gamma radiation is considered safe and strictly controlled to avoid significant nutritional or sensory harm.

Gamma irradiation is an effective and safe technology for food preservation, with minimal effects on nutritional value and organoleptic properties. The main concerns are the possible loss of certain vitamins and fat oxidation, which can be minimized using appropriate doses and optimal packaging techniques [12], [16].

1.2. Gamma irradiation: wheat samples

Starch is a key component of wheat and has been widely studied in its pure form to understand how gamma irradiation affects its structure and properties. Research shows that irradiation causes changes like molecular breakdown and rearrangement, especially depending on the dose. Low doses tend to affect the less organized (amorphous) regions, while higher doses disrupt the crystalline parts. These changes can reduce viscosity, increase solubility, and make starch more easily digestible by enzymes.

However, in real wheat grains—which are complex systems made of starch, proteins, fats, and other molecules—the effects of irradiation are harder to predict. Interactions between starch and other components, such as proteins, can either stabilize or destabilize the overall structure.

Most research so far has focused on how irradiation improves hygiene by reducing microbes, but less attention has been given to the molecular-level changes in starch and proteins. There's also a lack of fast, non-destructive tools and protocols to assess these changes, which limits industrial use. Finally, public concern about food irradiation remains a challenge, and more studies proving its safety at the molecular and nutritional levels could help improve acceptance.

2 Methods

The wheat samples are characterized by means of different experimental techniques (as described in the following paragraphs) before and after gamma exposure to evaluate the changes induced by the radiation treatment.

2.1 Calliope gamma irradiation facility

The Calliope gamma irradiation facility is a pool-type plant equipped with a Cobalt-60 (mean energy ≅ $1{,}25\ MeV$) radioisotope source, who emits two photons, in coincidence, of $1{,}17$ and $1{,}33\ MeV$, array in a high volume shielded cell [17], [18], [19].

Figure 1 shows the Calliope Cobalt-60 source shielded by the water (see Fig. 1a, source in the pool) and

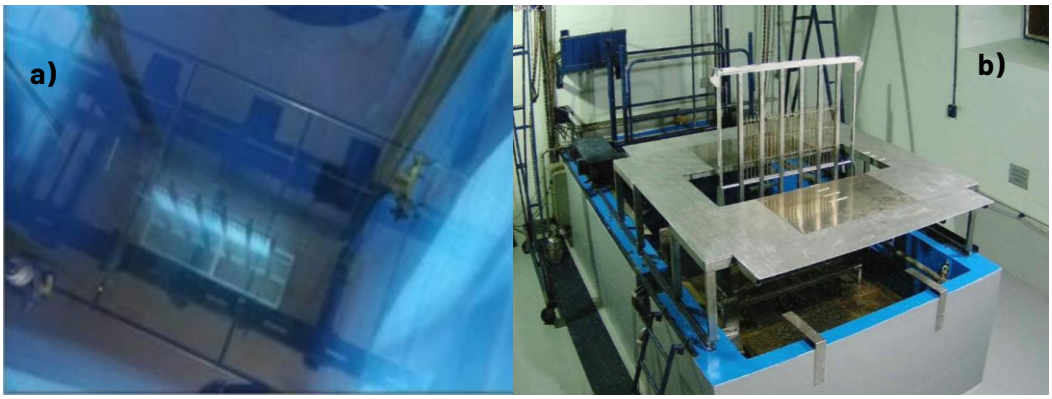

Figure 1-Photos showing a) Calliope source rack in the pool and b) source rack within the irradiation cell (picture acquired by remote camera).

the bunker during an irradiation test (Fig. 1b, source outside the pool). This source is configured in a plane geometry with 25 source rods (active area: $41\ cm\ \times 90\ cm$) arranged within a large irradiation cell measuring $7\ m \times 6\ m \times 3{,}9\ m$.

The maximum licensed activity for the Calliope plant is 3.70 x 10^{15} Bq (100 kCi). Currently (May 2025), the facility offers a wide range of dose rates, from low levels around $0{,}1\ Gy/h$ to high intensities reaching 5 kGy/h. These values can be experimentally controlled by variyng the position of the samples within the irradiation cell and/or with a suitable lead shielding.

At Calliope facility, several dosimetric systems are used, depending on the absorbed dose range of interest (e.g., for sterilization, mutation induction, or preservation):

Fricke solution (20 – 200 Gy), Red Perspex (5 – 50 kGy) and radiochromic (1 kGy – 3 MGy), alanine-ESR (1 Gy – 500 kGy), Thermo Luminescent Dosimetry TLD (0.1 mGy – 100 Gy) and electronic RADFET (0.01 – 1000 Gy) dosimeters [19].

2.2 Characterization Techniques

The wheat samples characterization procedure described in this technical report is performed by the following experimental techniques.

2.2.1 FTIR-ATR spectroscopy

Fourier Transform InfraRed (FTIR) spectroscopy is useful to investigate the chemical composition and the oxidation states variation of samples. This technique uses the interaction between infrared light and matter and allows to obtain the frequencies of light that are absorbed by the sample to identify and quantify different chemical species [12]. Infrared spectra were collected using a PerkinElmer spectrometer equipped with a horizontal Attenuated Total Reflectance (ATR) with a crystal of zinc selenide ($ZnSe$), with an angle of incidence of the beam of infrared light on the crystal of 45°.

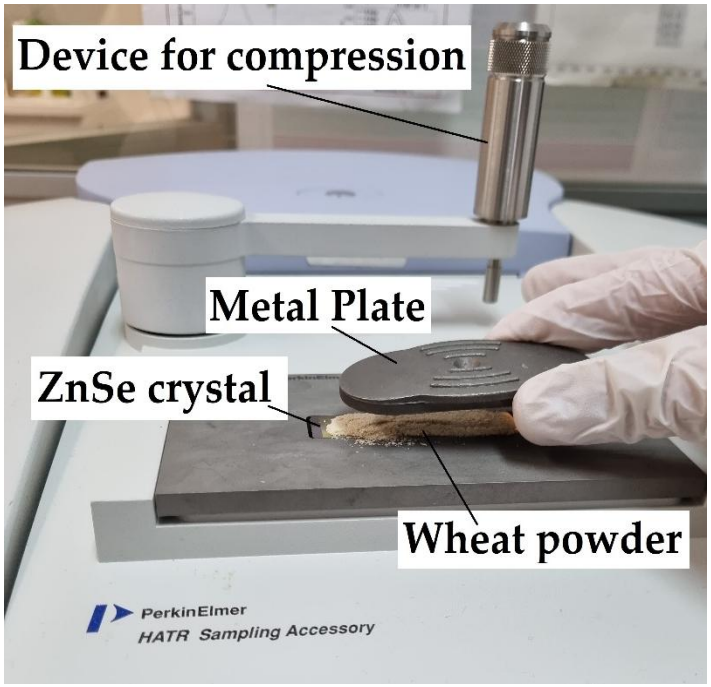

Figure 2-Photo showing the experimental setup for the FTIR-ATR measurements of wheat samples.

Spectra were recorded in the range of $650\,cm^{-1}$ to $4000\,cm^{-1}$. Before each acquisition, the spectrometer performed a background measurement, which was then automatically subtracted from the sample measurements to have a precise and clean signal.

Then for each sample have been acquired the spectres, this process was applied to each sample and to each irradiation dose included the reference ones.

The sample of grain in form of powder was positioned along the crystal, the quantity was not calculated but was enough to cover the crystal for a length of $\approx 3\,\mathrm{cm}$, and then to prevent the possibility of falsifying the results, a metal plate was positioned on top of the sample. During the measurement, a

force is applied on the top of the metal plate.to compress the sample on the crystal. All the measurements were done with the same value of compression Gauge Force (equal to 75).

The pressure applied to the sample helped spread the grain across the crystal surface, allowing the analysis of a uniform surface.

2.2.2 micro-Raman spectroscopy

The HORIBA XploRA PLUS Micro Raman spectrometer is a confocal microscope, which can operate in both transmission and reflection modes. It was used for imaging and collect vibrational modes of samples.

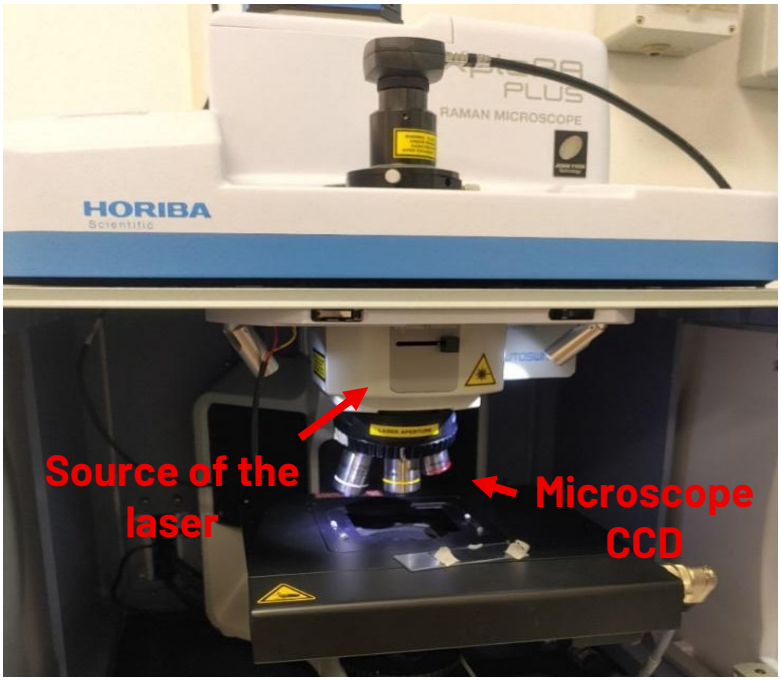

Figure 3-Micro Raman spectrometer (HORIBA) at Calliope facility.

For the Raman analysis protocol, the procedures for image acquisition and spectral collection were optimized. Optical images of the samples were captured using a 10x objective microscope. Raman spectra were recorded with 10x objective magnification, covering the spectral range of $200\ cm^{-1}$ to $3100\ cm^{-1}$. The analysis was conducted using a $785\ nm$ laser excitation with a power of 50 mW for an acquisition time of 20 seconds.

To analyse the wheat powders, the samples were homogeneously distributed on a glass slide before starting the measurements. Figure 3 shows the Micro Raman spectrometer apparatus.

2.2.3. EPR spectroscopy

Electron Paramagnetic Resonance (EPR) spectroscopy technique is useful to investigate the behavior of free radical species. The EPR spectra are the first derivative of the physical signal (absorption of photons which allows the transition of the unpaired electron of a molecule from one energy state to another [13]). The EPR signal is proportional to the free radicals' number presents in the samples. In

this study, ESR spectra were obtained by using an ESR Bruker *e*-scan spectrometer operating in the X-band with a frequency of 9.4 GHz, microwave power of 0.14 mW and magnetic field in the range from 3385 G to 3590 G.

These parameters were settled, as shown in Figure 4, by using a certain amount of the unirradiated samples (around 100 mg).

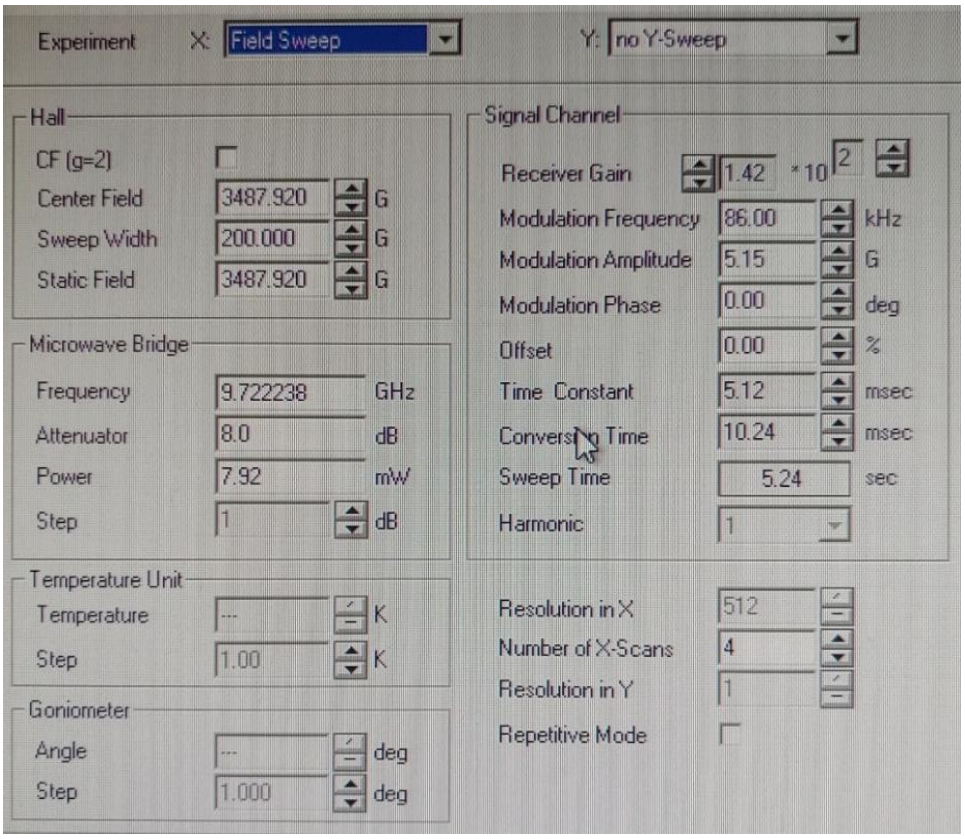

Figure 4-Set of parameters for EPR spectroscopy for wheat samples.

3 Procedures and Results

The sample preparation and the optimized measurements procedures are described in the following with the specific results for each technique.

3.1 Sample preparation

Four types of wheat matrices were processed milling to achieve a fine, uniform powder. These milled wheat samples were used as received for further analyses, without additional modifications. Each

matrix differs in its chemical composition and agricultural origin. The specific details of the wheat samples, including names and descriptions, are summarized in Table 1.

Table 1-Description of the four grain samples.

Sample	Type of grain	Varieties	Origin and Region
M06	Durum Wheat	Iride	Italy-Basilicata
M23	Durum Wheat	Iride	Italy-Puglia
M28	Organic soft wheat	/	Italy-Toscana
M30	Soft Wheat	Sofru	Croatia

Wheat samples were irradiated at 0.1, 1, 4.5, and 10 kGy absorbed dose at a dose rate value of 0.5 kGy/h. All absorbed dose and dose rate values are referred to as water. The dose rate values were experimentally determined by the alanine–EPR system. The irradiation tests were performed in air at room temperature.

Before the irradiation test, the various unirradiated grain samples were studied using three spectrometric techniques: ATR-FTIR, Micro Raman and EPR spectroscopies.

This was done to analyse the samples and record their reference spectrum, which were compared with the spectra of irradiated samples, to find changes in the peaks amplitude that led to degradation of the corresponding bond.

3.2 ATR-FTIR analysis

ATR-FTIR analysis was employed to evaluate the effects produced by gamma irradiation on the chemical composition of wheat samples after irradiation.

Table 2 shows the list of the functional groups useful to evaluate samples' chemical composition degradation after irradiation.

Table 2-Set of wavenumbers of the reference functional groups used on FTIR spectrometry.

Functional Group	Wavenumbers (cm^{-1})
$C-O, C-C$	~ 1030
$C=C$	~ 1600
$C=O$	~ 1730
$C-H$	~ 2900
$O-H$	~ 3500

The Figure 5 shows the four spectra of the wheat sample, in which the structural composition of the samples with all the main functional group were highlighted (details in Table 2).

Independently on wheat crop origin and nature, each sample displayed similar spectral features, suggesting comparable chemical compositions with slight variations that could be unique to each sample.

Figure 6 shows the FTIR-ATR spectra for each typology of wheat sample before and after irradiation at 0.1, 1, 4 and 10 kGy.

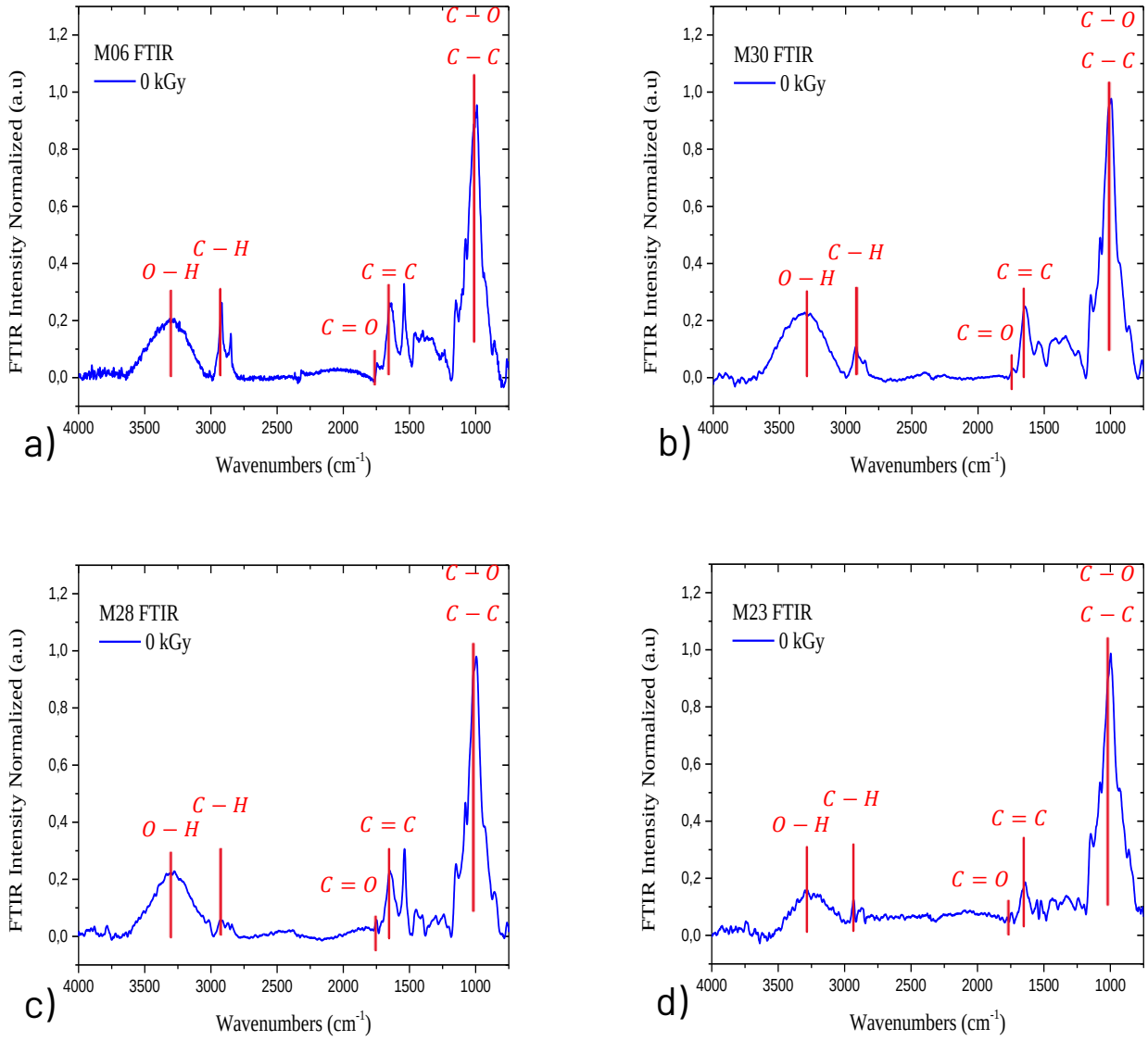

Figure 5-FTIR Spectres of 0 kGy wheat samples a) M06, b) M30, c) M28 and d) M23, with characteristic peaks highlighted.

The spectra reported in Figure 6 highlight the presence of specific chemical bonds or functional groups within the wheat samples. From the analysis of the peaks, it is possible to observe in Figure 6 that there is no significant change in the spectra of the M30 and M28 samples after irradiation, while for samples M23 and M06, small variations in the $O-H$ functional group peak can be observed.

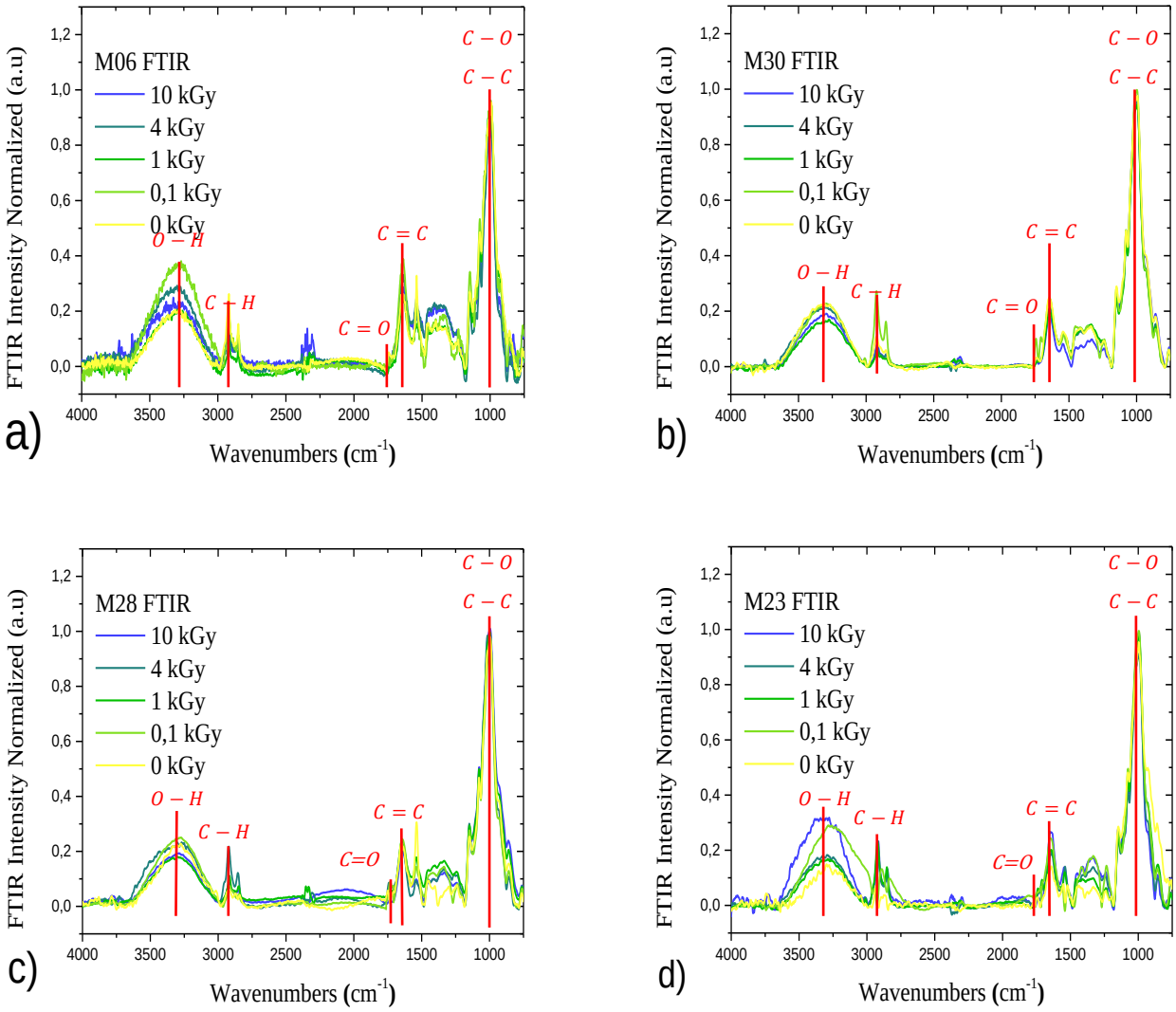

Figure 6-FTIR Spectres of the four wheats a) M06, b) M30, c) M28 and d) M23, highlighting the main peaks of the wheat.

The observed changes in the FTIR signals associated with OH groups can reasonably be attributed to the breaking of bonds caused by gamma irradiation. This phenomenon is consistent with the known effects of ionizing radiation, which induces structural modifications by disrupting hydrogen bonds and cleaving molecular linkages, particularly in hydroxyl-rich regions.

Furthermore, the potential contribution of adsorbed water molecules interacting with radical species produced on polar groups by irradiation cannot be excluded. These interactions could amplify or shift the FTIR signals in regions related to OH stretching and bending vibrations.

FTIR spectra enable the evaluation of gamma irradiation effects on the chemical composition of wheat samples by analyzing changes in the intensity of key functional group peaks:

- *Hydroxyl Groups* (O-H Stretching, ≈ 3400 cm^{-1}): a decrease in intensity at higher doses (4 kGy and 10 kGy) could be attributed to degradation of hydroxyl-containing compounds or to bond breakage induced by irradiation;
- *Carbonyl Groups* ($C = O$ Stretching, $\approx 1700\ cm^{-1}$): variations in peak intensities for certain wheat types, such as M23 and M06, indicate potential oxidative effects leading to carbonyl

compound formation. These effects align with the generation of free radicals under ionizing radiation;

- $C-H$ *Vibrations* ($\approx 2900\ \mathrm{cm}^{-1}$): minimal changes suggest that the hydrocarbon backbones of macromolecules remain intact, preserving the fundamental molecular structure of carbohydrates, proteins and lipids.

Overall, the FTIR results confirm that gamma irradiation at doses below regulatory limits does not significantly alter the fundamental chemical composition of wheat. This finding is crucial for the maintenance of wheat's nutritional quality.

3.3 Micro-Raman analysis

Figure 7 shows the optical microscope images collected on the four sample of wheat with a 10x objective.

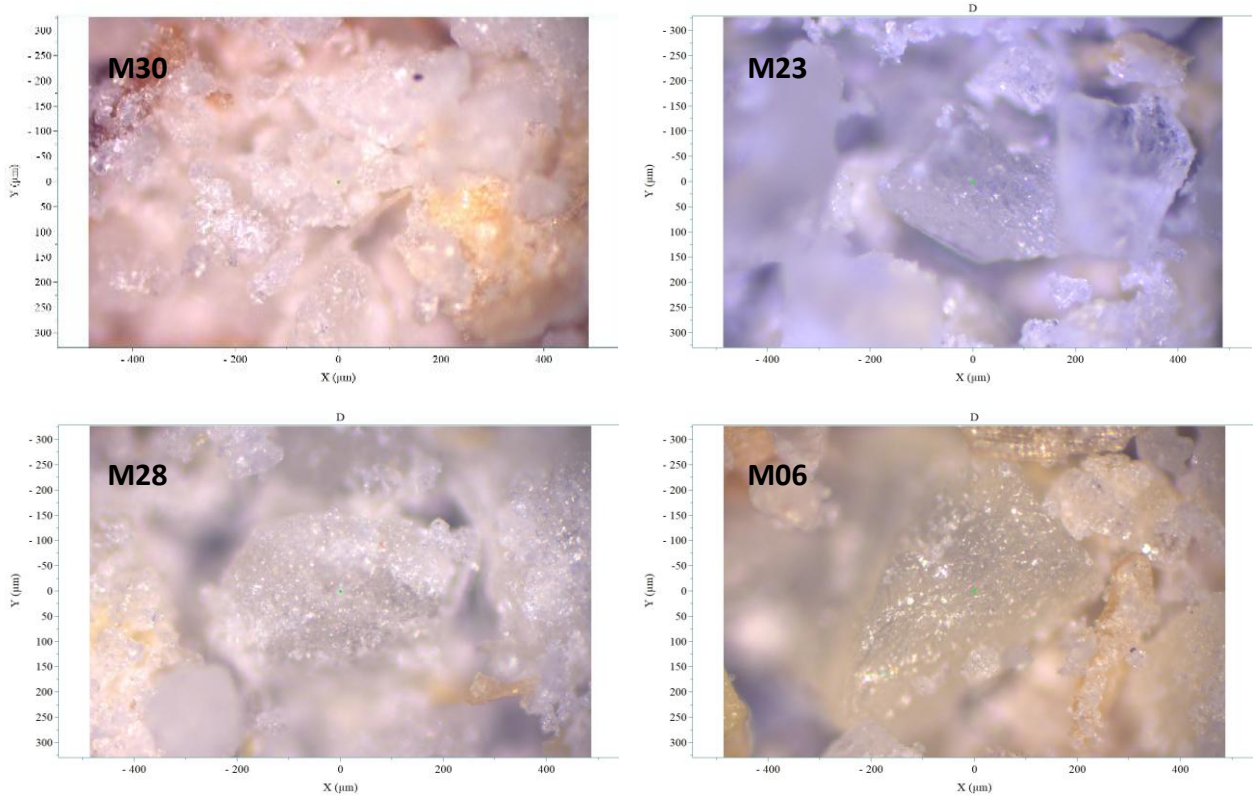

Figure 7-Wheat samples optical images acquired with the 10x objective of the microscope of the HORIBA XploRA PLUS.

The 10x objective was selected to capture the Raman spectra while also providing sufficient morphological detail of the samples. This magnification allowed for effective targeting of areas free from larger aggregates, which could otherwise produce fluorescence and interfere with or distort the spectral signal.

To maximize the information obtained from the Raman spectra, the spectral range of $200 - 3000\ cm^{-1}$ was selected, as it includes all the main diagnostic peaks relevant to starch-based samples.

In Table 3 are reported the main vibrational bands of wheat samples and their assignments.

Table 3-Vibrational bands and their assignments in Raman spectra of wheat kernels [20].

Band (cm^{-1})	**Vibrational Mode**	**Assignment**
480	δ(C-C-C) + τ(C-O) scissoring of C-C-C and out-of-plane bending of C-O	Carbohydrates: Starch line fingerprint
536	S-S gauche-gauche-trans	Protein
576	δ(C–C–O) + τ(C–O)	Carbohydrates
616	δ(C-C-O)	Carbohydrates
716	δ(C-C-O)	Carbohydrates; related to glycosidic ring skeletal deformations
764	δ(C-C-O)	Carbohydrates
864	δ(C-C-H) + δ(C-O-C)	Carbohydrates; glycosidic bond; anomeric region; (C-O-C) skeletal mode of α-anomers
940	Skeletal modes; δ(C-O-C) + δ(C-O-H) + ν(C-O)	Carbohydrates; α-1,4 glycosidic linkages
1004	ν3(C-CH3 stretching)	Carotenoids; phenylalanine; Proteins
1088	ν(C–O) + ν(C–C) + δ(C–O–H)	Carbohydrates
1124	ν(C–O) + ν(C–C) + δ(C–O–H)	Carbohydrates
1264	ν(C–O) + ν(C–C) + δ(C–O–H)	Carbohydrates; Guaiacyl ring breathing,
1342	ν(C–O); δ(C–O–H)	Carbohydrates
1380	δ(C–O–H)	Carbohydrates
1460	δ(CH) + δ(CH2) + δ(C–O–H)	Carbohydrates; aliphatic molecules; Lignin
1556	–C=C– (in plane)	Carotenoids
1600	ν(C–C) aromatic ring + σ(CH)	Gluten; Proteins
1632	C=C–C (ring) or C=O stretching, amide I	Gluten; Proteins

For each sample at every irradiation dose, three spectra were acquired from different areas to ensure the reliability and reproducibility of the results. The Raman spectra of samples are reported in Figure 8

before and after irradiation at 0.1, 1, 4 and 10 kGy. For a better visualization, the Raman spectra were background subtracted and smoothed by means of the LabSpec6 software.

Figure 8 shows the samples Raman spectra, which exhibit the specific characteristic peaks of wheat:

- the C-O-C associated with polysaccharides, indicating presence of starch components;
- the starch line, representing the starch content in the samples.

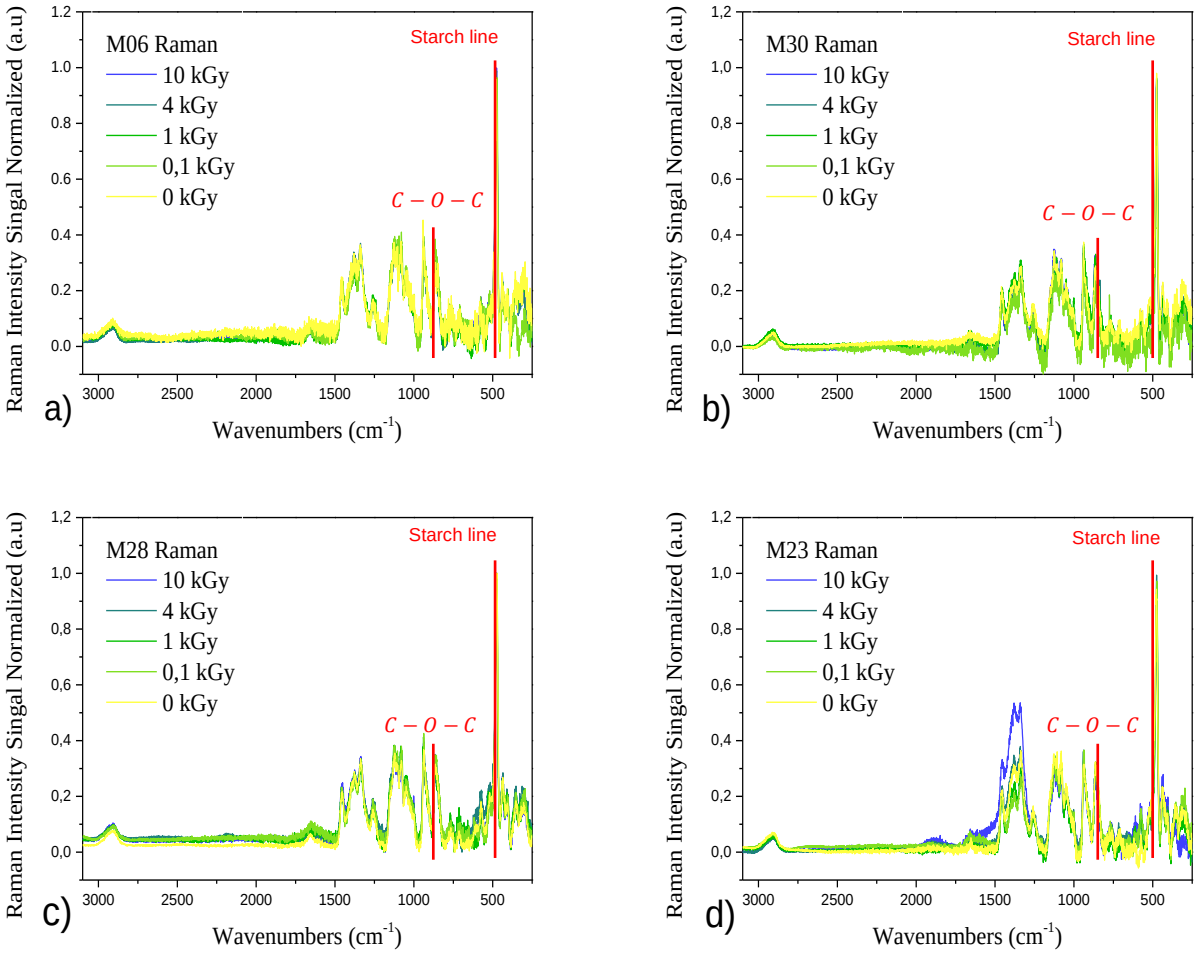

Figure 8-Raman Spectres of the four wheats M06, M30, M28 and M23, highlighting the main peaks of the wheat.

From the spectra in Figure 8, it is possible to identify the presence of specific molecular structures within the wheat samples. As expected, each spectrum has similar features, indicating that starch is the main component for each typology of samples.

The highlighted peaks correspond to these functional groups are C-O-C and to the starch line, which corresponds to ring deformation and skeletal vibrations of the glucose units in starch. The negligible variation observed in the Raman spectra after gamma irradiation suggests that the structural integrity of the molecular components in wheat is preserved under the experimental conditions used.

To evaluate and compare the variation of the wheat structural features, the Raman spectra were deconvolved by several Lorentz line functions by means of LabSpec 6 software to derive the peaks

parameters of position, area and amplitude. In Figure 9 representative deconvolved Raman spectra of wheat samples after irradiation at 10 kGy are reported.

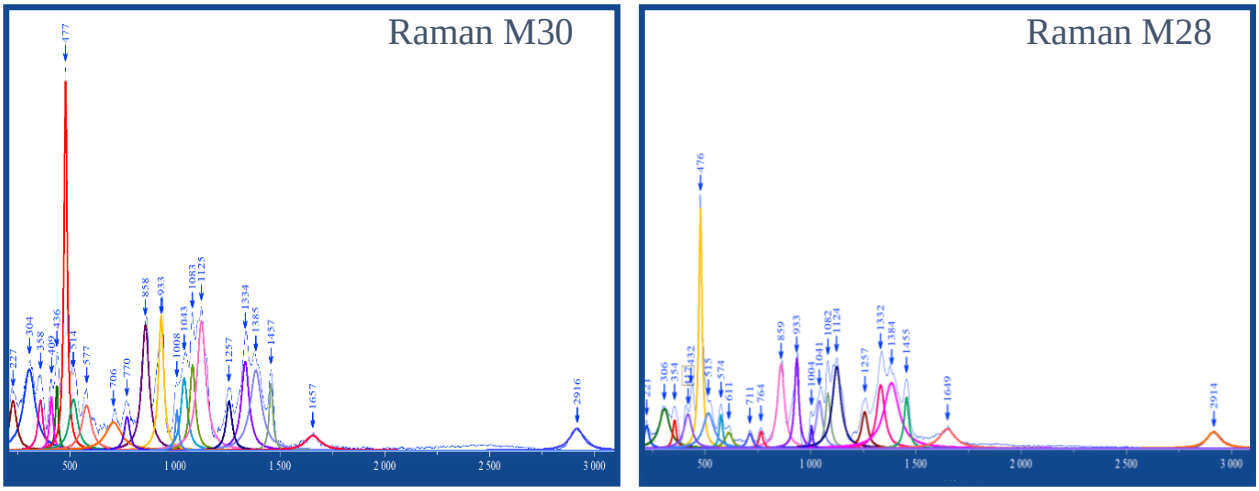

Figure 9-Deconvolutions of the Raman spectres M30 and M28 irradiated at 10 kGy, done by using the software LabSpec 6.

To evaluate the possible degradation of the wheat molecular structural features, the ratio between the area of the peaks at $476\ cm^{-1}$ (starch line) and at $858\ cm^{-1}$ (C-O-C glycosidic bonds) was used as an indicator. As a general trend, no significant changes in the molecular structure of the wheat samples were observed up to 10 kGy. Therefore, the peak parameters derived from the deconvolution are reported in the table 5 only for the non-irradiated samples and those irradiated at 10 kGy. In particular, Table 4 shows the values of the area of the peak at $476\ cm^{-1}$ (starch line) and the peak at $858\ cm^{-1}$ (C-O-C) and the ratio between these area values for the samples before and after irradiation at 10 kGy.

Table 4-Evaluation of the characteristics peaks of wheat by Raman Spectroscopy rate and variation of rate of the peaks area derived for the wheat samples at 0 kGy and at 10 kGy.

Raman	**Area of peak 476 cm^{-1} *Starch Line***	**Area of peak 858 cm^{-1} *C-O-C***	**Rate of areas of the peaks**	**Variation of rate of areas after irradiation**
M23-0kGy	238653	140478	1,69889	-
M23-10kGy	53106,7	46671,4	1,13789	1,493000989
M06-0kGy	33824,1	24443,3	1,38378	-
M06-10kGy	74620,2	60349,8	1,23646	1,119143658
M28-0kGy	117582	87450,7	1,34455	-
M28-10kGy	63591,8	48848,2	1,30182	1,032820858
M30-0kGy	66638,2	49638,2	1,34248	-
M30-10kGy	73790,1	56472,8	1,30665	1,02742104

The ratio values around 1 for each type of samples corroborates that molecular structural integrity of the wheat components is preserved under the adopted irradiation conditions. The M23 sample exhibits a ratio of approximately 1.5, indicating a greater susceptibility to molecular structure degradation upon irradiation. Since M23 corresponds to a durum wheat sample, this higher sensitivity is likely linked to the specific molecular interactions within its matrix. Durum wheat is known to possess a denser gluten protein network than soft wheat, which may favor the formation of reactive species upon irradiation. These species, originating from gluten or other proteins, could interact with surrounding starch molecules, promoting their structural degradation or rearrangement.

3.4 EPR analysis

EPR spectroscopy, based on the phenomenon of spin electron resonance (or paramagnetic electron resonance), allows to study free radicals, such as atoms or molecules containing an unpaired electron. In particular, EPR spectroscopy is crucial to identify the types of paramagnetic species present in the samples and measure their concentrations. The variation of free radicals' concentration over time allows to study the and radical content decay before and after the irradiation.

The free radicals produced by gamma irradiation in wheat matrices are carbon-based radicals, located along the units of the samples' macromolecules.

The spectra were acquired right after the end of the irradiation tests. Then, the spectra were recorder at regular interval of times, such as at 7, 11, 18 and 25 days after the irradiation. Only for the samples irradiated at $4\ kGy$ and $10\ kGy$ the measures were performed at 125 days after the irradiation.

In Figure 10 are reported the EPR spectra for each sample at 0, 0.1, 1, 4 and 10 kGy and the EPR signal's decay for samples irradiated at 10 kGy.

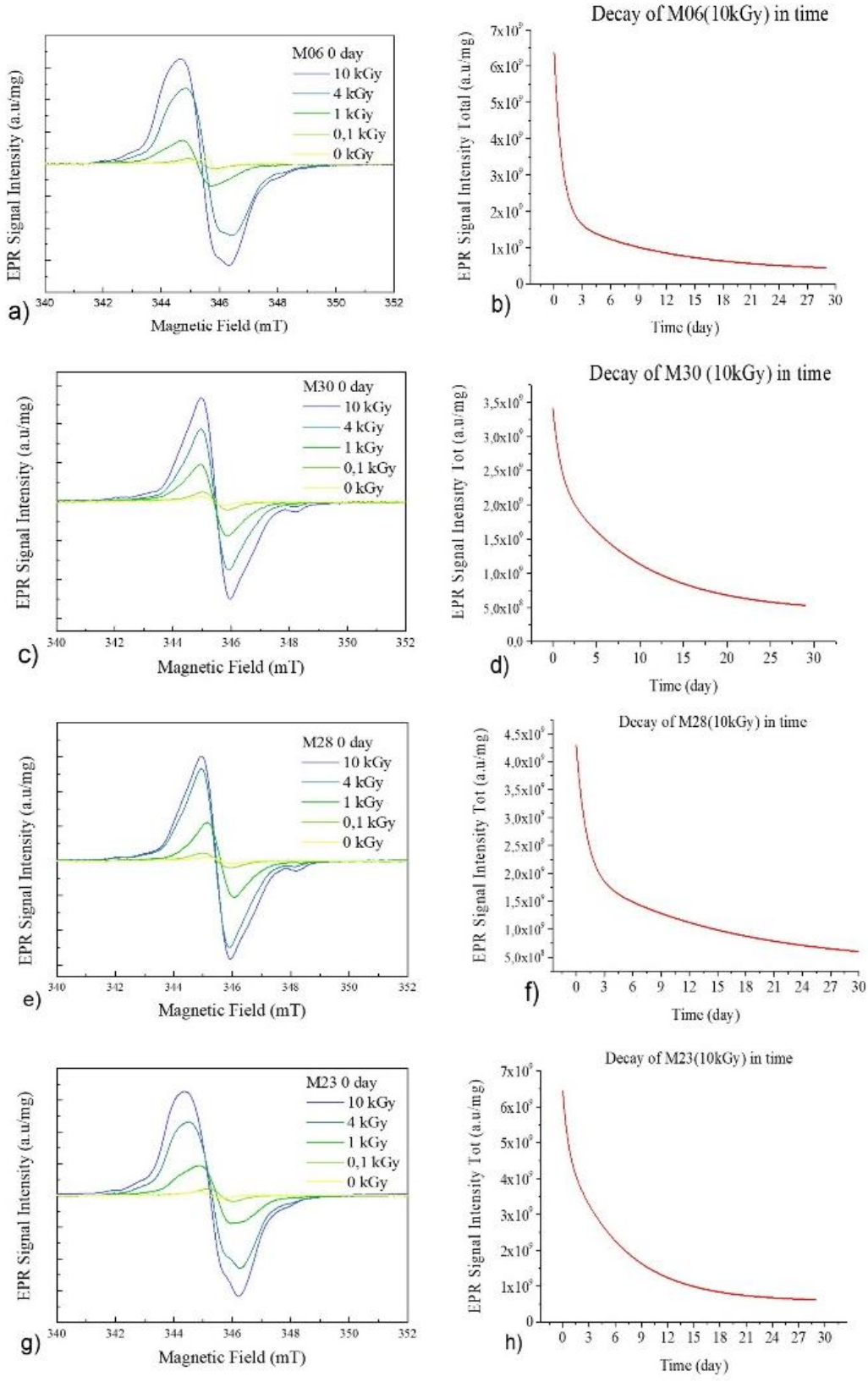

Figure 10-EPR spectra for (a) M06, (c) M30, (e) M28, (g) M23 samples at 0, 0.1, 1, 4 and 10 kGy; EPR signal's decay for (b) M06, (d) M30, (f) M28, (h) M23 samples irradiated at 10 kGy.

From the spectra in Figure 10 a-c-e-g, it's possible to observe that the intensity of the EPR signal increases as a function of the irradiation dose for each typology of wheat samples. Independently on the nature and origin of the wheat matrix, this finding indicates a direct relationship between radiation dose and the concentration of radiation-induced free radicals in the wheat samples. The position of the EPR peaks indicates the presence of organic radicals, reasonably formed via radiolysis of water and oxidation of organic molecules in wheat samples.

The EPR spectra general shape for both varieties appears similar, suggesting that the type of free radicals generated by irradiation is the same in both. However, there might be slight differences in the relative intensities or fine structure of the spectra, which could indicate differences in the chemical environment of the free radicals or the presence of different radical species in different proportions.

The decay plots in Figure 8 b-d-f-h show the decreasing of the EPR signal intensity over time for only the samples irradiated at $10\ kGy$. The EPR signal intensity decreases over time for each typology of wheat crop variety, suggesting that the radical recombination mechanisms are mainly associated to the starch radicals. This decay highlights the temporary nature of radical-induced changes, reducing concerns about long-term chemical instability.

4 Conclusion

This technical report is focused to develop a simple and effective protocol for studying the effects produced by gamma irradiation within the 0.1–10 kGy range for treating wheat-based food products. Through the combined use of non-destructive approach based on combining FTIR-ATR, Raman, and EPR spectroscopic techniques, we carried out an in-depth analysis of structural, conformational, and radical-related changes across four different wheat matrices (soft, durum, soft integrated, and soft biological). The results demonstrate that the performed irradiation tests do not significantly alter their chemical composition and molecular structures of wheat matrices.

For microbial control, gamma irradiation at around $10\ kGy$ are expected to reduce microbial loads, enhancing food safety and extending shelf life without compromising structural or nutritional integrity. For the nutritional preservation, instead minimal impact on carbohydrates, proteins, and lipids underscores the nutritional stability of irradiated wheat. Also, for the consumer perception, the minor changes in visual properties suggest high acceptance potential for irradiated wheat products.

The experimental findings point out that gamma irradiation up to $10\ kGy$ produces no dramatic secondary effects on wheat matrices. In particular, EPR spectroscopy demonstrated a direct relationship between absorbed dose and radical formation, with the molecular composition and intrinsic organization of the wheat samples modulating this behavior.

The obtained results convincingly disclose gamma irradiation technologies as reliable methods for enhancing the safety and longevity of wheat and other food matrices. At doses below regulatory limits, irradiation preserves the structural and nutritional integrity of wheat while offering additional benefits such as microbial reduction and anti-germinative effects. Future research may explore optimizing irradiation parameters for specific applications, addressing consumer perceptions, and expanding the application of this technology to a broader range of food products.

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