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Impact of irradiation on metabolomics profile of ground meat and its implications toward food safety

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ABSTRACT

Irradiation is a reputed efficient sterilizing method that demonstrated valuable effects on meat preservation. This research, based on qualitative untargeted HPLC-Orbitrap metabolomics approach, was intended to estimate the variations in global metabolome profile of irradiated chicken, turkey, and mixed (chicken, turkey, and pork) ground meat to assess the possible presence of a food safety issue concerning the metabolome alteration. Overall, 402 metabolites were identified, and all three matrices exhibited a specific metabolome profile that was not influenced remarkably by the application of five different levels of irradiation intensity. In particular, all three meat categories displayed the following common features: 1) free amino acids pool remained unaffected by irradiation 2) taurine appeared as the most important differentiator for all three category 3) amount of glutathione decreased 4) characteristic although minor, oxidative modifications of polyunsaturated free fatty acids occurred 5) intensified adenosine nucleotide degradation. The presented analytical approach highlighted the usefulness of meat metabolome profiling in distinction of irradiated meat from non-treated one. Metabolomics analysis did not diagnose any relevant negative impact of irradiation on meat safety issues, but contemporary has demonstrated alternations in few metabolic pathways.

1. Introduction

The meat industry has grown substantially due to the increasing demand for meat products and their excellent nutritional properties. Increasing interest has been given to chicken and turkey meat, which presents a precious nutritional composition of proteins and lipids. However, meat is susceptible to microbial contamination from different sources (Nerin, Aznar, & Carrizo, 2016), which is a challenge for the preservation of this foodstuff. Food processing with ionizing radiation represents a preservation technology that aims to protect the hygienic quality of food, extending its shelf-life. As one of the most exhaustive non-thermal decontamination techniques in the food industry, irradiation is reported as a safe and effective method to extend the fresh meat and meat products shelf-life. During this process, the food is treated with well-defined doses of ionizing radiation that can inactivate the genetic material of microbial cells (Reddy, Jayathilakan, & Pandey, 2015). Therefore, it is applied to prevent/delay sprouting of tubers and bulbs, to reduce the saprophytic microbial load in fresh meat, poultry, and fish,

to inactivate insect pests, including larval and parasitic states, and pathogenic bacteria in perishable and frozen foodstuffs (Panseri et al., 2018; Kume, Furuta, Todoriki, Uenoyama, & Kobayashi, 2009).

The World Health Organization (WHO) reported that an irradiation dose minor or equal to 10 kGy is generally considered irrelevant in changing the foodstuff safety (Ravindran & Jaiswal, 2019). When applied in compliance with the current regulations, irradiation processing is considered harmless and provides for the obligation of labelling, control of the plants, and control of the foodstuffs during the marketing phase. In Italy, food irradiation treatment is governed by EU Directives 1999/2/EC and 1999/3/EC (European Union, 1999a, 1999b).

Regarding meat products, several methods have been developed for the determination of markers to identify possible irradiation treatments. The most important are following two: 1) screening method based on microgel electrophoresis of suspected DNA fragments formed by the application of ionizing radiation; 2) confirmatory one based on headspace solid-phase microextraction coupled to Headspace Solid-Phase

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Microextraction Gas Chromatography Mass Spectrometry (HS-SPME GC-MS) for the search and quantification of 2-alkylbutanones recognized as radiation markers, of which 2-Dodecylcyclobutanone derived from palmitic acid is the most investigated (Ministry of Health, 2020; Panseri et al., 2014; Panseri et al., 2015; Soncin et al., 2012).

Although great attempts have been made to evaluate the biochemical constituents in irradiated meat, studies concerning the status of metabolites after irradiation treatment are just a few (Zhao et al., 2018; Reddy et al., 2015). The irradiation-induced changes in the meat components occur via primary radiolysis impacts, due to the direct absorption of energy, and by secondary indirect effects (Zanardi, Caligiani, & Novelli, 2018). The high reactivity of the free radicals and excited molecular ions produced by the radiolysis of water and/or oxygen form very reactive intermediates leading to stable chemical products. In general, the extent of chemical reactions induced by irradiation in food components depends on many variables; the most important are the irradiation treatment conditions like the absorbed dose, facility type, and presence or absence of oxygen and temperature. The composition of meat and its physical state also influence the extent of the reactions induced by the treatment and the nature of the formed products (Sommers & Fan, 2006).

Metabolomics as a modern analytical methodology has been applied for examining metabolic alterations in different foodstuffs (Utpott et al. 2021; Yu, Cooper, Sobreira, & Kim, 2021; Li et al., 2020; Muroya, Ueda, Komatsu, Miyakawa, & Ertbjerg, 2020; Tomassini et al., 2019). Metabolomics is expected to be a tool to monitor the impact of irradiation, but it needs to be a non-time-consuming, non-invasive, and reproducible method (Zanardi et al., 2018). In this regard, metabolomics based on NMR spectroscopy followed by chemometric evaluation appears appropriate for such purpose, and, consequently, has been used in attempts to investigate the influence of irradiation on beef where glycerol, lactic acid esters, and tyramine were found to be important biomarkers for the differentiation (Zanardi et al., 2015). However, to the best of our knowledge, the studies that examined the effect of irradiation on chicken, turkey, and mixed (chicken, turkey, and pork) ground meat using a metabolomic analytical platform approach have not been reported yet.

With the present work, we intended to estimate in which extend the treatment with ionizing radiation affects the global metabolome of the investigated matrices. For this purpose, an experimental plan was prepared which included the evaluation of the impact of ionizing radiation on ground meat samples coming from the largescale distribution and retail market, treated at different levels of intensity. Chicken, turkey, and mixed (chicken, turkey, and pork) ground meat preparations chosen for the present study were selected as they were proven as the most frequently irradiated in violation of the European Union indications. Therefore, the comprehensive identification of small polar molecular species in irradiated ground meat based on untargeted metabolomics approach by High Pressure Liquid Chromatography Q-Exactive Orbitrap High Resolution Mass Spectrometry (HPLC-HRMS-Q-Orbitrap) was conducted to monitor the dynamic changes in amino acids, monosaccharides, nucleotides, and free fatty acid profiles. Additionally, since irradiation can provoke oxidative modifications that could be potentially hazardous, we were aimed to explore the presence of any oxidative products that could be formed due to irradiation.

2. Materials and methods

2.1. Chemicals and reagents

All reagents (water, methanol, ammonium formate, amino acids mix 9906, AMP, IMP, inosine, xanthine, hypoxanthine, guanosine, Proline D3) were provided from Sigma Aldrich.

2.2. Irradiation treatment

This study was conducted on the three meat matrices: chicken, turkey and mixed (chicken, turkey and pork) ground meat for sausages preparation and were collected from local supermarkets. Respective pools were divided into five groups for irradiation treatment, with two biological replicates in each group. The irradiation was performed using an X-ray irradiator (RS-2400, Radsource Inc., Texas, USA). The anode-cathode voltage of the X-ray tube was 150 kV and the current was 45 mA at room temperature and the dose rate of the treatment was approximately 2 kGy h^{-1}. Fresh meat samples were put in plastic bags and subsequently exposed at irradiation doses of 0.5, 1, 3, and 5 kGy in five replicate (Campaniello et al., 2020). Immediately after treatment, the samples were stored at – 80 °C until analysis.

2.3. Sample preparation

After lyophilization, 100 mg of meat sample were extracted by 2.5 mL of a cold mixture (formic acid 0.1% water solution: methanol = 20:80). Subsequently, samples were vortexed, centrifuged and filtered, and diluted in the mobile phase (1:10). Lastly, 10 μL for each sample was injected.

2.4. HPLC Q-Exactive Orbitrap High Resolution Mass Spectrometry analysis

Chromatographical separation was accomplished on Vanquish HPLC instrument (Thermo Fisher Scientific, San Jose, CA, USA) using a Raptor ARC-18 5 μm, 120 × 2.1 mm column (Restek, Bellefonte, United States).). The mobile phase consisted in water (A) and methanol (B) both acidified with 0.1% formic acid. At first, the gradient (flow rate at 0.25 mL/min), started with 98% of eluent A with a linear reduction to 50% in 5 min, that was constant in the next 3 min. The initial conditions returned at 8 min, tracked by a 5-min re-equilibration period. The column department temperature was set at 50 °C and while autosampler was kept at 5 °C.

The HPLC-HRMS-Q-Orbitrap (Thermo Scientific, San Jose, CA, USA) worked in both positive mode and negative mode, each one performed with predetermined acquisition parameters. The full scan (FS) with resolving power 140.000 (scan range of m/z 70–1000) was used for the screening and statistical evaluation of the chromatographic profiles. Full scan data-dependent acquisition (FS-dd-MS^2) with resolving power 70.000 and 17.500 for FS and dd-MS^2, respectively, was employed for fragmentation of pseudo-molecular ions detected in FS mode. Fragmentation of precursors was executed with stepped, normalized collision energy (NCE) set at 20 eV and 30 eV.

2.5. HPLC-Q-exactive-orbitrap-MS untargeted metabolomics approach with Compound Discoverer™ workflow

As extensively presented in our previous works (Castrica et al., 2021; Chiesa et al., 2020), the raw data from Q Exactive Orbitrap analysis were elaborated by Compound Discoverer (CD) 3.2 software (Thermo Fisher, MA, USA), that enabled the programmed compound identification and statistical evaluation. The procedure was based on a series of steps that include following workflow nodes: spectra selection, alignment of retention time, the precursor ions collection consulting CD integrated databases (https://www.mzcloud.org and https://www.chemspider.com), and normalization of the chromatographical peaks area. Criteria for metabolites' putative identification by CD workflow were chosen as a combination of few different assets: an mzCloud match score higher than 80% and the same identification being proposed by at least one external web databases (i.e. Human Metabolome platform HMDB (https://hmdb.ca/), Kyoto Encyclopedia of Genes and Genomes (KEGG), (https://www.genome.jp/kegg), Pubchem (www.pubchem.com) or Small Molecule Pathway Database (SMPDB) (http://smpdb.ca)). In case of no

correspondence between metabolites' mass fragmentation pattern with none of the web databases, manual verification of fragmentation pattern program was realized using ChemDraw software.

To ensure an adequate permanence of sequence analysis, all samples run in duplicate along with quality controls (QC) which were employed randomly through the analytical batch. The QC samples were prepared by pooling the same volume from the real samples for each experiment. Also, a procedural blank sample was involved in each batch to identify background signals.

2.5.1. Statistical evaluation

Descriptive, univariate, and multivariate statistical analysis was performed as an integral part of Compound Discoverer workflow throughout the Differential Analysis node. It consisted of: Hierarchical Clustering (heat map) Analysis (HCA) for the Box-Whisker charts (BWC), and Volcano Plot (VP) processing. HCA view was used to visualize the correlation between detected compounds in involved samples in a two-dimensional array of color-coded heat map where each rectangle represents the relative amount (by area) of a specific compound in a specific sample. HCA uses an agglomerative (bottom-up) approach to find the similarities between samples regarding the identified. The analysis proceeds iteratively, at each stage joining the two most similar clusters into a new cluster, continuing until there is one overall cluster represented by a dendrogram. BWC presents median, first and third quartile, upper and bottom whiskers of compound of interest from each sample groups in regard to the irradiation dosage applied. Multi-group comparison hypothesis test performed was by a one-way ANOVA model with Tukey as post-hoc test. P-values are adjusted by the Benjamini-Hochberg algorithm. For the VP, hypothesis test was performed by a multivariate paired t-test (assuming equal variance). VPs created on Log_2 (FC) and $-log_{10}$ (P-value) was employed to filter metabolites of interest and to individuate the main differentiators between non irradiated and irradiated samples groups.

3. Results and discussion

Irradiation of meat and meat products for sanitary purposes is not permitted in the European Union, except for certain categories of chicken and poultry preparations in The Netherlands and France (European Commission, 2001). However, the possible presence on the market of irradiated foods coming from other countries is constantly monitored in Italy. European regulatory framework on food irradiation is not harmonized with the non European countries: a substantial difference in irradiation dosage and types of processed food, as well as variations in the labeling of imported products, have been lately registered (Zanardi et al., 2018). Therefore, it is likely that on the Italian national market may be found imported products that do not carry the appropriate indication of eventual irradiation processing (Mangiacotti et al. 2013). The current EU regulation leaves to the individual national health authorities to carry out checks on irradiated products using standardized/validated methods. A qualitative analytical strategy based on metabolome profiling could be valuable in food inspection purposes, especially when the samples from animal origin are suspected on irradiation treatment. Also, metabolomics can contribute to a better understanding of the nutritive status of irradiated meat (Utpott et al., 2022).

3.1. General metabolomics profiling of irradiated ground meat preparations

A comprehensive metabolomic analysis of irradiated ground meat was performed by the HPLC-HRMS-Q-Orbitrap platform that has been proven as a consistent technique for the exploration of key compounds that endorse the physicochemical properties and sensory characteristics of food, and thereby it contributes to the monitoring of food quality and safety (Yu et al., 2022; Muroya et al., 2020). In this project, an

HRMS-based metabolomics investigation was conducted in both polarization modalities (positive and negative) in separate analytical runs, to achieve reliable, sensitive, and double-confirmed identification of key metabolites in complex meat matrices. Overall, a total of 331 compounds were detected: 298 in positive ionization mode while 33 were characterized exclusively in negative polarity. Although the majority of compounds were detectable in both modes, the positive acquisition was much more specific in identification of free amino acids, peptides, nucleotides, nucleosides, amines, monosaccharides, and their derivates (amines and phosphates) and short and long chain fatty acids (Table 1S). The negative mode was more specific for some nucleotides (AMP and IMP) and phosphated monosaccharides. Some acids either organic (lactic, fumaric, maleic, succinic, etc.) or amino (aspartic and glutamic) were detected exclusively in this ionization type. The number of metabolites reviled by our platform surpasses those reviled by NMR techniques applied for the characterization of irradiated chicken (Xiao et al. 2019), beef (Zanardi et al., 2015) and pork meat (García-García et al. 2019). Anyway, the metabolomic analytical platform employed herein exhibited a moderate capability to identify all present compounds (402 recognized among 1618 signal registered), and thus some of metabolites reported earlier (Zanardi et al., 2015) as main discriminant regarding the irradiation treatment were not found significant in this study.

The metabolic pathways reconstructed from the identified compounds are the following (Table 1S): glycine, serine and threonine metabolism, alanine and aspartate metabolism, glutamate metabolism, histidine metabolism, lysine metabolism, phenylalanine metabolism, tyrosine metabolism, tryptophan metabolism leucine, isoleucine and valine metabolism, methionine, cysteine, SAM and taurine metabolism, urea cycle, arginine and proline metabolism, creatine metabolism, polyamine metabolism, glutathione metabolism, gamma-glutamyl amino acid, dipeptide, pentose metabolism, glycogen metabolism fructose, mannose and galactose metabolism, aminosugar metabolism TCA cycle, oxidative phosphorylation, fatty acid synthesis, medium chain fatty acid, long chain saturated fatty acid, long chain monounsaturated fatty acid, long chain polyunsaturated fatty acid (n3 and n6) fatty acid, dicarboxylate fatty acid metabolism, fatty acid metabolism (acyl carnitine, short, medium, long chain saturated), hydroxy, peroxyl and epoxy fatty acid, inositol metabolism, phospholipid metabolism, glycerolipid metabolism, purine metabolism ((hypo)xanthine/inosine, adenine, containing, purine guanine containing, pyrimidine metabolism and vitamins metabolism. for the majority of recognized pathway there were no alterations provoked by irradiation, with exception of two that will be disused in down text.

The heatmap (hierarchical cluster) analysis was used to identify differential metabolites (Fig. 1). This type of data presentation provides an intuitive visualization of global metabolome differences between three meat matrices. As shown in the heatmap, after taking the intersection of all groups, each ground meat type expressed a distinct metabolome profile. This was not surprising considering that contents of skeletal muscle metabolites are affected by species and type, animal genetic background, feeding, muscle type, postmortem aging, and meat processing (Muroya et al., 2020). Only in chicken meat, the clustering corresponds linearly to irradiation intensities which was not a coincidence with turkey and mixed preparations. For these two meat types, there were no substantial differences between nontreated samples, and the lowest irradiation dose (0.5 kGy) applied. Also, clustering for the irradiation intensity applied was random either for turkey or for mixed preparations which led us to search for the isolated compound or investigate defined metabolic pathway that are common for all three matrices and that emerged as statistically significant differentiators.

Glutathione was only one significantly downregulated compound that was found in all three matrices which candidates this tripeptide as universal marker for irradiation treatment. The differential analysis between pooled irradiated vs. pooled controls showed following statistical parameters for this naturally occurring tripeptide: Ratio = 0.121, log2Fold Change = 1.89 and P value = $5.8*10^{-4}$. Glutathione acts as an
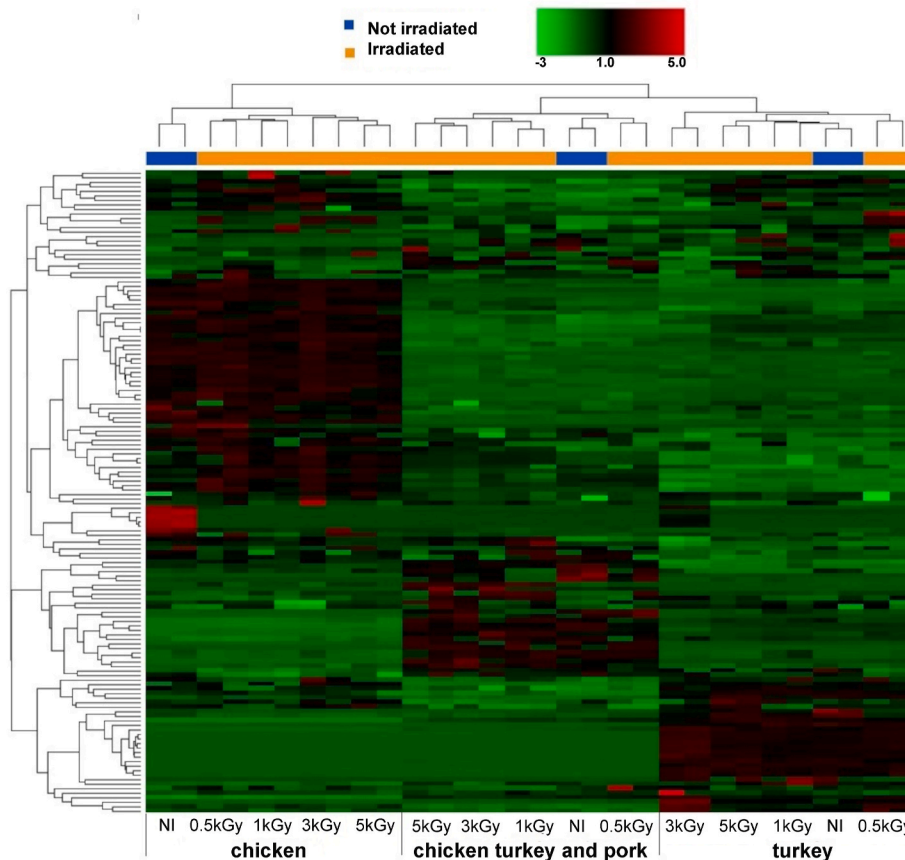
Fig. 1. Hierarchical cluster analysis for the 298 species identified in positive mode: heat-map reflecting the differences between relative amount in respect to meat typologies and irradiation dosage applied; z-color scale indicates normalized peak area value: red and green indicates more and less abundant, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

antioxidant in various pathways (Domínguez, Gagaoua, Barba, Zhang, & Lorenzo, 2019). Its reactivity may include combination with reactive oxygen species (ROS) like hydroxyl, peroxy or alkoxy radicals that can be eventually formed by irradiation. This antioxidant action provokes glutathione depletion in irradiated samples. Also, it is possible that radiation causes its breakdown: glutathione contains free SH-group that is proven to be highly susceptible to cessation due to adsorbed irradiation energy (Martin, Batzer, Landmann, & Schweigert, 1962).

Anyway, the common upregulated differential metabolites for all three categories that can be usd as irradiation markers were: taurine, oxidized cis,cis-1,4-pentadiene fatty acids, and compounds involved in purine nucleotides 5′-monophosphate pathway. Results on differential analysis between pooled irradiated *vs.* pooled controls revealed that taurine is a major differentiator as it was upregulated by irradiation (Ratio = 2.013, log2Fold Change = 1.03 and P value = $1.9*10^{-4}$). This finding has its validity in the fact that taurine is present abundantly in the turkey and chicken meat (Wójcik, Koenig, Zeleniuch-Jacquotte, Costa, & Chen, 2010), therefore it can be easily quantified. Also, taurine emerged as a common substance for all three matrices which candidates this compound as general meat irradiation marker.

The second important chemical alteration regards the lipid oxidation (Feng & Ahn, 2016). The susceptibility of irradiated muscle tissues to lipid oxidation is closely related to the nature, proportion, degrees of saturation in fatty acids, and the composition of phospholipids in the cell membrane (Domínguez, Gagaoua, Barba, Zhang, & Lorenzo, 2019; Xiao, Zhang, Lee, Ma, & Ahn, 2011). Anyway, the extend of the lipid oxidation has been measured indirectly by the TABS method or evaluation the VOCs profile (Al-Bachir & Zeinou, 2014; Panseri et al., 2015), but the data regarding the oxidized free fatty acid as important oxidation

products, to the best of our knowledge, was not reported for the irradiated meat. The reason of this may lay down in the analytical gap that specially regards two common free polyunsaturated fatty acid (PUFA), namely linolic and α-linolenic acid. Their oxidative products are occasionally studied in lipidomics research, but for food processing, they were not considered important due to their modest abundance (Xiao et al., 2011). A small amount does not explicitly indicate a low rate of their production, but it may be a consequence of poor extraction rate during sample preparation procedure applied in lipidomic research (Hu, Wang, & Han, 2017). It involves multiphase extraction with non-polar solvents in combination with a small amount of polar once which is not the best solution for the peroxyl-, hydroxy- or epoxy- PUFA derivates (Aoyagi, Ikeda, Isobe, & Arita, 2017). Anyway, with metabolomics approach the great portion of lipids' fraction cannot be followed, but PUFA and their derivates are the exception: in this study they were detected with the quantity and characteristics that allowed to speculate about lipid oxidation pathway triggered by irradiation.

Generally, lipid oxidation mechanism in irradiated meat is not fully understood but are likely to be similar to those in nonirradiated meat (Feng, Moon, Lee, & Ahn, 2017; Panseri et al., 2015). There are two possible events: 1) non-enzymatic, initiated by the free radicals (reactive oxygen species, ROS) produced during the irradiation treatment; 2) enzymatic, comprises lipoxygenases (LOXs) that catalyze the oxidation of PUFA and lipids containing a *cis,cis*-1,4-pentadiene structure (Domínguez, Gagaoua, Barba, Zhang, & Lorenzo, 2019). Both reactions produce lipid hydroperoxides, which decompose and form secondary oxidation products that give strong undesirable flavors, causing food deterioration (Wang & Hammond, 2010). Examining integrated results of metabolomic analysis it was possible to construct a metabolic

pathway that involves oxidation either of linoleic or α-linolenic acids (Fig. 2). The compounds that were upregulated in irradiated meat are highlighted in red. Linoleic acid was significantly oxidized by irradiation giving both mono-peroxyl oxidation products: 9 S- and 13 S-hydroperoxyl octadecadienoic acids (9 S- and 13 S-HPODE). On the contrary, hydroperoxyl octadecatrienoic acids (9 S- and 13 S-HPOTE) where not altered by irradiation what leads to the conclusion that proxy-oxidative modification is not preferable route of α-linolenic acid response to irradiation. At the moment, from results obtained in this study it is not possible to establish whether irradiation provokes the development of oxidated polyunsaturated fatty acid derivatives by ROS action, or their production is stimulated by LOX catalytic activity. Anyway, the proposed metabolic pathway (Fig. 2) has certain similarities to that executed by LOXs, as two exclusive LOXs products (colenleate and colenelnate) were significantly upregulated in the irradiation meat preparations. On the other hand, the ROS mechanisms despite explaining many of the changes observed in irradiated meat (Zanardi et al., 2009), does not provide a detailed and complete description of the changes produced in PUFA and developed products during the oxidation process (Králová, 2015). Therefore, the main challenge is to complete the scheme that can fully explain all the agents, intermediate products and reactions involved (Ghnimi, Budilarto, & Kamal-Eldin, 2017).

The second metabolic pathway affected by irradiation is the adenosine-5′-monophosphate degradation. The proposed degradation pathway of nucleotides by irradiation was shown in Fig. 2, with irradiation upregulated compounds highlighted in red. The study on impact of irradiation on purine nucleotides/nucleotides interconversion in turkey meat has been recently published: it was revealed that the rate of AMP breakdown contributes to the sensory properties of meat (Feng et al. 2017). Our results are in the line of this finings as irradiation has expressed a significant effect on the degradation of AMP in all treated samples regardless the dosage applied. The hypoxanthine emerged as the most important purine base due to its pronounced accumulation with following statistical parameters for pooled irradiated vs. pooled controls: Ratio = 3.4, log2Fold Change = 2.13 and P value = $1.5*10^{-5}$). Once hypoxanthine is formed, it can produce bitter taste, and thus contributes to off flavor (Ozogul, Ozden, Ozogul, & Erkan, 2010). Also, increased production of inosine and inosine-5′-monophosphate (IMP) in irradiated samples might be considered important since inosine and IMP contributes to the generation of meat odor and flavor, as well (Castrica et al., 2021). However, as it can be seen in Fig. 3., there are two possible pathways of AMP transformation to inosine and IMP: 1) IMP was formed direct deamination from AMP 2) The deamination and dephosphorylation began simultaneously, in which inosine was generated directly from AMP without an intermediate, IMP. It seems that first option is more probable as NH₂-purine heterocycles has relatively high hydrophilicity and low volatilities, therefore the deamination of nucleotides could notably increase due to reaction of AMP and ROS produced during the irradiation of meat matrices. In this point, the role of AMP-deaminase (AMPD) enzyme (3.5.4.6) that catalyzes the irreversible hydrolysis of AMP to IMP could not be ecluded. Being the integral enzyme of purine nucleotide cycle, AMPD participates in deamination of amino acids and their involvement into energetic metabolism (England, Matarneh, Scheffler, Wachet, & Gerrard, 2015).

Having a substantial differentiation in metabolomics profiles of three ground meat type we proceed analyzing datasets for the individual groups to find main irradiation markers for each of samples' batch.

3.2. Differential metabolites related to individual ground meat preparations

The Volcano plot comparison between the relative intensity of 316 compounds chromatographic peaks from non-treated controls and pooled irradiation groups is presented in Fig. 4. This is followed by the Box Whiskers chat with the most important differentiators. Unexpectedly, the significant downregulated compounds that were almost completely depleted at the irradiated samples were two flavonoids' structures: pinocembrin and 5,7-dihydroxy-3,8-dimethoxy–phenyl-4H-chromen-4-one. Those compounds, most probably belongs to the plant extracts that are usually used for the ground meat preparations. Although the information in form of recipes was not available, the strong signals of two flavonoids whit statistically important differences between controls and all four irradiation doses confirmed their beneficial role as natural antioxidants in protection against the oxidative stress caused by irradiation (Trindade, Mancini-Filho, & Villavicencio, 2010). The dose dependent increase of O-arachidonoyl ethanolamine and acetyl-L-carnitine, both belonging to the class of fatty acids physiological products, needs further elucidations as there is not any literature note in their regard. Dose dependent increase in hypoxanthine is in the line with general trend observed in AMP degradation pathway (Fig. 3).

For the mixed preparation (chicken, pork and turkey) (Fig. 5) the involvement of pork fraction most probably caused the increase of two common phosphatidylcholine species. On the other hand, downregulation of dihydrothymine point towards the alterations in pyrimidines metabolism that has not been studied so far. Also, the decrease in 3-methylhistidine concentration remains to be studied more profoundly. 3-Methylhistidine is formed by the posttranslational methylation of histidine residues of the main myofibrillar proteins actin and myosin. During protein catabolism, 3-methylhistidine is released but cannot be reutilized. That is reason why it is used as a urinary marker of red meat consumption (Cross, Major, & Sinha, 2011).

For the turkey meat the most important result regards the increasing in the ortho-tyrosine concentration (Fig. 6). This amino acid has been already proposed as a marker for the identification of irradiated protein-rich food because it is formed by the reaction between radiolytically produced hydroxyl radicals and the aromatic ring of phenylalanine and
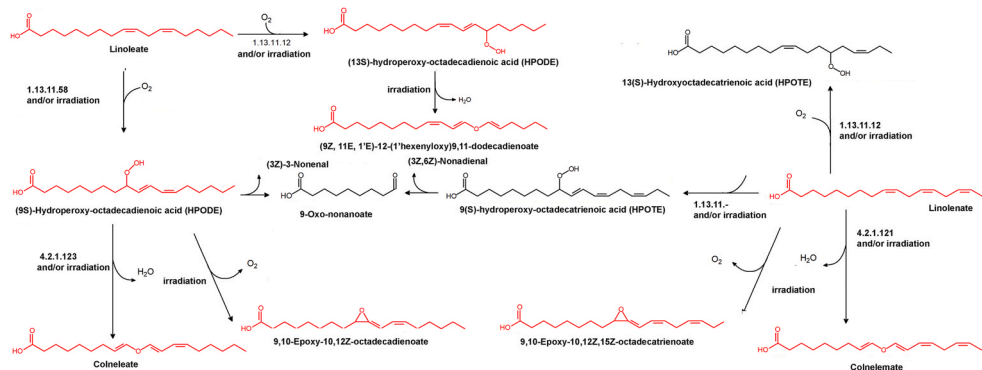

Fig. 2. Proposed linoleic and α-linolenic acids oxidative modifications caused by irradiation (compounds highlighted in red are upregulated in irradiated samples). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
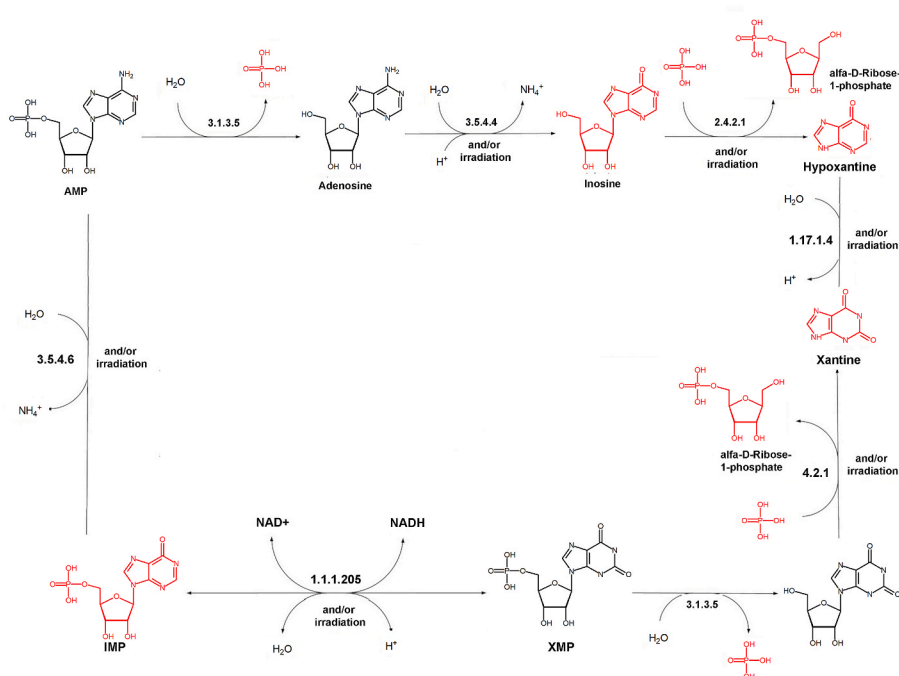
Fig. 3. Proposed AMP modification caused by irradiation (compounds highlighted in red are upregulated in irradiated samples). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
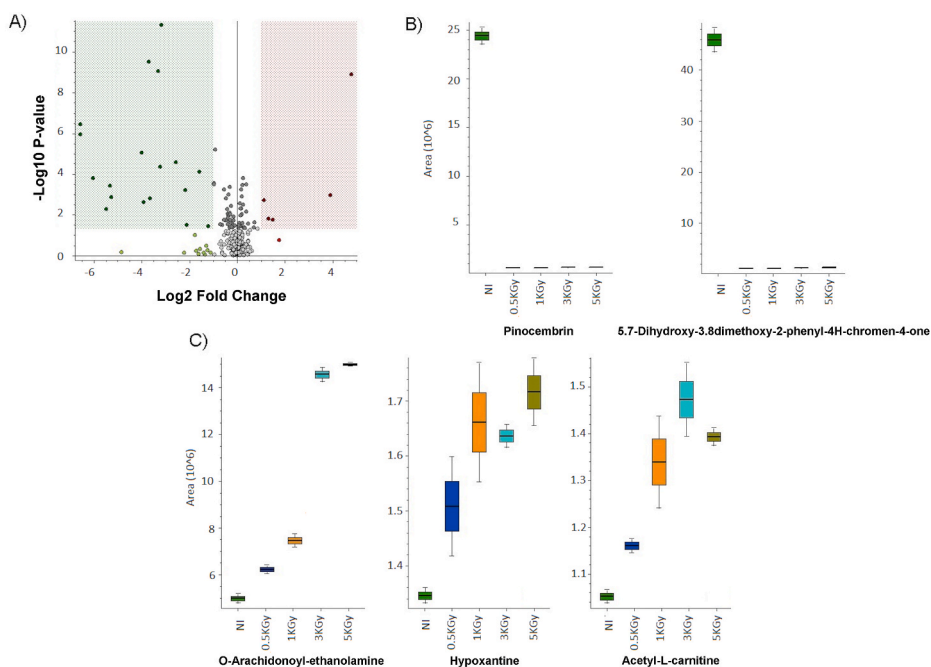

Fig. 4. Chicken ground meat preparations **A)** Volcano plot comparison between the relative intensity of 316 chromatographic peaks acquired in positive ionization mode from non-treated controls and pooled irradiation groups. The left region contains down-regulated signal with intensities from non-treated that are significantly lower than those from pulled irradiated samples and are greater than the upper fold-change (FC) threshold. The right region includes up-regulated peaks where the intensities from controls was significantly higher than those from pulled ones and was less than the lower FC threshold. P-value (PV) = 0.05. **B)** and **C)** Box–whisker plots of the most important down- and up-regulated compounds, respectively (NI-Non Irradiated); Boxes represent 1st and 3rd quartiles; the median is indicated by the horizontal line inside the box; whiskers represent the upper and lower 25% of the distribution.

phenylalanine containing proteins (Chen, Fan, Song, Liu, & Zhang, 2013). Regarding the other two upregulated substances (serine and 2-(14,15)-epoxyeicosatrienoyl) glycerol) no hypothesis can be formulated or supported by literature unless if they are not considered as lipid derivatives that follow a general trend observed for lipids' metabolites. Finally, the decreasing in the xanthine 5′-monophosphte (XMP) corresponds to its position in the proposed AMP degradation pathway (Fig. 3). XMP behavior should be counted as a turkey meat particularity as for two other matrices the differences in its content between controls and irradiation samples were not found.

4. Conclusions

The chemical changes in meat affected by irradiation are of concern to consumers, and the meat industry is having difficulties in using this technology to achieve its food safety benefits. Therefore, this study represents a step forward in the metabolic profiling of irradiated meat. An extensive assignment of HRMS signals/fragmentations in correlation with chromatographical separation was carried out to interpret metabolic changes occurring because of the irradiation treatment. Also, the results of this study focused on an innovative strategy able to provide
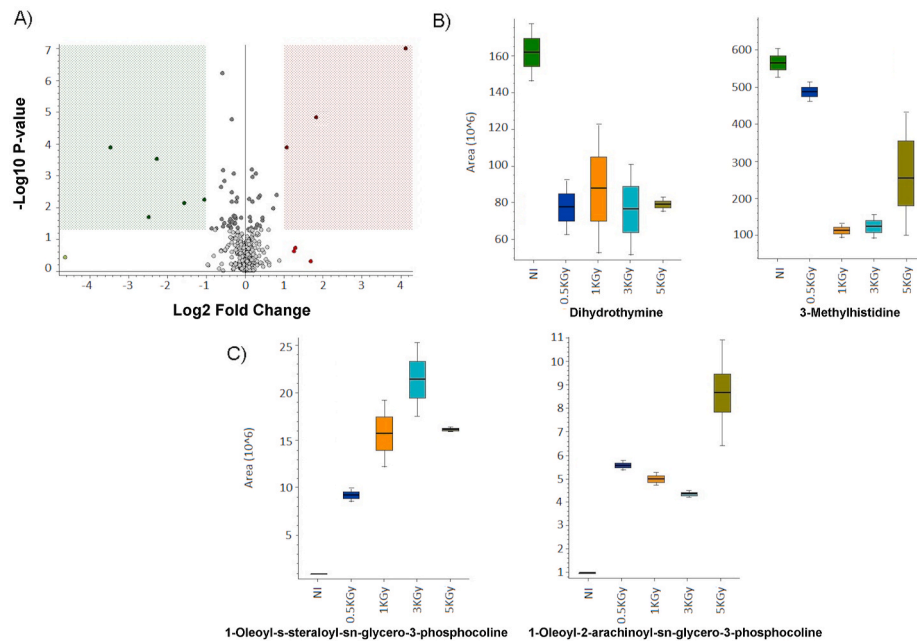
Fig. 5. Mixed (chicken, pig and turkey) ground meat preparations **A)** Volcano plot comparison between the relative intensity of 291 chromatographic peaks acquired in positive ionization mode from non-treated controls and pooled irradiation groups. The left region contains down-regulated signal with intensities from nontreated that are significantly lower than those from pulled irradiated samples and are greater than the upper fold-change (FC) threshold. The right region includes up-regulated peaks where the intensities from controls was significantly higher than those from pulled ones and was less than the lower FC threshold. P-value (PV) = 0.05.**B)** and **C)** Box–whisker plots of the most important down- and up-regulated compounds, respectively (NI-Non Irradiated); Boxes represent 1st and 3rd quartiles; the median is indicated by the horizontal line inside the box; whiskers represent the upper and lower 25% of the distribution.
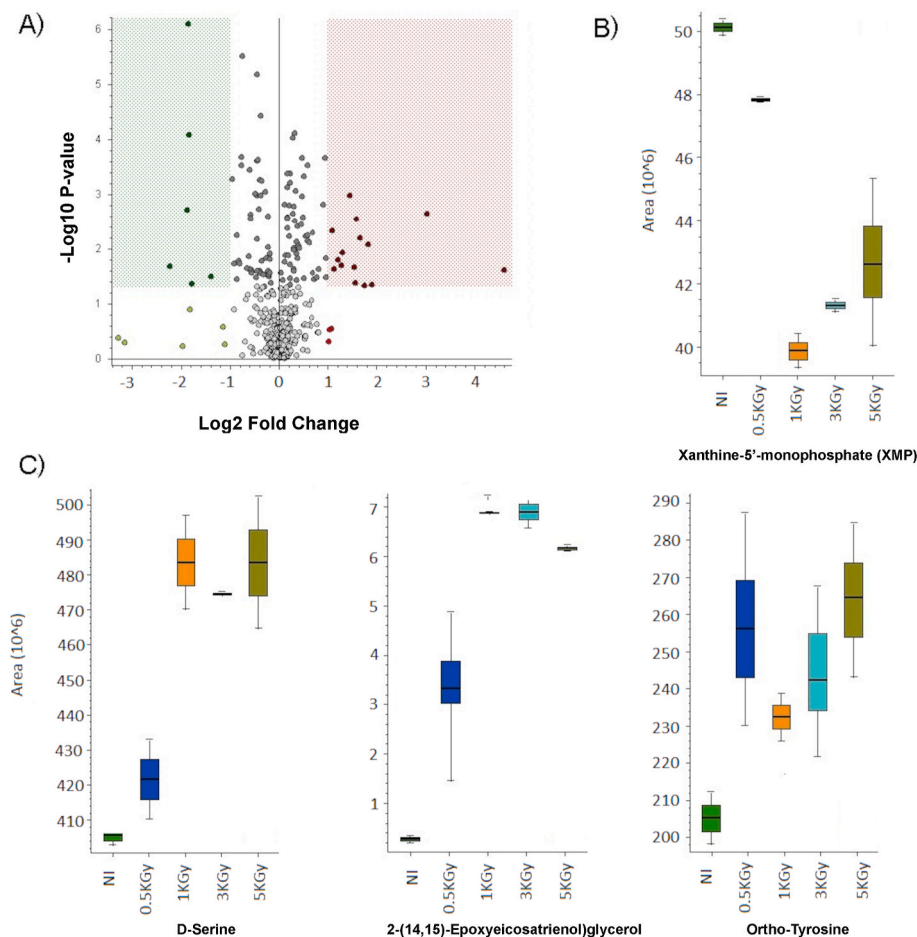

Fig. 6. Turkey ground meat preparations **A)** Volcano plot comparison between the relative intensity of 303 chromatographic peaks acquired in positive ionization mode from non-treated controls and pooled irradiation groups. The left region contains down-regulated signal with intensities from nontreated that are significantly lower than those from pulled irradiated samples and are greater than the upper fold-change (FC) threshold. The right region includes up-regulated peaks where the intensities from controls was significantly higher than those from pulled ones and was less than the lower FC threshold. P-value (PV) = 0.05. **B)** and **C)** Box–whisker plots of the most important down- and up-regulated compounds, respectively (NI-Non Irradiated); Boxes represent 1st and 3rd quartiles; the median is indicated by the horizontal line inside the box; whiskers represent the upper and lower 25% of the distribution.

recognition of metabolic pathways altered due to the exposition of food to ionizing radiation and distinguish between irradiated and nonirradiated food are introduced and discussed for potential future applications. Overall, the irradiation did not cause the changes in main food ingredients such as free amino acids pool, therefore the original quality of meat is maintained. This is a valuable finding that establishes irradiation as a beneficial tool for meat preservation. Furthermore, our data may provide important information for the application of irradiation in

meat preservation therefore significant for the food safety issue.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Sara Panseri: Conceptualization, Project administration. **Francesco Arioli:** Writing – review & editing. **Radmila Pavlovic:** Conceptualization, Methodology, Data curation, Writing – original draft. **Federica Di Cesare:** Methodology, Writing – original draft. **Maria Nobile:** Resources, Investigation, Writing – review & editing. **Giacomo Mosconi:** Formal analysis, Writing – original draft. **Roberto Villa:** Resources, Writing – original draft. **Luca Maria Chiesa:** Conceptualization, Writing – review & editing. **Elisabetta Bonerba:** Visualization, Supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113305.

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