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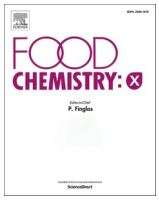

Exploring the impact of irradiation on the sensory quality of pork based on a metabolomics approach

Bo Yao [a], Dong Zhang [a,c], Xinyu Wu [a], Ruiyan He [a], Hui Gao [a], Kailan Chen [a], Dan Xiang [e], Yong Tang [a,b,d,*]

[a] College of Food and Bioengineering, Xihua University, Chengdu 610039, China
[b] Sichuan Yiyang Modern Agricultural Development, Ltd, Chengdu 610000, China
[c] Food Industry Collaborative Innovation Center, Xihua University, Chengdu 610039, China
[d] Chongqing Key Laboratory of Speciality Food Co-Built by Sichuan and Chongqing, Chengdu 610039, China
[e] Chengdu Xiwang Food., Ltd, Chengdu 610000, China

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ABSTRACT

The effects of irradiation on pork quality characteristics were investigated by combining sensory experiments, pork color, TBARS, volatile components, and differential metabolites. Pork irradiated at a dose of 1 kGy received the highest sensory scores, whereas pork irradiated at doses of 3 and 5 kGy obtained lower sensory scores, particularly with regard to odor. Irradiation makes pork more ruddy and promotes fat oxidation, leading to increased a^* and TBARS values. The main volatile substances in irradiated pork were hydrocarbons, aldehydes, and alcohols, and hexanal, heptanal, and valeric acid were considered as important substances responsible for the generation of radiation-induced off-flavors. 65 differential metabolites were identified. L-pyroglutamic acid, L-glutamate, L-proline, fumarate acids, betaine, and L-anserine were considered as the main substances contributing to the differences in pork quality. In addition, metabolic pathways such as arginine biosynthesis, alanine, aspartate and glutamate metabolism were found to be considerably affected by irradiation.

Introduction

Pork is a commonly consumed red meat worldwide, providing humans with a significant source of energy, macronutrients, and micronutrients (Pluske, Murphy, & Dunshea, 2024). China is the largest producer and consumer of pork worldwide, the safety of pork is essential for consumers' health. However, the long-term preservation of fresh meat has always been a problem. Fresh meat is susceptible to microbial contamination during production, transportation, and sales (Kanatt, Chander, & Sharma, 2005). Thus, the inhibition of microbial proliferation and the extension of pork shelf life are currently hot topics.

Irradiation, as a non-thermal processing technology offers advantages such as eco-friendliness, absence of chemical residues, low cost, strong penetration capability, rapid sterilization speed compared to alternative methods (Fan et al., 2024; Wang, Liang, et al., 2022) considered the safest and most effective method for extending the shelf life of meat products. The U.S. Food and Drug Administration has already approved irradiation for use in meat products to control foodborne pathogens and extend the shelf life of the product.

After irradiation, a large number of free radicals are generated in the meat, which can damage chromosomal DNA, leading to the inactivation of microorganisms and parasites. However, (Jia, Wang, Zhang, Shi, & Shi, 2023) indicated that many of the chemical and biological changes in irradiated meat are associated with free radical reactions, particularly the oxidation of lipids and proteins, resulting in changes in sensory quality of the meat, and these adverse changes in pork quality caused by irradiation have remarkably limited the application of this technology in pork preservation.

In recent years, most current research on the effects of irradiation on pork is based on studies of changes in physicochemical indicators (color, texture, TBARS) and volatile flavor compounds in pork. Some studies have reported that the free radical chain reactions induced by irradiation can lead to lipid oxidation, resulting in changes in the types and relative contents of compounds such as aldehydes, alcohols, ketones, and hydrocarbons, cause adverse changes in the odor of meat (Guo et al., 2021). Irradiation can increase the content of fresh-tasting amino acids while reducing the content of bitter and sweet amino acids (Chen et al., 2023). In addition, irradiated pork has been observed to impart a more

* Corresponding author at: College of Food and Bioengineering, Xihua University, Chengdu 610039, China.
E-mail address: yongtang@mail.xhu.edu.cn (Y. Tang).

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vivid red color and enhanced hardness of texture (Chen et al., 2023; Kanatt, Chawla, & Sharma, 2015; Li, Jin, He, & Xiao, 2020). Nevertheless, few studies have investigated the formation mechanism of pork quality changes by combining the changes in volatile substances with pork metabolites from the perspective of substance content.

Metabolomics is the application of systematic methods for the high-throughput identification of small-molecule metabolites (Wu et al., 2022), it can analyze multiple metabolites and their metabolic pathways within intricate biological systems, has been extensively utilized in food testing (Setyabrata et al., 2021). Through metabolites, our understanding of the biological dynamic processes that regulate meat quality can be enhanced, providing a visual explanation of the principles behind the changes in important evaluation indicators after irradiation, including meat color, texture, and flavor attributes.

Therefore, this study aims to use UPLC-Q-TOF MS and apply metabolomics to compare the metabolites before and after meat irradiation and analyze the differences in metabolic pathways, combined with GC–MS to interpret the underlying causes leading to changes in sensory quality of pork after irradiation, to provide theoretical basis for the direction of changes in the preservation quality of pork after irradiation.

Materials and methods

Sample preparation

The samples were made using tenderloin meat from Yorkshire pigs purchased at the Walmart supermarket (Pidu, Chengdu). The pigs were fed for approximately 180 days and were stunned using an electric shock in a commercial centralized slaughterhouse. Then, they were bled using standard industry protocols, and the facilities of the slaughterhouse met the requirements of relevant government departments. The meat had also been inspected, and a certificate of compliance was obtained. To mitigate the influence of extraneous experimental variables, all pork samples were obtained from the carcass approximately 36 h post-slaughter. After being freshly cut, they were promptly transported back to the laboratory in insulated boxes equipped with ice packs for processing. The purchased pork was divided into approximately 3 cm^3 (2 cm × 2 cm × 0.75 cm) cubes after removing excess fat, mixed thoroughly, and then randomly sampled and packaged in vacuum-sealed polyethylene bags. The vacuum-packed meat samples labeled with the irradiation dose, were stored in an incubator with ice cubes and then sent to the Sichuan Atomic Energy Research Center for irradiation treatment (^{60}Co irradiation source, FJX-432G2 mode) at 1, 3, and 5 kGy. Based on equipment and facility conditions, pork samples were irradiated with doses of 1 kGy, 3 kGy, and 5 kGy, experiencing exposure durations of 1 h, 2 h, and 6 h, respectively. After irradiation, the samples placed into an incubator and returned to the laboratory immediately for experimentation.

Sensory evaluation

The sensory evaluation criteria were developed in accordance with the method of (Zhang, 2023) with slight modifications. Ten food professionals with sensory evaluation experience (five males and five females) were trained and scored the odor, appearance, texture, and overall acceptability of the products in the sensory laboratory. Each index was assigned a maximum score of 100 points, and the higher the score, the more acceptable the index was to the sensory evaluators. The evaluation was performed under normal lighting and room temperature (25 °C). The experimental protocol has been approved by the Institutional Review Board (College of Food and Bioengineering, Xihua University), and the individuals who participate in sensory experiments are informed of the experimental protocol and volunteer to participate. The rights and privacy of all participants are well protected, and they consent to the collection and use of their personal information and relevant experimental data.

Changes in TBARS

Based on the method described by (Wang et al., 2021) with slight modifications, 10 g of minced pork was accurately weighed and mixed with 50 mL of 7.5 % trichloroacetic acid (containing 0.1 % ethylenediaminetetraacetic acid disodium salt). The mixture was homogenized at 6000 rpm for 1 min and transferred to a 100 mL conical flask. The solution was filtered using double-layer qualitative filter paper and 5 mL of the filtrate was pipetted into a 20 mL graduated tube. Then, 5 mL of 0.02 mol/L thiobarbituric acid solution was added, and the mixture was shaken well and heated in a boiling water bath for 30 min. A blank experiment was also performed simultaneously. After cooling for 1 h, 20 μL of each sample were pipetted into the microtiter plate, and the absorbance was measured at 532 nm and 600 nm, with the blank as a control.

$$TBARS\ (\mathrm{mg/kg}) = \frac{(A_{532} - A_{600}) \times 72.6 \times 100}{155 \times 10}$$

Changes in pork color

Color measurements were taken using a precision colorimeter (CR-400, Konica Minolta Investment Co., Ltd., Shanghai, China). The calibrated precision colorimeter was employed to determine the lightness (L^*), redness (a^*), and yellowness (b^*) values of meat samples (Li et al., 2017). Six replicate measurements were conducted at different locations on the pork. During the measurement, the D-65 light source was used, and a white calibration plate was applied for correction prior to measurement. The probe was kept in close contact with the sample to ensure accurate readings, while the indoor environment remained free from direct sunlight or other strong light sources.

Headspace solid-phase microextraction (HS-SPME)

Three different irradiation dose groups and the non-irradiated group of pork were separately chopped, 2 g of minced pork was precisely weighed and transferred into a 15 mL SPME vial. The aged SPME needle was inserted into the headspace of the sample, which was equilibrated at 50 °C for 30 min (Wang, Dong, et al., 2022). The extraction needle was inserted into the GC injection port using manual injection mode, and the sample was desorbed at 250 °C for 3 min.

Volatile compound analysis

GC–MS was performed on a Shimadzu QP2020 NX (Shimadzu Corporation, Kyoto, Japan) equipped with an HP-5 quartz capillary column (30 m × 0.32 mm, 0.25 μm) at the following conditions: helium flow rate, 1.00 mL/min; the injection port temperature, 250 °C, and the unsplit mode was used. The automatic temperature program was as follows: the first stage was 40 °C, held for 3 min; the second stage was heated at 5 °C/min to 90 °C, held for 0 min; the third stage was heated at 10 °C/min to 240 °C, held for 5 min. The MS conditions were as follows: ionization mode was EI; electron impact energy, 70 eV; interface temperature, 220 °C; ion source temperature, 230 °C, and mass scanning range, 35–500 *m/z* (Chen et al., 2023).

Metabolite extraction from irradiated pork

Pork at different irradiation doses was crushed and homogenized. Afterward, 2.0 g of pork was accurately weighed in a 50 mL centrifuge tube. Subsequently, 10 mL of methanol–water solution (1:1, v/v) was added, vortexed for 1 min using a vortexer, ultrasonicated for 10 min, then vortexed for 1 min, and ultrasonicated for 10 min. The mixture was then incubated at 1 °C for 3 h and centrifuged at 4000 r/min for 15 min.

The supernatant was filtered through a 0.22 μm PTFE filter and collected in a 2 mL brown vial for analysis (Cao et al., 2020). HPLC-grade formic acid, acetonitrile, and methanol were obtained from Fisher Scientific (Shanghai, China), and the remaining reagents used in this study were of analytical grade.

In testing the stability and reliability of the instrument, 20 μL of each sample was aspirated and mixed homogeneously as quality control (QC) samples, and one QC sample was inserted after every five injections of the actual sample.

UPLC-Q-TOF MS

The samples were placed in a 4 °C automatic sampler, and the SHIMADZU-LC30 ultra-high-performance liquid chromatograph system (UHPLC) equipped with an Ultimate UHPLC XB-C18 (1.8 μm, 2.1 mm × 100 mm) column was used. LC–MS analysis was was performed using a Q-TOF (SCIEX Co., Framingham, MA, USA). The injection volume was 5 μL; the column temperature was 40 °C, and the flow rate was 0.3 mL/min. Mobile phase A was 0.1 % formic acid water solution, and mobile phase B was 0.1 % formic acid acetonitrile (Cao et al., 2020).

The gradient elution procedure was as follows: 0–1 min, 0 % B; 1–7 min, 0 %–40 % B; 7–9 min, 40 %–85 % B; 9–14 min, 85 %–100 % B; 14–17 min, 100 % B washing the column; 17–17.1 min, 100 % B–0 % B; 17.1–20 min, 0 % B rebalancing the column.

The MS conditions were as follows: The samples were analyzed by UHPLC-Q-TOF in both positive and negative ionization modes, with MS precursor ion MS2 ion scanning from 50 to 700 Da. The parameters for data acquisition were as follows (Li et al., 2023): nebulizer gas (nitrogen) pressure at 2 bar. The positive and negative ion modes were applied with capillary voltages of 5.5 and 4.5 kV, respectively. The ion source temperature was set at 450 °C, and the dry gas flow was set at 9 L/min. The resolution of the TOF system was 32,000 FWHM at 200 *m/z*. The data acquisition mode used was information-dependent acquisition, with the top ten most intense selected ions per spectrum being fragmented (MS/MS) using collision-induced dissociation energy at 35 eV.

Data processing and statistical analysis

The raw data exported from UPLC-Q-TOF MS were imported into Analysis BaseFile Converter for format conversion and analyzed using MSDIAL version 4.9 software. The peak area data of each substance obtained were then imported into Metaboanalyst 5.0 online data analysis software for normalization. Afterward, the standardized data were imported into SIMCA 14.1 for multivariate statistical analysis, including PCA analysis and PLS-DA model construction. Finally, variables with a VIP of >1 and *P* of <0.05 were selected as differential metabolites between the experimental group and the control group. The remaining metabolites were visualized using Origin 2022 software, while significance analysis was conducted with SPSS 22 software. Differences were considered statistically significant when $P < 0.05$. Results were presented as the mean ± standard error (SE).

The measurement data of UPLC-Q-TOF MS were obtained through five replicate experiments. Analysis of Variance (ANOVA) was employed to significant differences analysis.

Results and discussion

Sensory evaluation

As shown in Fig. 1a, different irradiation doses had a significant impact on the sensory properties of pork. With regard to color, the score of pork color increased with the increase of irradiation dose. Irradiation could make the pork color more ruddy and enhance its commercial attributes, which was attributed to the fact that irradiation treatment reduced the redox potential and produced carbon monoxide, which could serve as the sixth coordination group of myoglobin, forming more stable CO-myoglobin than oxygenated myoglobin (Nam & Ahn, 2002). With regard to smell, the scores decreased gradually with the increase of irradiation dose, which was opposite to the color scores. This result may be due to the fact that irradiation causes the oxidation of fats, producing unpleasant special radiation flavors (Li et al., 2017). The pork tissue toughened with the increase of irradiation dose. The score of pork tissue state also increased accordingly probably because the myofibrillar units in meat skeletal muscle decreased in size, causing contraction of the sarcomere width, another feasible explanation can be the possibility of the aggregation effect of proteins caused by irradiation, accompanied by the generation of high molecular protein groups and the decrease in protein solubility (Yoon, 2003). The overall acceptability score did not have a linear relationship with the irradiation dose, with the scores from highest to lowest being 70.8 points for the 1 kGy group, 68.9 points for the non-irradiated group, 56.9 points for the 3 kGy group, and 36.1 points for the 5 kGy group. Although irradiation can bring advantages with regard to pork color, the increase in irradiation flavor with the increase of irradiation dose makes it unacceptable. Combining the indicators of color, smell, and tissue state, irradiated pork at 1 kGy is the best among the four groups of pork, indicating that appropriate irradiation treatment can increase the acceptability of fresh pork and enhance its commercial attributes.

TBARS

TBARS values can accurately evaluate the degree of lipid oxidation (Wang, Dong, et al., 2022). As shown in Fig. 1b, the TBARS value for the non-irradiated group is 0.12 mg/kg, with the increase of irradiation dose, the TBARS value of pork also shows an increasing trend, the TBARS values of pork at 1 kGy, 3 kGy, and 5 kGy are 0.16 mg/kg, 0.19 mg/kg, and 0.21 mg/kg, indicating that irradiation generates a large number of free radicals that accelerate lipid oxidation.

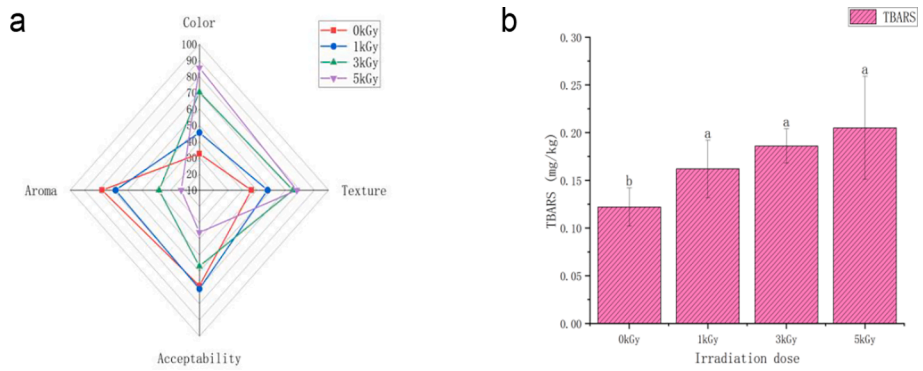

Fig. 1. (a) Sensory evaluation chart of different irradiated pork meat, (b) Effects of different irradiation doses on TBARS of pork.

Pork color

As shown in Table 1, L^* and b^* values did not exhibit significant changes with irradiation dose treatment, whereas a^* values showed a positive correlation with increasing irradiation dose. The higher the irradiation dose, the greater the a^* value, resulting in pork appearing more ruddy, this observation is consistent with the findings of other researchers' studies on raw meat (Li et al., 2017; Nam & Ahn, 2002) discovered that there is a positive correlation between the production of CO gas and the irradiation dose. An increase in the amount of carbon monoxide forming compounds with myoglobin has led to a rise in the a^* value of pork as the irradiation dose increases.

Changes in volatile compounds

As shown in Table 2, 76 volatile components were detected in pork samples treated with different irradiation doses, including 28 types of hydrocarbons, 17 types of alcohols, 15 types of aldehydes, 8 types of esters, 2 types of ketones, 2 types of phenols, 2 types of acids, and 2 types of others. Fig. 2 (a) shows that the categories of hydrocarbons, alcohols, and aldehydes account for the largest proportion.

As shown in Table 3, compared with the irradiated group, the unirradiated group contained 40 detected volatile compounds, whereas the 1 kGy group had 45, the 3 kGy group had 42, and the 5 kGy group had 56. This result is similar to that reported by (Wang, Dong, et al., 2022), indicating that the three irradiated treatment groups all experienced an increase in the variety of volatile flavor compounds, with significant increases in the number of hydrocarbons, aldehydes, and ketones being derived from fat oxidation.

Hydrocarbons are the major radiolytic products in fat and are related to the fatty acid composition of fats (Li et al., 2017). Hydrocarbons have a high aroma threshold. They do not contribute much to the flavor of meat products, but they may be important intermediates in the formation of heterocyclic compounds, which can enhance the overall flavor to some extent (Wu, Zhan, Tang, Li, & Duan, 2022). In the experiment, 11 kinds of hydrocarbons were detected in the pork sample without irradiation treatment, while 13, 15, and 18 kinds of hydrocarbons were detected in the 1 kGy, 3 kGy, and 5 kGy experimental groups, respectively. Therefore, the increase in irradiation dose promotes the cleavage of the chemical bonds of the substance.

Aldehydes are mostly derived from the oxidative degradation of unsaturated fatty acids, and they have low odor thresholds. They are major components of volatile compounds in meat products (Feng, Moon, Lee, & Ahn, 2017). In the experiment, the relative contents of the non-irradiated group and the 1 kGy, 3 kGy, and 5 kGy dose groups were 58.38 %, 68.07 %, 68.82 %, and 68.43 % respectively, indicating that aldehyde compounds have the greatest contribution to the flavor of irradiated meat and are positively correlated with the irradiation dose. Benzaldehyde was detected in non-irradiated pork, which has a fragrance similar to hyacinth and naturally occurs in meat. However, benzaldehyde was not detected in irradiated pork, indicating that it may be a characteristic flavor substance of fresh pork (Meng, 2018). Hexanal, which has a pungent odor, has a high correlation with the degree of meat oxidation (Feng et al., 2017). In the experiment, the relative content of hexanal increased from 13.197 % in the non-irradiated group to 18.134 % in the 1 kGy group, 20.06 % in the 3 kGy group, and 20.303 % in the 5 kGy group, confirming that radiation causes accelerated lipid oxidation.

Table 1
Effects of Different Irradiation Doses on Pork Color.

	0 kGy	1 kGy	3 kGy	5 kGy
L^*	5.86 ± 0.61A	5.88 ± 0.78A	5.58 ± 0.29A	5.67 ± 0.89A
a^*	1.68 ± 0.19D	2.27 ± 0.52C	2.86 ± 0.47B	3.92 ± 0.48A
b^*	1.06 ± 0.31A	1.32 ± 0.35A	1.41 ± 0.29A	1.31 ± 0.39A

$^{A–D}$ represent differences in the same value under different irradiation doses ($p < 0.05$).

In general, alcohols are the products of lipid oxidation, and they can be generated by reducing aldehydes with free hydrogen (Brewer, 2009). They also exert a remarkable impact on the formation of meat flavor, especially unsaturated alcohols, which have lower odor threshold values and have a greater impact on flavor. Heptanol (fruity wine aroma with waxy aroma), 2,4-dimethylcyclohexanol (fresh and fragrant smell), 1-nonanol (slightly rose-like aroma), and 1-octadecanol are specific alcohol flavor substances in the irradiated group, which may give irradiated pork a special flavor.

Ketones are often formed by the decarboxylation of two carboxylic acids to form a ketone group (Renz, 2005). Only two ketone substances, namely, 2-heptanone and geranylacetone, were detected in the 5 kGy irradiation group, which is consistent with the results of (Feng et al., 2017). Therefore, large doses of irradiation can result in differences in flavor.

Acids may originate from the oxidative degradation of fatty acids or from the oxidation of aldehydes (Chen et al., 2021), and they usually have a pungent odor. In this experiment, only two types of acids have a low relative content, namely, pentanoic acid and phosphonoacetic acid. Pentanoic acid was only present in the irradiated group, and it had a pungent odor.

Compared with the non-irradiated group, the types of aldehydes, hydrocarbons, and acids in the irradiated group increased significantly, indicating that irradiation promotes fat oxidation. Given the production of new flavor compounds and the increase in the content of some substances, the irradiated flavor is generated. As the irradiation dose increases, the high-dose group of pork produces substances that do not exist in the low-dose group, such as aldehydes (E, E)-2,4-nonadienal, heptadecanal), hydrocarbons (pentyl cyclopropane), acids (pentanoic acid), and ketones (2-heptanone, geranylacetone), or changes in the relative content of some flavor compounds, making its unique irradiated flavor more evident.

Qualitative results of untargeted metabolomics of irradiated pork meat

Non-targeted metabolic analysis was performed between the three irradiated samples and the control using UPLC-Q-TOF MS. A total of 266 metabolites were detected in ESI (+) mode, and 97 metabolites were detected in ESI (−) mode, for a total of 363 metabolites.

In distinguishing the changes in pork metabolites treated with different doses of irradiation, we used unsupervised PCA and supervised PLS-DA multivariate analysis to investigate the relationship between metabolites and the quality changes in irradiated pork. As shown in Fig. 3 (a, d), t1 represents principal component 1, and t2 represents principal component 2. In the positive (Fig. 3a) and negative (Fig. 3d) ion modes, the five group samples form independent regions in the entire space, and the five parallel samples among groups are closely clustered together, indicating that the five group samples have significant differences and can be well distinguished. The clustering of QC samples can reflect the repeatability of this experiment. As shown in the figure, the QC samples are tightly clustered in the positive and negative ion modes, indicating that this experiment has good repeatability.

Based on effective data, the impact of irradiation on pork metabolites was analyzed by PLS-DA to obtain differential metabolites among pork samples with different irradiation intensities. As shown in Fig. 3 (b, e), the classification parameters (R2Y) under the positive ionization mode and negative ionization mode were 0.984 and 0.994, respectively. The Q^2 values of the positive ion mode and negative ion mode were 0.966 and 0.985, respectively, indicating that both modes have a good fitting ability and prediction ability. As shown in Fig. 3(c) and Fig. 3(f), a permutation test of 200 iterations showed that the intercept values of R^2 and Q^2 were (0, 0.361) and (0, −0.532) in the positive mode and (0, 0.206) and (0, −0.484) in the negative mode, indicating that the PLS-DA model did not overfit and was reliable. Then, a VIP of >1 and P value of <0.05 were used as the screening conditions for differential metabolites.

Table 2
Effects of different irradiation doses on volatile flavor compounds of pork.

Name	CAS NO	Retention Time	Relative amount/%			
			0 kGy	1 kGy	3 kGy	5 kGy
Hydrocarbons						
3-Ethyl-3-methylheptane	17302-01-1	12.824	——	——	——	0.318 ± 0.024
Pentylcyclopropane	2511-91-3	13.409	——	——	——	0.891 ± 0.058
5-Methyl-5-propylnonane	17312-75-3	13.961	——	0.042 ± 0.004	——	——
Dodecane	112-40-3	16.587	0.096 ± 0.017	0.054 ± 0.018	0.114 ± 0.005	0.054 ± 0.007
1,3-Di-*tert*-butylbenzene	1014-60-4	17.521	——		0.147 ± 0.024	0.039 ± 0.004
Heneicosane	112-95-8	18.003	0.228 ± 0.026	0.261 ± 0.078	——	0.249 ± 0.056
1-Iodoeicosane	34994-81-5	18.007	0.219 ± 0.044	0.069 ± 0.006	——	0.129 ± 0.035
3-Methyl-5-propylnonane	31081-18-2	18.152	0.147 ± 0.001	——	——	0.132 ± 0.021
1-Iodooctadecane	629-93-6	18.251	——	——	0.063 ± 0.008	——
2,6,11-Trimethyldodecane	31295-56-4	18.392	——	0.210 ± 0.033	——	0.129 ± 0.004
Tetradecane	629-59-4	18.809	——	——	0.327 ± 0.084	——
6-Methyltridecane	13287-21-3	18.814	0.525 ± 0.025	——	——	——
4,6-Dimethyldodecane	61141-72-8	18.979	0.249 ± 0.010	——	0.210 ± 0.006	0.480 ± 0.160
2,6,10-Trimethyltridecane	3891-99-4	19.11	——	——	0.150 ± 0.051	——
Hexadecane	544-76-3	19.6	0.39 ± 0.046	0.315 ± 0.027	0.228 ± 0.073	0.150 ± 0.031
Pentadecane	629-62-9	21.508	0.231 ± 0.013	0.108 ± 0.009	0.132 ± 0.025	——
Eicosane	112-95-8	21.963	——	——	——	——
6-phenylundecne	4537-14-8	23.22	——	0.036 ± 0.006	——	0.009 ± 0.002
5-Phenylundecane	4537-15-9	23.272	——	0.048 ± 0.002	0.048 ± 0.006	0.021 ± 0.001
Icosan-4-ylbenzene	2400-03-5	23.41	——	0.090 ± .0.12	——	——
Heptadecane	629-78-7	24.079	0.111 ± 0.003	——	0.069 ± 0.011	0.084 ± 0.004
6-Phenyldodecane	2719-62-2	24.425	——	0.036 ± 0.004	0.039 ± 0.002	0.021 ± 0.003
5-Phenyldodecane	2719-63-3	24.482	——	0.030 ± 0.005	——	0.030 ± 0.002
4-Phenyldodecane	2719-64-4	24.635	——	——	——	0.015 ± 0.001
3-ethyl-2-methylhexa-1,3-diene	61142-36-7	11.945	——	0.360 ± 0.068	0.168 ± 0.004	0.237 ± 0.030
Longifolene	475-20-7	20.353	0.081 ± 0.007	——	——	——
Beta-Cedrene	546-28-1	20.57	0.093 ± 0.010	——	——	——
1,7-Hexadecadiene	125110-62-5	22.503	——	——	0.099 ± 0.011	0.048 ± 0.005
Aldehydes						
Hexanal	66-25-1	4.664	39.591 ± 4.977	54.402 ± 1.630	60.204 ± 6.958	60.909 ± 3.48
Heptanal	111-71-7	7.645	0.522 ± 0.067	0.555 ± 0.046	0.561 ± 0.036	0.825 ± .0.032
Octanal	124-13-0	11.072	2.847 ± 0.420	2.385 ± 0.365	1.368 ± 0.138	1.089 ± 0.110
Benzeneacetaldehyde	122-78-1	12.435	2.253 ± 0.282	——	——	——
Trans-2-Octenal	2548-87-0	12.926	0.597 ± 0.093	0.405 ± 0.057	0.342 ± 0.041	0.288 ± 0.030
Nonanal	124-19-6	14.311	11.397 ± 1.738	8.754 ± 1.147	5.466 ± 0.259	4512 ± 0.197
Trans-2-Octenal	18829-56-6	15.704	0.180 ± 0.025	0.138 ± 0.018	0.102 ± 0.009	0.081 ± 0.007
Trans-4-Decen-1-al	65405-70-1	16.441	——	0.072 ± 0.007	——	0.045 ± 0.002
Decanal	112-31-2	16.7	0.777 ± 0.156	0.699 ± 0.117	0.510 ± 0.049	0.255 ± 0.024
Trans-2-Decenal	3913-81-3	17.801	0.237 ± 0.007	0.189 ± 0.011	——	0.102 ± 0.002
Pentadecanal	2765-11-9	18.07	——	0.180 ± 0.009	0.084 ± 0.004	——
Undecanal	112-44-7	18.592	——	0.123 ± 0.023	0.105 ± 0.007	0.078 ± 0.001
Tridecanal	10486-19-8	20.215	——	0.153 ± 0.032	0.081 ± 0.004	0.141 ± 0.009
(E,E)-2,4-Nonadienal	629-90-3	24.281	——	——	——	0.117 ± 0.017
Heptadecanal	5910-87-2	16.921	——	——	——	0.063 ± 0.006
Alcohols						
1-Pentanol	71-41-0	3.952	6.831 ± 2.163	5.037 ± 0.678	5.43 ± 0.811	6.408 ± 0.495
1-Hexanol	111-27-3	6.785	0.819 ± 0.032	0.786 ± 0.098	0.330 ± 0.037	0.651 ± 0.131
1-Heptanol	111-70-6	10.078	——	0.255 ± 0.044	0.273 ± 0.039	0.228 ± 0.003
1-Octen-3-ol	3391-86-4	10.39	5.661 ± 0.776	4.890 ± 0.364	4.338 ± 0.222	3.81 ± 0.284
2-Ethylhexan-1-ol	104-76-7	12.007	0.573 ± 0.012	——	——	——
4-Ethylcyclohexanol	4534-74-1	12.249	0.705 ± 0.023	0.561 ± 0.036	0.378 ± 0.025	0.333 ± 0.020
2,4-Dimethylcyclohexanol	69542-91-2	12.58	——	0.231 ± 0.022	0.144 ± 0.010	0.132 ± 0.009
Trans-2-Octen-1-Ol	18409-17-1	13.309	0.795 ± 0.0470	0.612 ± 0.071	0.510 ± 0.031	0.627 ± 0.062
1-Octanol	111-87-5	13.416	2.427 ± 0.298	2.49 ± 0.077	1.020 ± 0.044	——
3,4-Dimethylpent-2-en-1-ol	1623076-33-4	14.048	0.729 ± 0.098	0.495 ± 0.021	0.459 ± 0.025	0.561 ± 0.055
Linalool	78-70-6	14.229	0.369 ± 0.005	——	——	——
Isoborneol	124-76-5	15.81	0.093 ± 0.003	——	——	——
Trans-2-Dodecen-1-ol	22104-81-0	15.928	0.183 ± 0.033	——	0.186 ± 0.028	0.051 ± 0.003
1-Nonanol	143-08-8	16.038	——	0.096 ± 0.001	——	0.042 ± 0.001
Trans-2-Undecen-1-ol	75039-84-8	18.072	0.462 ± 0.035	——	——	——
1-Octadecanol, TMS derivative	18748-98-6	19.937	——	——	——	0.150 ± 0.003
1-Dodecanol	112-53-8	21.192	0.216 ± 0.001	0.111 ± 0.001	0.423 ± 0.030	0.060 ± 0.009
Ketones						
2-Heptanone	110-43-0	7.275	——	——	——	0.210 ± 0.005
Geranylacetone	689-67-8	20.78	——	——	——	0.033 ± 0.002
Phenols						
2-Bromo-4,6-di-*tert*-butylphenol	20834-61-1	23.024	0.684 ± 0.083	0.459 ± 0.013	0.240 ± 0.008	0.19 ± 0.005
4-*sec*-Butyl-2,6-di-*tert*-butylphenol	17540-75-9	23.168	——	0.042 ± 0.006	——	——
Esters						
Vinyl hexanoate	3050-69-9	10.493	15.87 ± 2.101	12.180 ± 0.497	4.65 ± 0.504	3.838 ± 0.716
Pentadecafluorooctanoic acid, 2-ethylhexyl ester	62185-54-0	17.894	——	——	——	0.030 ± 0.008
N-Methyl-dithiocarbonimidic acid dimethyl ester	18805-25-9	17.961	0.186 ± 0.004	——	——	——

(*continued on next page*)

Table 2 (*continued*)

Name	CAS NO	Retention Time	Relative amount/%			
			0 kGy	1 kGy	3 kGy	5 kGy
Sulfurous acid, butyl dodecyl ester	959095-65-9	18.81	——	0.252 ± 0.041	——	——
Carbamodithioic acid, diethyl-, methyl ester	686-07-7	19.797	0.408 ± 0.073	0.258 ± 0.013	0.036 ± 0.019	0.034 ± 0.007
Butyric acid, 2-phenyl-, dec-2-yl ester	170899-22-6	22.374	——	0.081 ± 0.003	——	0.015 ± 0.004
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	6846-50-0	22.699	0.084 ± 0.015	0.054 ± 0.004	0.017 ± 0.012	0.008 ± 0.001
Dibutyl phthalate	84-74-2	26.997	——	——	——	0.028 ± 0.017
Acids						
Pentanoic acid	109-52-4	10.944	——		——	0.061 ± 0.005
Phosphonoacetic Acid	4408-78-0	17.228	1.497 ± 0.044	0.444 ± 0.077	0.268 ± 0.063	0.130 ± 0.078
Others						
Methyl *N*-hydroxybenzimidate	67160-14-9	8.539	0.576 ± 0.093	0.828 ± 0.257	0.242 ± 0.086	0.804 ± 0.194
1,1,3-Trimethyl-3-phenyl-2,3-dihydro-1H-indene	2613-76-5	24.346	——	——	——	0.016 ± 0.010

Notes: Values represent the average ± SEM, SEM stands for standard error of mean,(——) means not detected.

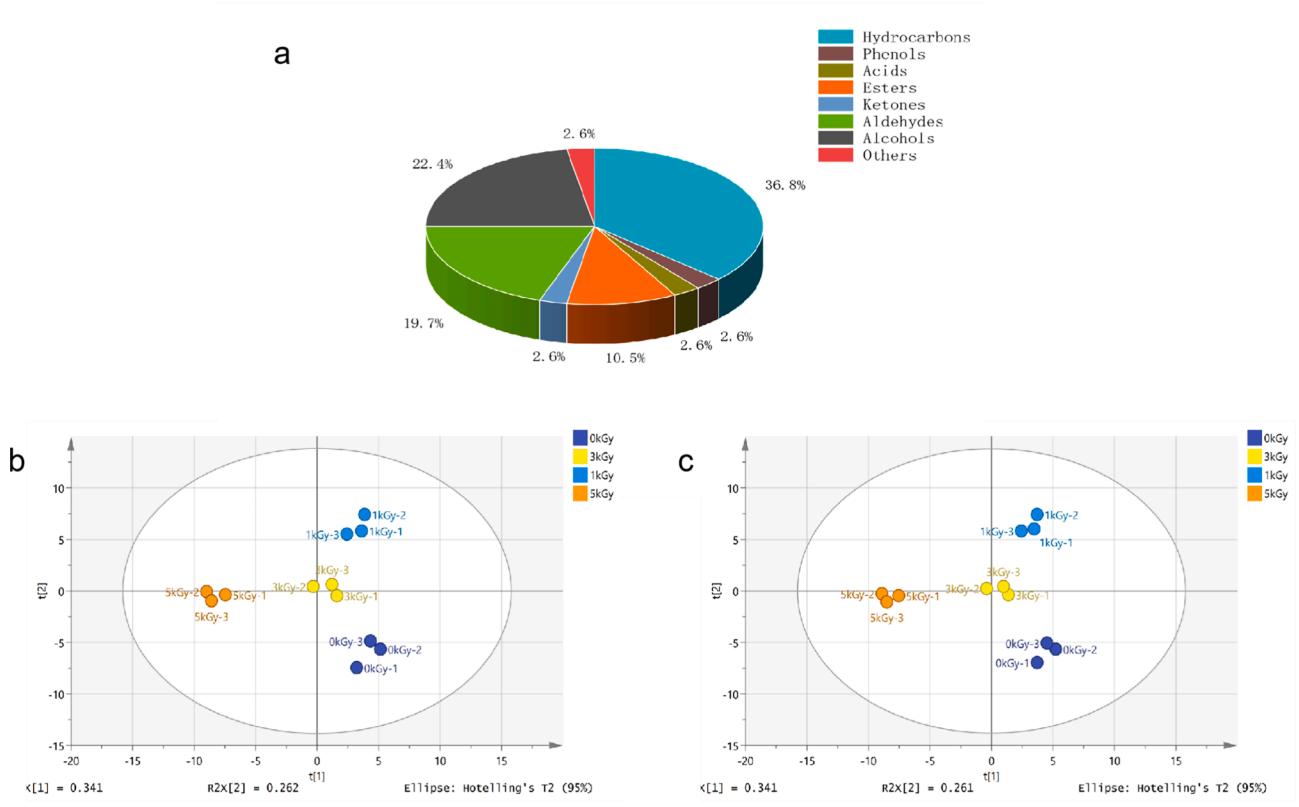

Fig. 2. (a) Analysis of volatile components species distribution, (b) PCA plot, (c): PLS-DA plot.

Table 3
Relative contents of volatile substances and total number of substances in pork at different irradiation doses.

Volatile components	relative content (Substance Total)			
	0 kGy	1 kGy	3 kGy	5 kGy
Hydrocarbons	2.40 % (11)	1.65 %(13)	1.79 %(15)	3.03 %(18)
Aldehydes	58.38 %(9)	68.07 % (12)	68.82 % (10)	68.49 % (12)
Alcohols	19.86 % (13)	15.57 % (11)	13.50 % (11)	13.08 % (13)
Ketones	0 %(0)	0 %(0)	0 %(0)	0.24 %(2)
Phenols	0.66 %(1)	0.51 %(2)	0.24 %(1)	0.18 %(1)
Esters	16.59 %(4)	12.84 %(5)	14.11 %(3)	11.91 %(6)
Acids	1.50 %(1)	0.45 %(1)	0.81 %(1)	0.60 %(2)
Others	0.57 %(1)	1.20 %(1)	0.24 %(1)	2.46 %(2)
Total	100 %(40)	100 %(45)	100 %(42)	100 %(56)

As shown in Table 4, 44 differential metabolites were screened in the positive ion mode, whereas 21 differential metabolites were screened in the negative ion mode, for a total of 65 metabolites. These metabolites included 7 amino acids, 1 pyridine, 5 alcohols, 3 dipeptides, 1 nucleotide, 9 flavonoids, 7 organic acids, 7 alkaloids, 2 sugars, 2 ketones, 3 vitamins, 2 amides, 1 indole, 5 esters, 1 pyrazine, and 9 other compounds.

Changes in differential metabolites with different irradiation doses

Cluster heat map (Fig. 4a, Fig. 4b) analysis was utilized to visualize the differences directly in metabolites of pork at different irradiation intensities. Each row in the plot represents a differential metabolite, and the darker the square is, the higher its content in that sample.

In general, the quality changes in pork before and after irradiation are primarily due to changes in non-volatile substances, as these non-volatiles are precursors of volatile flavor compounds. Unsaturated fatty acids, free amino acids, inosine monophosphate, inorganic salts,

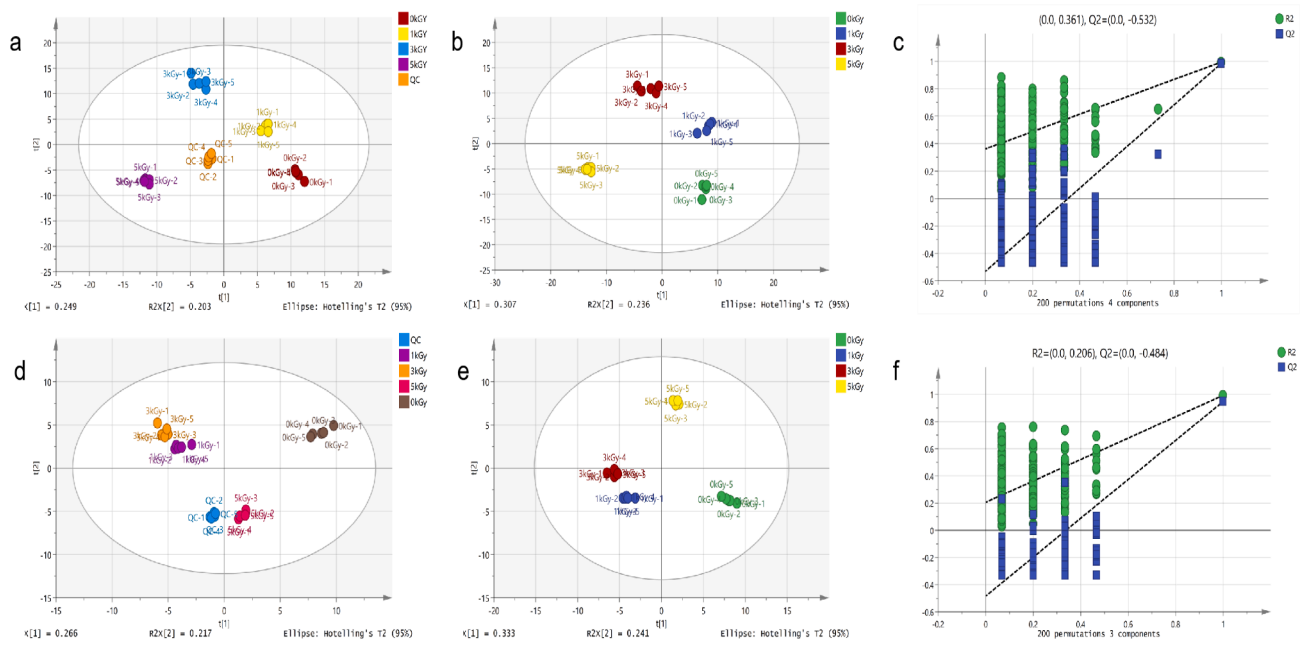

Fig. 3. Multivariate statistical analysis of identified metabolites in three irradiation doses. (a) PCA score plots of samples acquired in positive mode. (b) PLS-DA score plots of samples acquired in positive mode. (c) The validation of the PLS-DA model by permutation testing (200 iterations) in positive mode. (d) PCA score plots of samples acquired in negative mode. (e) PLS-DA score plots of samples acquired in negative mode. (f) The validation of the PLS-DA model by permutation testing (200 iterations) in negative mode.

ribose, polypeptides, and organic acids are common compounds that can cause changes in taste presenting (Wang, Dong, et al., 2022). Based on the heat map, significant changes were observed in the levels of amino acids and polypeptides such as L-pyroglutamic acid, isoleucine-leucine, isoleucylvaline, L-aspartic-acid-L-phenylalanine, L-glutamic acid, L-proline, kynurenine, L-anserine, *N*-acetylornithine, and *N*-methylhistidine. The contents of kynurenine, L-anserine, *N*-acetylornithine, and *N*-methylhistidine were upregulated, whereas the contents of L-pyroglutamic acid, L-aspartic-acid-L-phenylalanine, isoleucine-leucine, isoleucylvaline, L-glutamic acid, and L-proline were downregulated.

Amino acids, polypeptides and thier derivatives are also the precursors of many flavor substances and are crucial to the overall flavor formation of irradiated pork (Ardö, 2006; Jia et al., 2021).

L-Pyroglutamic acid is a metabolite of the glutathione cycle converted to glutamate by 5-oxoprolinase and is a natural amino acid derivative, have salty, umami, and sour flavors (Eom et al., 2023). L-Glutamic acid has a umami taste, and it is considered as the main contributor to chicken flavor. L-Glutamic contributes to the flavor of meat, including the "umami" and "brothy" descriptors, and is one of the important taste-active components in meat (Watanabe et al., 2017). The content of L-pyroglutamic and L-glutamic acid in irradiated pork were significantly reduced, so irradiation treatment may have a greater impact on meat flavor. The significant downregulation of proline content in irradiated pork may be related to muscle tissue damage and denaturation, as radiation-induced proline residues can also oxidize myofibrillar proteins in pork, leading to protein aggregation and other problems (Wang, Dong, et al., 2022), thereby affecting the quality and taste of meat, which is also related to the texture score of the irradiated group in sensory experiments. In addition, we discovered *N*-acetylglutamine, which is an intermediate in arginine synthesis. Upregulation of its content may promote arginine synthesis, thereby influencing the quality of pork.

Dipeptides such as L-anserine, L-aspartic acid-L-phenylalanine, isoleucine–leucine, and isoleucylvaline showed significant differences between irradiated and non-irradiated pork. Maehashi, Matsuzaki, Yamamoto, and Udaka (1999) demonstrated that dipeptides can improve the taste and flavor of pork, which may also contribute to the difference in pork flavor before and after irradiation. This result may also contribute to the difference in pork flavor before and after irradiation. L-Anserine is a type of carnosine with a bitter and umami taste. Liu et al. (2021) pointed out in the experimental results that L-Anserine represent umami, and the increased L-anserine in the MG group can improve the taste of meat. It is also a key precursor for the formation of flavor-related components in chicken and meat soup. Upregulation of L-Anserine contributes to the improvement of the taste of chicken and meat soup. It was also found in a study that the overall preference of consumers in China and New Zealand for lamb meat is positively correlated with amino acids and L-Anserine (Pavan, Subbaraj, Eyres, Silcock, & Realini, 2022). Furthermore, L-Anserine also has antioxidant properties and can scavenge hydroxyl radicals.

Betaine, the trimethyl derivative of glycine, is a naturally occurring compound widely distributed in plants and animals. Betaine can indirectly affect myoglobin synthesis and meat color by increasing levels of glycine and succinyl coenzyme A. It also enhances fatty acid β-oxidation in muscles, leading to increased levels of creatine and creatinine, effectively improving the flavor of pork (Fu et al., 2022).

The variation of organic acids is also one of the reasons for the change in pork flavor. Fumaric acid, a natural organic acid, possesses the ability to inactivate foodborne pathogens. Among organic acids used as antimicrobial agents in meat, fumaric acid exhibits stronger antibacterial effects compared to acetic acid and lactic acid. The upregulation of fumaric acid detected in irradiated pork may contribute to extending the pork's shelf life. Song, Lee, and Song (2011) treated the ham slices inoculated with microorganisms with 0.5 % fumaric acid. Compared to the control group, fumaric acid treatment reduced the population of Listeria monocytogenes and Salmonella typhimurium by approximately 1 log CFU/g. Although fumaric acid is effective in controlling microorganisms, it significantly affects the quality and sensory characteristics of meat, even in cooked samples.

In addition, vitamins K and niacin, as essential nutrients for the human body, are widely present in animal tissues, and they play important roles in promoting blood clotting, protecting bone health, and promoting growth. The contents of vitamins K and niacin in irradiated pork were significantly increased, indicating that irradiation can also

Table 4
Differential metabolites identified in irradiated pork.

Name	Formula	RT (min)	*m/z*	VIP	P value	CAS NO	Class	Mode
Zataroside B	C16H24O7	15.80345	329.1595	1.264	2.65E−11	95645-52-6	Flavones	Pos
2-[(2-hydroxy-3-methylbutanoyl)amino]-4-methylpentanoic acid	C11H21NO4	3.066767	232.1542	1.46853	1.49E−07	70134-19-9	Organic acids	Pos
2-acetoxy-4-pentadecylbenzoic acid	C24H38O4	0.686733	413.2657	1.0352	1.23E−10	79688-39-4	Organic acids	Pos
2-Aminopyridine	C5H6N2	1.62275	95.06013	1.46951	2.27E−07	504-29-0	Pyrimidines	Pos
(+)-Vestitol	C16H16O4	1.755267	295.0893	1.3792	1.85E−08	20879-05-4	Flavones	Pos
3′,5′-Cyclic dAMP	C10H12N5O5P	1.56355	314.063	1.109	1.12E−14	1157-33-1	Nucleotides	Pos
3,6,9,12-Tetraoxatetracosan-1-ol	C20H42O5	17.48653	363.3104	1.1911	1.01E−09	5274-68-0	Alcohols	Pos
3-Formylindole	C9H7NO	3.1169	146.0598	1.0107	1.86E−10	487-89-8	Indoles	Pos
L-Pyroglutamic	C5H7NO3	2.760217	130.05	1.4657	4.62E−07	98-79-3	Amino acids	Pos
8-acetamido-2-methyl-7-oxononanoic acid	C12H21NO4	3.0999	244.1546	1.4245	3.70E−13	407627-97-8	Organic acids	Pos
L-asparticacid-L-phenylalanine	C13H16N2O5	3.101833	281.1125	1.5274	6.30E−11	13433-09-5	Peptides	Pos
Betaine	C5H11NO2	2.692667	118.0854	1.76	1.21E−06	107-43-7	Alkaloids	Pos
Butyryl carnitine	C13H23ClNO6	10.43457	232.154	1.7351	2.59E−14	25576-40-3	Organic acids	Pos
C14-homoserine lactone	C18H33NO3	12.08168	312.2513	1.1636	6.79E−11	98206-80-5	Organic acids	Pos
Catechin Tetramethylether	C19H22O6	16.3871	347.1964	1.1089	2.71E−10	51079-25-5	Flavones	Pos
Colchicine	C22H25NO6	17.73145	417.1994	1.4422	5.98E−08	64-86-8	Alkaloids	Pos
Decaethylene glycol	C20H42O11	10.89638	481.2623	1.0426	2.37E−10	5579-66-8	Alcohols	Pos
Desferrioxamine B	C25H48N6O8	17.40938	561.3984	1.5046	3.19E−12	70-51-9	Others	Pos
Dihydrocapsaicin	C18H29NO3	10.46552	330.2022	1.0986	3.72E−11	19408-84-5	Others	Pos
Erucamide	C22H43NO	15.94488	338.3425	1.4734	0.00015582	112-84-5	Acylamides	Pos
Estrone	C18H22O2	12.83772	309.1266	1.0671	1.83E−12	53-16-7	Others	Pos
Glucose 6-Phosphate	C6H13O9P	3.367333	283.0189	1.6249	2.68E−14	56-73-5	Carbohydrates	Pos
Glycerol	C3H8O3	18.87607	331.2847	1.1942	1.46E−06	56-81-5	Alcohols	Pos
Isoleucine-leucine	C12H24N2O3	3.063017	245.1852	1.3592	3.76E−09	26462-22-6	Peptides	Pos
Isoleucylvaline	C11H22N2O3	10.52928	231.1702	1.4485	4.59E−16	41017-96-3	Peptides	Pos
Kynurenine	C10H12N2O3	1.60825	209.1034	1.7014	1.37E−07	343-65-7	Amino acids	Pos
L-Anserine	C10H16N4O3	1.72595	241.1296	1.4574	2.43E−09	584-85-0	Amino acids	Pos
L-Glutamic acid	C5H9NO4	2.822517	148.0599	1.4175	6.94E−10	56-86-0	Amino acids	Pos
Methyl-1-oxo-4-(1H-pyrrol-2-yl)-2H-isoquinoline-3-carboxylate	C15H12N2O3	2.61285	307.0443	2.4326	7.89E−15	920020-07-1	Others	Pos
Santonin	C15H18O3	10.4974	247.1286	1.5243	3.39E−15	481-06-1	Others	Pos
Mono-isobutyl phthalate	C12H14O4	13.94127	245.0784	1.1489	1.91E−14	30833-53-5	Esters	Pos
N-2-Fluorenylacetamide	C15H13NO	1.755017	224.1028	1.1908	6.89E−07	53-96-3	Acylamides	Pos
N-Acetylornithine	C7H14N2O3	2.606917	175.0015	1.2736	1.16E−08	6205/8/9	Amino acids	Pos
3-(5,6-dihydroxyheptyl)-4-methyl-2H-furan-5-one	C12H20O4	16.2029	479.2612	1.1582	3.18E−10	6066-49-5	Ketones	Pos
3-(5,7-dimethoxy-4-oxochromen-2-yl)propanoic acid	C14H14O6	1.678333	279.0854	1.5821	1.80E−09	853749-52-7	Alkaloid	Pos
Nicotinic acid	C6H5NO2	1.596817	125.045	1.0856	4.31E−12	59-67-6	Vitamin	Pos
N-Methylhistidine	C7H11N3O2	1.69665	170.0921	1.4904	1.37E−16	332-80-9	Amino acids	Pos
Nonaethylene glycol	C18H38O10	10.8804	437.2352	1.075	1.50E−13	3386-18-3	Alcohols	Pos
Ononin	C22H22O9	13.2861	453.1162	1.2852	9.36E−06	486-62-4	Flavones	Pos
Proline	C5H9NO2	2.70725	229.1544	1.1341	0.000235	344-25-2	Amino acids	Pos
Tetradecanoylcarnitine	C21H41NO4	12.71657	372.312	1.1268	7.81E−09	25597-07-3	Others	Pos
Theophylline	C7H8N4O2	3.021883	219.0178	1.7091	0.000835	58-55-9	Alkaloids	Pos
Vitamin B12	C63H88CoN14O14P	14.70422	678.2984	1.2879	4.04E−15	13408-78-1	Vitamins	Pos
Vitamin K1	C31H46O2	17.5021	473.3452	1.7381	1.33E−12	84-80-0	Vitamins	Pos
8-Hydroxycarapinic Acid	C26H30O8	1.6449	509.152	1.522	2.67E−16	85775-57-1	Organic acids	Neg
Adonitol	C5H12O5	1.285683	190.9283	1.7557	1.69E−08	84709-28-4	Carbohydrates	Neg
Amarogentin	C29H30O13	1.6151	587.1689	1.1547	1.31E−07	21018-84-8	Esters	Neg
Argopsin	C18H14Cl2O6	1.474783	429.05	1.4067	2.44E−08	52809-10-6	Others	Neg
Batatasin III	C15H16O3	2.546217	245.1146	1.0674	3.40E−10	56684-87-8	Others	Neg
Bis(2-Ethylhexyl) Phthalate	C24H38O4	13.60517	391.2849	1.5088	8.60E−13	117-81-7	Esters	Neg
Catalposide	C22H26O12	2.654467	195.0509	1.4698	3.77E−09	6736-85-2	Flavones	Neg
Dihydromyristicin	C11H14O3	2.732383	195.0511	1.2006	9.78E−12	52811-28-6	Others	Neg
Erythraline	C18H19NO3	2.421833	265.9952	1.7376	7.38E−14	466-77-3	Alkaloids	Neg
Fumaric acid	C4H4O4	3.052133	117.0193	1.5117	2.60E−13	110-17-8	Origanic acids	Neg
Gluconolactone	C6H10O6	2.36145	179.0556	1.1402	8.85E−13	4253-68-3	Esters	Neg
Glycocholic Acid	C26H43NO6	1.066117	504.271	1.4522	5.68E−06	475-31-0	Flavones	Neg
Karanjin	C18H12O4	1.688783	293.0809	1.4534	2.49E−08	521-88-0	Alcohols	Neg
Khellin	C14H12O5	1.51985	283.0578	1.2427	5.69E−08	82-02-0	Alkaloids	Neg
Methionine conjugated chenodeoxycholic acid	C29H49NO5S	1.101317	524.3362	1.2922	2.20E−08	88046-01-9	Bile acids	Neg
Methyl-3-aminopyrazine-2-carboxylic acid	C6H7N3O2	1.709633	154.0622	1.7343	2.96E−09	16298-03-6	Pyrazines	Neg
Mevalolactone	C6H10O3	2.056583	283.1132	1.4637	8.04E−09	503-48-0	Ketones	Neg
6,7-dihydroxychromen-2-one	C9H6O4	12.91265	379.0437	1.4989	1.93E−11	305-01-1	Flavones	Neg
Ovalitenin B	C19H18O4	1.2864	310.8582	1.408	9.06E−07	64280-21-3	Flavones	Neg
Rutilantinone	C22H20O9	13.24055	429.119	1.416	5.41E−07	21288-61-9	Alkaloids	Neg
Vinyl Carbamate	C3H5NO2	2.193383	88.04051	1.5036	5.26E−12	15805-73-9	Esters	Neg

lead to changes in the nutritional composition of pork.

Differential metabolic pathway analysis

The metabolic pathway refers to the interaction network among metabolic products in living organisms, which reflects the path of

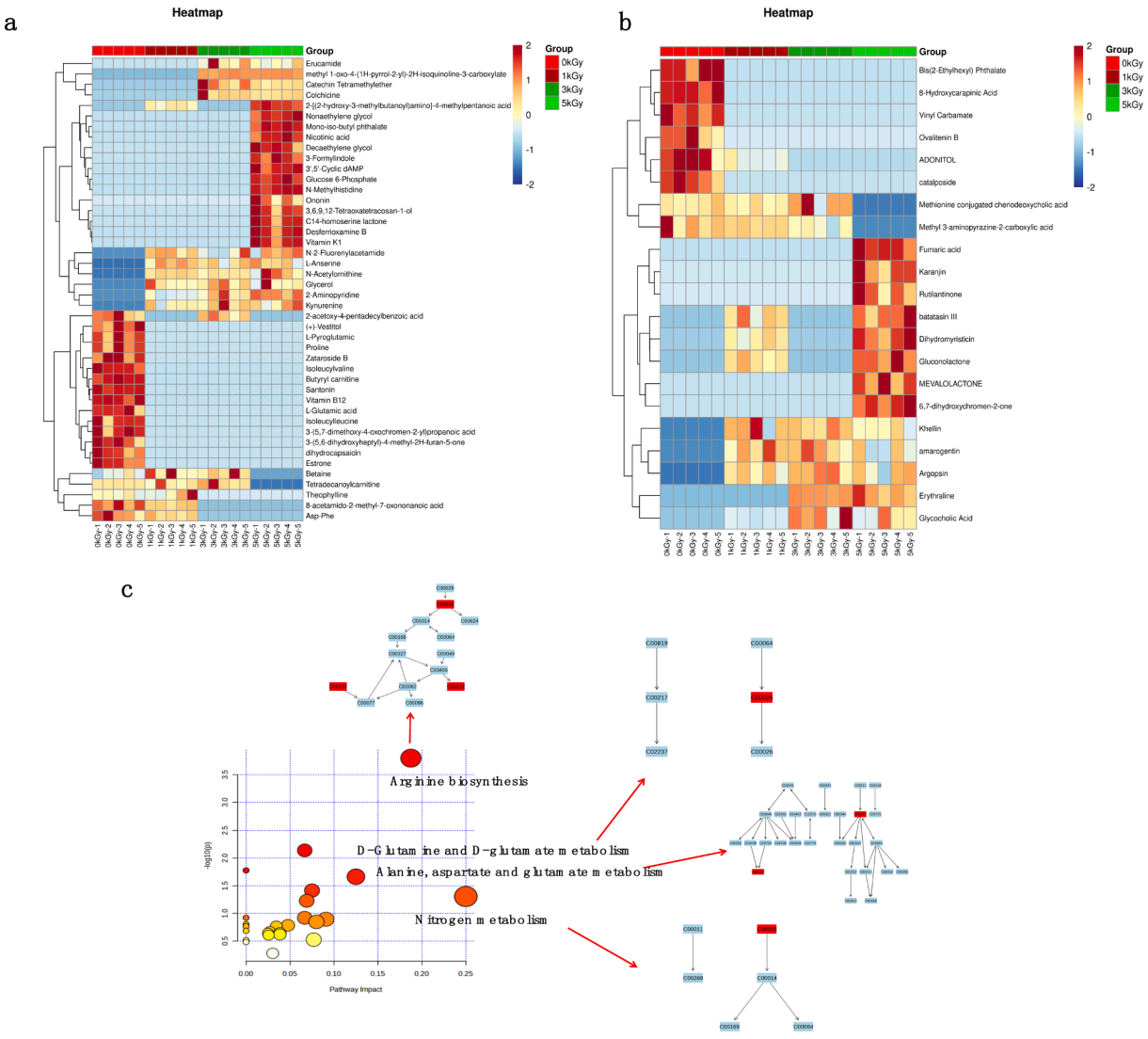

Fig. 4. (a) Heat map visualization of differential metabolites in positive mode. (b) Heat map visualization of differential metabolites in negative mode. (c) Significant metabolic pathways.

compound synthesis, decomposition, or transformation into certain final compounds through key intermediates. Using the Metaboanalyst 5.0 metabolic analysis tool and metabolic pathways reported by the KEGG database, 26 metabolic pathways were screened out in pork after irradiation treatment. As shown in Figure (Fig. 4c), the pathway analysis overview diagram is based on the −log (*P*) value of enrichment analysis as the vertical axis and the impact value of topological analysis as the horizontal axis. Each bubble represents a metabolic pathway, and the deeper the bubble color, the lower the *P*-value and the more significant the enrichment. Using a *P* value of <0.05 and an impact value of >0.1 as the screening criteria for significant metabolic pathways, four significant pathways were obtained, namely, arginine biosynthesis, alanine, aspartate and glutamate metabolism, D-glutamine and D-glutamate metabolism, and nitrogen metabolism. This finding indicates that irradiation treatment of pork affects the metabolic pathway.

Arginine is an essential amino acid in the body that can be used in conjunction with L-lysine and L-cysteine to improve the color of cured products (Ning et al., 2019). This amino acid can also give pork a reddish color in the presence of a nitric oxide synthase enzyme (Zając, Zając, & Dybaś, 2022). Moreover, arginine in meat increases the amount of flavor substances and enriches the diversity of flavor substances (Dou et al., 2023).

Aspartic acid metabolism produces fumaric acid, resulting in the upregulation of the relative content of fumaric acid in irradiated pork. Fumaric acid has certain antibacterial and antioxidant effects, and it can prolong the shelf life of pork. In addition, studies have shown that fumarate and L-aspartic acid can help improve the flavor and taste of pork (Hou et al., 2023). Moreover, considering that the umami flavor of meat is closely related to free amino acids, aspartic acid, and glutamic acid, the metabolic pathways of alanine, aspartic acid, and glutamic acid have a certain impact on the formation of meat flavor (Ge et al., 2023).

Wang, Dong, et al. (2022) found that the metabolism of alanine, aspartic acid, and glutamic acid had the greatest impact on the flavor of yak meat. Aspartic acid and glutamic acid significantly influenced the flavor of yak meat. The generation of free radicals due to increased irradiation intensity led to the oxidative degradation of these umami amino acids, thus affecting the meat flavor. Their conclusion is similar to the effects of irradiation on pork observed in this experiment. Zhao et al. (2022) found significant metabolic pathways between Hu sheep and Dorper sheep, including lipid transport, arginine biosynthesis, as well as alanine, aspartic acid, and glutamic acid metabolism. These differences resulted in significantly higher levels of Asp, Glu, Ala, and Arg in Hu sheep compared to Dorper sheep, indicating that the increase in amino acid levels in the muscles enhances the flavor of the meat.

Conclusion

This experiment was based on sensory experiments, pork color and TBARS to study the changes in pork quality, combining GC–MS and UPLC-Q-TOF MS of pork after irradiation at doses of 1, 3, and 5 kGy to explore the factors affecting pork quality changes. The results showed that different doses of irradiation treatment could considerably affect fresh pork, with the scores of pork color and texture increasing with the increase of irradiation dose, but the score of flavor was opposite. The overall acceptability was the highest in the 1 kGy treatment group. As the irradiation dose increases, the TBARS value of pork also shows an upward trend due to fat oxidation, reaching a maximum of 0.21 mg/kg at 5 kGy. A total of 76 volatile compounds were identified by GC–MS, with hydrocarbons, alcohols, and aldehydes accounting for the largest proportion of species. Hexanal, heptanal, and valeric acid, among other fatty acid oxidation products, are the primary causes of off-flavors in irradiated pork. UPLC-Q-TOF MS combined with metabolomics analysis methods revealed that differential metabolites, including L-pyroglutamic acid, L-glutamate, L-proline, fumarate acids, betaine, vitamin K, and nicotinic acid, as well as polypeptides such as L-anserine, L-aspartic acid-L-phenylalanine and isoleucine-leucine, were related to the quality of irradiated pork. In addition, four significant metabolic pathways were also discovered, including arginine biosynthesis, alanine, aspartic acid, and glutamic acid metabolism, D-glutamine and D-glutamic acid metabolism, as well as nitrogen metabolism, may be the pathways affecting the quality of irradiated pork. The synthesis of arginine is associated with changes in pork color and enriches the diversity of flavor substances. And given the interconnected nature of free amino acids, aspartic acid, and glutamic acid, which influence the umami taste of meat, the metabolism of alanine, aspartic acid, and glutamic acid is a pathway that leads to variations in pork flavor. This study has explained the internal causes of changes in pork quality after irradiation from two perspectives of volatile flavor compounds and metabolites. It provides some reference and evidence for future research on the impact of irradiation on pork quality and lays a theoretical foundation for regulating the negative effects of irradiation on pork quality.

Statement

All subjects have obtained informed consent for the sensory evaluation experiment in the article. We promise that all sensory experiments related to humans were carried out in accordance with the Code of ethics of the World Medical Association (Declaration of Helsinki), and always abide by the privacy right of human subjects.

CRediT authorship contribution statement

Bo Yao: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dong Zhang:** Writing – review & editing, Validation, Supervision. **Xinyu Wu:** Investigation. **Ruiyan He:** Investigation. **Hui Gao:** Investigation. **Kailan Chen:** Investigation. **Dan Xiang:** Investigation. **Yong Tang:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101460.

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