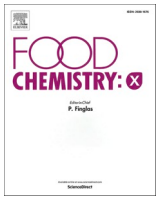
Food Chemistry: X 25 (2025) 102124

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Effects of X-ray and electron beam irradiation on wine quality: Emphasizing phenolic compounds and aroma profiles

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ARTICLE INFO

Keywords:
Wine quality
X-ray
Electron beam
Phenols
Volatile compounds

ABSTRACT

The content of flavor compounds in wine is limited by factors such as climate warming and the resistance of cell walls to maceration. This study used X-rays (ionizing radiation) and electron beams (particle radiation) at 0.5, 2, and 7 kGy for grape pre-treatment before winemaking. Scanning electron microscopy showed varying degrees of grape skin damage. Results indicated irradiation significantly enhanced phenolic compound extraction, with DPPH and ABTS scavenging activities increasing by up to 38.98 % and 38.70 %. Wines treated with 0.5 kGy electron beams exhibited the highest levels of esters and higher alcohols, enhancing fruity aromas. Irradiation reduced C6 compound content, decreasing green notes and improving color and complexity scores. This study demonstrates that X-ray and electron beam irradiation significantly enhance phenolic and aromatic compound extraction in wine, showing the potential of irradiation technology in the wine industry.

1. Introduction

Wine is one of the world's most popular beverages, with its quality primarily determined by taste, aroma, and color. A major contributor to these characteristics are phenolic compounds, including pigments (flavonoids and anthocyanins) and non-pigmented polyphenols (phenolic acids and polymeric polyphenols), which enhance attributes such as body, astringency, and color (Castro-López et al., 2016; Guler, 2023; Tu et al., 2022). Aroma, crucial for defining wine style, is composed mainly of higher alcohols, esters, aldehydes, ketones, and terpenes. These phenolic and aromatic compounds are predominantly found in grape skin cells, yet only a small fraction is extracted into the wine through solid-liquid diffusion during maceration (Wang et al., 2022; Xie et al., 2023). Additionally, climate change has led to earlier harvests, resulting in insufficient accumulation of phenolic compounds in grapes. Therefore, overcoming the resistance of cell walls and membranes to enhance the extraction of phenolic and aromatic compounds has become a significant focus in recent years (Tong et al., 2023; Wei et al., 2023).

Disrupting the cell structure of grape skins, particularly weakening the cell walls, is crucial during maceration (Hensen et al., 2022). Traditional maceration methods include thermal treatments, CO_2 maceration, and the use of pectinase (Chen et al., 2023; El Darra et al., 2016; Geffroy et al., 2018). However, these traditional methods have limited efficacy in extracting phenolic and aromatic compounds, and thermal treatments may adversely affect sensory characteristics. Emerging non-thermal processing technologies such as ultrasound, pulsed electric fields, high pressure, and irradiation have become increasingly attractive for sustainable wine production. These methods enhance and accelerate the extraction of phenolic compounds, increase volatile compounds, and reduce the need for sulfur as a preservative, with some techniques also inactivating polyphenol oxidase (Kumar et al., 2023; Morata et al., 2021). These techniques work by applying high-energy physical fields to disrupt cell structures, creating perforations in the cell walls and membranes, increasing intercellular gaps, and generating free radicals that degrade cell wall polymers such as pectin and cellulose, thereby extracting metabolites from the cells (Cholet et al., 2014; Gamonpilas et al., 2021).

Irradiation technology has been widely used in various aspects of food preservation, sterilization, and modification. It is a well-established, non-thermal processing technique that does not compromise the sensory qualities of food (Bliznyuk et al., 2022; Choi et al., 2009). Its safety has been confirmed by the Food and Agriculture

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https://doi.org/10.1016/j.fochx.2024.102124
Received 1 November 2024; Received in revised form 20 December 2024; Accepted 20 December 2024
Available online 25 December 2024

Organization (FAO), the International Atomic Energy Agency (IAEA), and the World Health Organization (WHO). This technology is approved for use in the food sector in over 50 countries (Ravindran & Jaiswal, 2019). Irradiation can be classified into X-rays, gamma rays, and electron beams based on the source. Gamma rays, produced from ^{60}Co or ^{137}Cs sources, still raise consumer concerns regarding potential radioactivity and environmental risks (Honda et al., 2015). In contrast, electron beams and X-rays generated by electron accelerators are cleaner and more acceptable to the food industry (Ihsanullah & Rashid, 2017). Electron beams primarily cause Compton scattering with some oxidation effects, while X-rays are highly penetrating electromagnetic radiation without radioactivity. Both methods are increasingly applied in the food industry (Lei et al., 2023; Ravindran & Jaiswal, 2019). Recent advancements in irradiation technology have also been made in the processing of grapes and wine. Gamma irradiation has been shown to enhance the fruity and floral aromas of Merlot wine and improve the extraction of colored compounds (Mihaljević Žulj et al., 2019). Sumit Gupta et al. (2015) reported that gamma irradiation at 1.5 kGy resulted in the best phenolic extraction from Cabernet Sauvignon and Syrah grapes, although 10 kGy electron beam pretreatment reduced anthocyanin content in wine (Morata et al., 2015). Beyond enhancing maceration, irradiation's sterilization capabilities show potential as a substitute for sulfites in winemaking (Błaszak et al., 2019; Błaszak et al., 2023; Morata et al., 2015). This technology has been widely reported to improve the extraction rates and bioactivity of plant materials such as green walnut shells and *Citri Sarcodactylis Fructus* (Shen et al., 2022; Villavicencio et al., 2018; Wang et al., 2023). It has also been used in the processing of juices from carrots, sour cherries, and other fruits and vegetables for sterilization and antioxidant activity enhancement (Ayseli et al., 2024; Kalaiselvan et al., 2018; Naresh et al., 2015; Patras et al., 2010).

Previous studies on optimizing wine quality through irradiation have primarily focused on gamma rays, while research on electron beam irradiation of grapes has mainly explored alternatives to sulfites. There is a relative scarcity of studies examining the application of X-rays and electron beams to enhance maceration, and a comparative analysis of different types of irradiation for pre-treating grapes remains underresearched. Therefore, the objective of this study is to investigate the effects of X-ray and electron beam irradiation at different doses on the phenolic compounds, volatile compounds, and sensory attributes in wine, and to compare the roles of these two irradiation methods in enhancing wine quality. This study aims to provide new insights into improving winemaking processes and enhancing wine quality, while offering guidance for the practical application of ionizing radiation in the wine industry.

2. Materials and methods

2.1. Grape samples

Grape berries of *Vitis vinifera* L. cv. Cabernet Sauvignon were handpicked from a commercial vineyard located in Qingtongxia City, Ningxia Province, China (38°02′N, 106°07′E). The sugar content of the berries was 217.6 g/L.

2.2. Irradiation treatment

After manual destemming, each grape sample (15 kg) was spread in a single layer within polyethylene bags with a total thickness of 1–2 cm. The grapes were then subjected to electron beam and X-ray irradiation treatments using a 10 MeV/20 kW high-energy electron linear accelerator and a 5 MeV/150 kW high-frequency high-voltage electron accelerator from Yangling Hesheng Irradiation Technology Co., Ltd. (Yangling, China). The samples were placed on a continuous conveyor belt with the dose rate set at 0.5 kGy per pass. Absorbed doses of 0, 0.5, 2, and 7 kGy were achieved by adjusting the cumulative number of passes. The irradiation process was monitored using potassium dichromate and silver dichromate dosimeters, calibrated by the National Institute of Metrology. Irradiation was performed under ambient conditions and at a chamber temperature of 18 °C.

2.3. Vinification process

After irradiation, the grapes were immediately transported to the laboratory for winemaking, following the small-scale container fermentation method by Xie et al. (2023) with slight modifications. Sulfur dioxide was added at a rate of 30 mg/L SO_2. The samples were labeled as follows: CK (control group), 0 kGy; EN, 0 kGy, with 30 mg/L pectinase enzyme (Lallzyme EX, Lallemand, France); X0.5, X2, X7: grapes irradiated with X-rays at 0.5, 2, and 7 kGy, respectively; E0.5, E2, E7: grapes irradiated with electron beams at 0.5, 2, and 7 kGy, respectively. Each winemaking process was conducted in triplicate. The wine was then bottled and stored in a temperature-controlled wine cellar at 14–16 °C for three months before sample analysis.

2.4. Scanning electron microscopy (SEM) analysis

After irradiation, grape skins were collected and subjected to a drying treatment. The dried skins were then coated with gold and platinum under vacuum conditions. The samples were analyzed using a scanning electron microscope (SEM) to observe the microstructural changes in the grape epidermis at a magnification of 50,000× (Shen et al., 2022). Microscopic observations were conducted using a Nova Nano SEM 450 (FEI Company, Czech Republic) at an accelerating voltage of 5.0 kV.

2.5. Analysis of basic physicochemical indicators of wine

In this study, ethanol content, pH, volatile acidity, and titratable acidity were determined according to the methods prescribed by the International Vine and Wine Organization (OIV, 2017).

2.6. Analysis of phenolic profile and antioxidant capacity

2.6.1. Total phenolic compounds and antioxidant capacity

Following previously described methods(Meng et al., 2012; Patel & Ghane, 2021), various phenolic compounds in the wine samples were quantified. Total phenols (TP) were determined using the Folin–Ciocalteu colorimetric assay, total anthocyanins (TA) by the pH differential method, total flavanols (TF) with the aluminum chloride colorimetric assay, total flavonoids (TFO) by the p-DMACA-HCl assay, and total tannins (TT) using the vanillin-HCl assay. The concentrations were expressed as follows: TP in milligrams of gallic acid equivalents per liter (mg GAE/L), TA in milligrams of malvidin-3-O-glucoside equivalents per liter (mg Mv/L), TF in milligrams of rutin equivalents per liter (mg RTE/L), and both TFO and TT in milligrams of catechin equivalents per liter (mg CTE/L). The antioxidant activity of the wines was assessed using DPPH and ABTS radical scavenging assays, following the modified methods of Cheng et al. (2020). Results were expressed as micromolar Trolox equivalents per liter (mM TE/L). Standards such as gallic acid, rutin, and catechin were procured from Yuanye Bio-Technology Co., Ltd. (Shanghai, China).

2.6.2. Determination of monomeric anthocyanins

Monomeric anthocyanins were analyzed using an HPLC system (LC-20 A, Kyoto, Japan) equipped with a photodiode array detector. Samples were filtered through a 0.22 μm organic membrane before analysis, and anthocyanins were separated on a Synergi Hydro RP C18 column (250 × 4.6 mm, 4 μm, Phenomenex, Torrance, CA, USA) at a flow rate of 1 mL/min (Wei et al., 2023). Mobile phase A consisted of formic acid, acetonitrile, and water in a ratio of 2.5:10:80 (*V*/V/V), while mobile phase B was composed of formic acid, acetonitrile, and water in a ratio

of 2.5:50:40 (V/V/V). The gradient elution program was as follows: 0–45 min, 0 %–35 % B; 45–46 min, 35 %–100 % B; 46–50 min, 100 % B; 50–51 min, 100 %–0 % B; and 51–55 min, 0 % B. The detection wavelength was set at 520 nm. The relative concentration of each anthocyanin was expressed as malvidin-3-*O*-glucoside equivalents. Malvidin-3-*O*-glucoside was purchased from Sigma-Aldrich (MO, USA).

2.6.3. Determination of non-anthocyanin monomeric phenols

The non-anthocyanin monomeric phenolic compounds were quantified using a triple quadrupole mass spectrometer (Triple QuadTM 5500+) coupled with an Exion LC AD system in negative ion mode. Data processing was performed using Analyst and OS-MQ software, both from SCIEX (USA). This method was adapted from a previously published approach with modifications (Chen et al., 2022). Liquid chromatography analysis employed an ACQUITY C18 UPLC column (2.1 mm × 50 mm, 1.7 μm, Waters, Mississauga, Ontario, Canada), with the column temperature maintained at 30 °C. The mobile phase consisted of 1 % acetic acid in water (A) and acetonitrile (B), with gradient elution at a flow rate of 0.2 mL/min as follows: 0–3 min, 0–6 % B; 3–7 min, 6–15 % B; 7–11 min, 15–30 % B; 11–13 min, 30 % B; 13–14 min, 30–95 % B; 14–17 min, 95 % B; 17–18 min, 95–3 % B; 18–23 min, 3 % B. A 5 μL injection volume was used for each analysis. The full scan range for mass spectrometry was set to *m/z* 100–2000, while the product ion scan range was m/z 50–2000. The electrospray ionization (ESI) source operated in negative mode, with a nebulizer gas pressure of 50 psi, an auxiliary gas pressure of 50 psi, and a curtain gas pressure of 35 psi. The ion source temperature was set to 550 °C, and the spray voltage to −4500 V. The quantification of target compounds was achieved by constructing standard calibration curves using the peak areas of standards at different concentration gradients. All wine samples were analyzed in duplicate.

2.7. Colorimetric analysis

The CIE coordinates of the wine samples (*L**: lightness, *a**: green-red axis, *b**: blue-yellow axis, *h**: hue, *C**: chroma) were directly obtained using a Wine Color Analyzer (W100, Haineng Instruments Co., Shandong, China). The wine samples were filtered through 0.45 μm polyethersulfone filter membranes, injected into cuvettes, and calibrated to zero with distilled water. All measurements were performed in triplicate.

2.8. Determination of volatile compounds

Volatile compounds in wines were analyzed by headspace-solid phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS) (7890B, Agilent, USA) following published methods (Zhang et al., 2020) with some modifications. To begin, 5 mL of wine was mixed with 10 μL of 4-methyl-2-pentanol (internal standard, 1.0083 g/L) and 1.0 g of NaCl. This mixture was quickly added to a 20 mL vial with a Teflon-insulated lid. The vials containing the prepared samples were equilibrated at 40 °C for 30 min. Then, SPME fibers were inserted into the headspace of the vials and stirred at 500 rpm for 30 min at 40 °C to extract the volatile compounds. GC–MS analysis was performed using an Agilent 7890B gas chromatograph and an Agilent 7890B mass spectrometer (Agilent Technologies, USA). Volatile compounds were separated on an HP-INNOWAX capillary column (60 m × 250 μm × 0.25 μm, J&W Scientific, Folsom, CA, USA) using helium as the carrier gas at a flow rate of 1 mL/min and a pressure of 16.909 psi. A 5:1 split injection mode was used with a flow rate of 5 mL/min, a sample volume of 0.5 μL, and an inlet temperature of 250 °C. The column chamber warming procedure was as follows: held at 50 °C for 1 min, then heated to 220 °C at a rate of 3 °C/min, and held for 5 min. The mass spectrometer used electron ionization (EI) at 70 eV to ionize the molecules of volatile compounds. The ion source and quadrupole temperatures were 230 °C and 150 °C, respectively, and the mass detector was in full scan mode (*m/z* 43–450 u). Volatile compounds were identified using the NIST 14 mass spectrum library and retention indices of authentic standards. The internal standard was 4-methyl-2-pentanol (1.0083 g/L). Calibration curves for volatile compounds were established by using the area ratio and concentration ratio of the target compound to the internal standard. Volatile compounds were quantitatively analyzed using these calibration curves.

2.9. Sensory analysis

Our institution does not mandate ethical approval for sensory evaluation studies involving human participants, so we adhere to rigorous protocols to protect participants' rights. These protocols ensure voluntary participation, provide clear information about the study's requirements and potential risks, obtain written or verbal consent, safeguard participant data from unauthorized disclosure, and allow participants to withdraw from the study at any time. All sensory evaluation procedures are conducted in accordance with applicable ethical standards, and participants provided informed consent prior to testing.

The sensory evaluation of the wine samples was conducted by 17 trained tasters (8 men and 9 women, aged 22–26) from the College of Enology at Northwest A&F University. All participants had completed a comprehensive wine tasting course, covering the theory and methodology of wine evaluation. The evaluation process followed the method described by Bai et al. (2024), with slight adjustments. Wine samples, labeled with random three-digit codes, were presented in ISO tasting glasses at room temperature (20 °C), and water and soda crackers were provided as palate cleansers between tastings. Panel members rated the appearance (clarity, color intensity), aroma (intensity, complexity, elegance), and taste (astringency, body, balance) of each sample using a 7-point scale. The intensity of seven pre-trained aroma descriptors (red fruit, black fruit, dried fruit, floral, spice, toasted, vegetal) was assessed using a 5-point scale. Aroma attributes were quantified using the M-value ($M = \sqrt{(F \times I)}$), where F represents the frequency of descriptor occurrence and I represents the intensity ratio (Tao et al., 2009).

2.10. Statistical analysis

Data processing and significance analysis were conducted using SPSS 22.0 (IBM, USA). Tukey's test was employed for statistical analysis at a significance level of $p < 0.05$. Experiments were repeated at least three times, with results presented as mean ± standard deviation. Column plots, radar plots, and correlation heat maps were generated using Origin 2021 (OriginLab Corporation, USA). Hierarchical clustering heat maps were created with Hiplot Pro (http://hiplot.com.cn). Principal component analysis (PCA), orthogonal partial least squares-discriminant analysis (OPLS-DA), and the 200 permutation test were performed using Simca 14.1 (Umetrics, Umeå, Sweden). Correlation and clustering heat maps were used to analyze the correlation levels and clustering effects of various parameters. Data were normalized during the analysis.

3. Results and discussion

3.1. The analysis of grape skin structure

To investigate the effect of irradiation on grape skin structure, scanning electron microscopy (SEM) was used to characterize the microstructure of both non-irradiated and irradiated grape skins (Fig. 1). The surface of the non-irradiated sample was intact and regular. In contrast, the grape skins subjected to irradiation exhibited significant structural changes, notably the porous and flaky structure caused by cell wall degradation and perforation. As the irradiation dose increased, the degree of damage to the grape skin became more pronounced, showing more fragmentation and porous structures. These observations are consistent with previous findings (Shen et al., 2022). The results demonstrate that both X-ray and electron beam irradiation can alter the

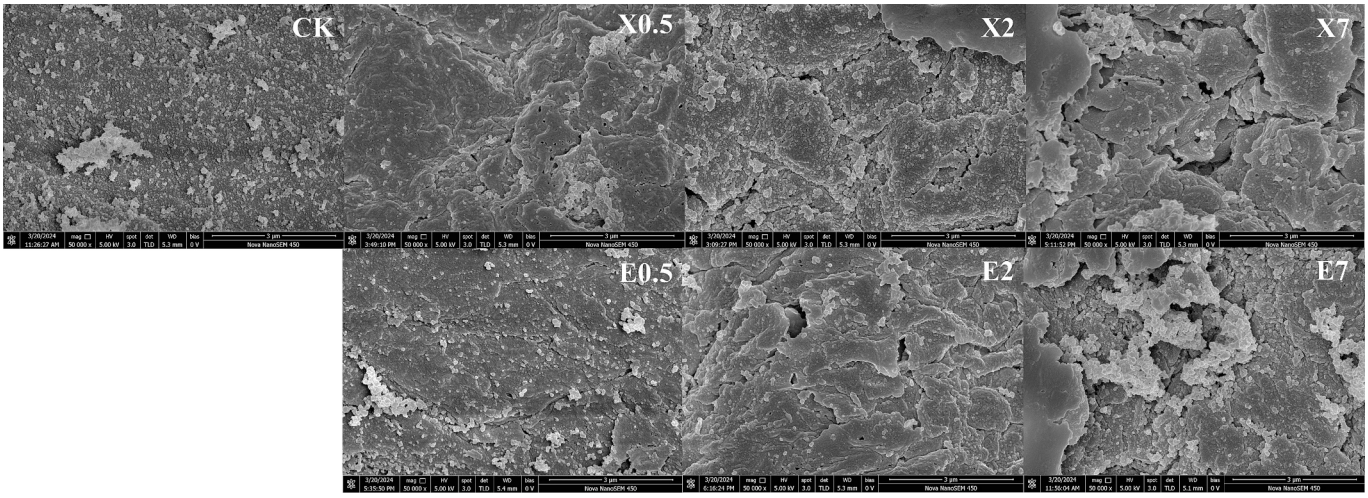

Fig. 1. SEM images of grape skins under different irradiation treatments.

internal and surface structures of grape skins. Such structural changes facilitate the migration of phenolic compounds within the cells, thereby enhancing the extraction efficiency of active substances (Wang et al., 2023).

3.2. The analysis of physicochemical parameters of the wine

The physicochemical parameters of each wine sample were analyzed, with the findings summarized in Table S1. Alcohol content, pH, titratable acidity, and residual sugars displayed no statistically significant differences when compared to the control group and remained within the parameters established by OIV (2017). This concurs with earlier reported findings (Mihaljević Žulj et al., 2019; Wei et al., 2023). Notably, a significant reduction in volatile acidity was observed in the 7 kGy X-ray treatment group (X7). Additionally, both E2 and E7 groups showed reduced volatile acidity compared to the EN control group ($p < 0.05$). Similar results have been reported in prior studies, likely due to the bactericidal effects of high-dose radiation (Gupta et al., 2015; Mihaljević Žulj et al., 2019).

3.3. The analysis of phenolic compounds in wine

3.3.1. Total phenolics and antioxidant activity

Fig. 2A–E illustrate the total amounts of various phenolic compounds across different treatments. All irradiation treatments significantly increased the content of total phenols (TP), total tannins (TT), total flavonoids (TF), and total flavonols (TFO) in the wine. Specifically, the TP, TT, TF, and TFO content increased by 38.04 %, 12.8 %, 52.14 %, and 66.13 %, respectively, with X7 treatment. Additionally, we observed that both X0.5 and X7 treatments had better extraction effects compared to the pectinase control group (EN). The TP, TT, TF, and TFO levels for both irradiation treatments showed a trend of first decreasing and then increasing with increasing doses ($p < 0.05$). Gupta et al. (2015) found that the extraction of phenolic compounds in grapes treated with gamma rays was positively correlated with doses below 1.5 kGy, with a decline observed at 2 kGy, similar to our findings. However, the trend for TA differed from the other four phenolic compounds, with a significant increase of 10.94 % observed in X0.5 compared to the control, while TA content in E2 significantly decreased, likely due to the high sensitivity of anthocyanins to irradiation ($p < 0.05$) (Błaszak et al., 2023). This contradicts the findings of Morata et al. (2015) and others, possibly due to differences in exposure systems, plant material types, and other conditions (Mihaljević Žulj et al., 2019). The accumulation of phenolic compounds in wine involves a balance between extraction and degradation. Irradiation treatment disrupts the cell membrane structure through ionization, while the primary effect is the generation of free radicals through water ionization, which degrades pectin and other polysaccharides in the cell wall, facilitating the extraction of bioactive compounds from the grape skin (Gamonpilas et al., 2021). However, the disintegration of cell wall structures increases the adsorption surface area, leading to more components being absorbed by cell wall materials (Castro-López et al., 2016). Free radicals induced by irradiation also oxidize phenolic compounds, such as anthocyanins, causing covalent bond breakage and degradation, thereby reducing the extraction efficiency (Błaszak et al., 2019). Low-dose treatment can disrupt the cell wall with minimal degradation effects on compounds, while at medium doses, the degradation effect increases, resulting in lower extraction efficiency at 2 kGy compared to 0.5 kGy. At high doses of 7 kGy, the cell structure disruption is significantly enhanced, achieving the best extraction efficiency for phenolic compounds other than TA. For both irradiation methods, X-rays showed better extraction enhancement capability at the same dose because of their stronger penetration and lower oxidation effect compared to electron beams, which reduces oxidative damage to biological components (Lei et al., 2023).

3.3.2. Monomeric anthocyanins

Anthocyanins are the primary color sources in wine and play a crucial role in influencing consumer preferences. To further analyze the impact of different doses of X-ray and electron beam irradiation pretreatment on the monomeric phenolic compounds in wine, HPLC-DAD was employed to quantify nine specific monomeric anthocyanins (Table S2). These included five non-acylated anthocyanins: delphinidin-3-*O*-glucoside (Dp), cyanidin-3-*O*-glucoside (Cy), petunidin-3-*O*-glucoside (Pt), peonidin-3-*O*-glucoside (Pn), and malvidin-3-*O*-glucoside (Mv); as well as four acylated anthocyanins: peonidin-3-*O*-(6-*O*-acetyl)-glucoside (Pn-ac), malvidin-3-*O*-(6-*O*-acetyl)-glucoside (Mv-ac), peonidin-3-*O*-(6-*O*-*p*-coumaryl)-glucoside (Pn-co) and malvidin-3-*O*-(6-O-*p*-coumaryl)-glucoside (Mv-co). Among the non-acylated anthocyanins, Mv had the highest content (102.76–153.57 mg/L), followed by Pt (3.94–5.33 mg/L), with the other three non-acylated anthocyanins being lower in concentration. This is consistent with previous reports on the anthocyanin content in Cabernet Sauvignon wines (Wei et al., 2023). In this study, 7 kGy X-ray treatment reduced Mv and Pt levels, as the high energy generated more free radicals that attacked the anthocyanin hydroxyl groups and ortho-diphenol structures, leading to the formation of quinones, double bond cleavage, and polymerization reactions, thus decreasing anthocyanin content (Błaszak et al., 2023; Patras et al., 2010). Previous studies also reported that total anthocyanin content in black carrot pomace decreased from 254.64 mg/g to 167.93 mg/g under 5 kGy gamma irradiation (Ayseli et al., 2024). Interestingly, except for

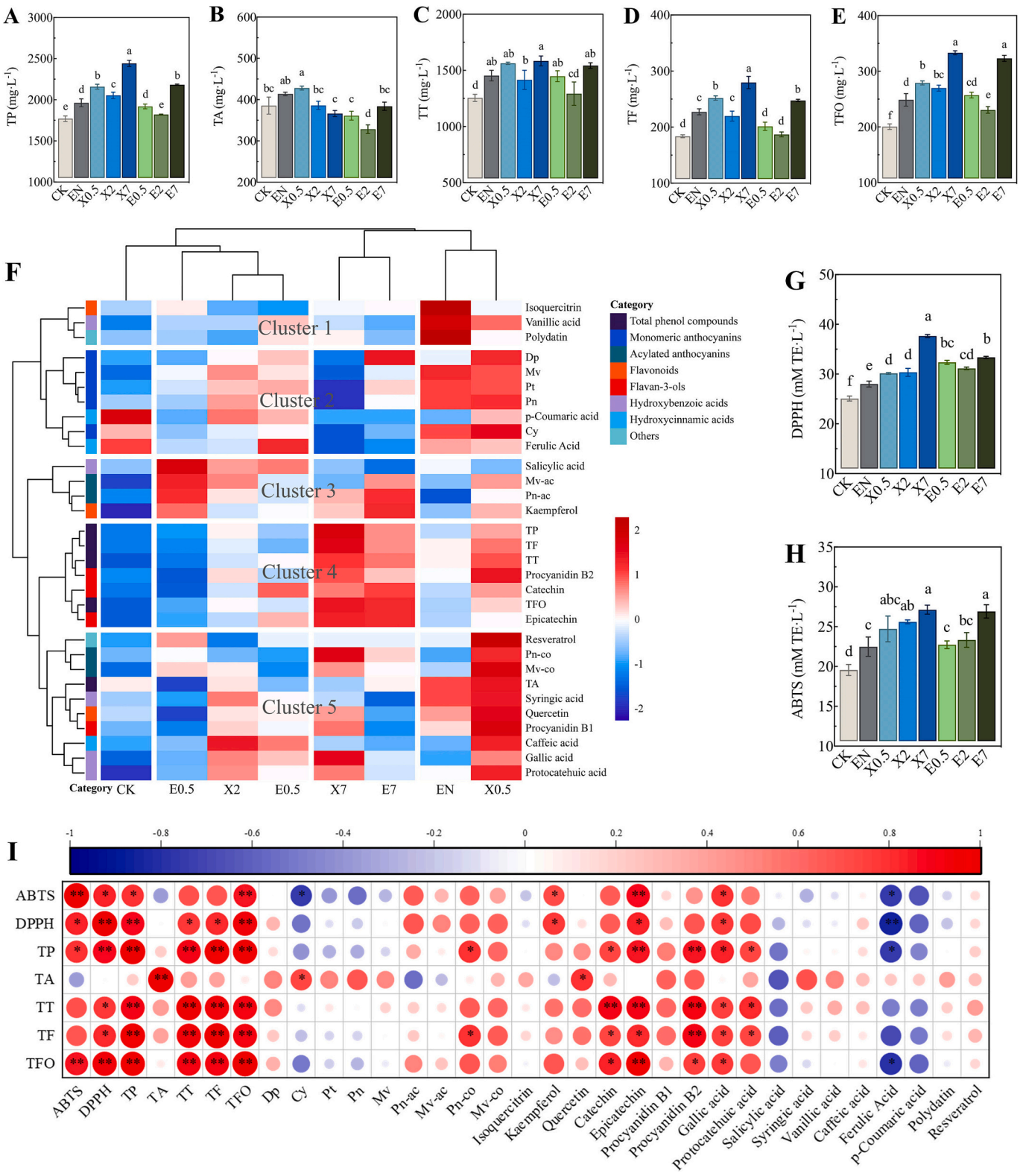

Fig. 2. Analysis of phenolic profile and antioxidant activity in wines under different irradiation pretreatments.

X7, all other irradiation treatments significantly increased the levels of Mv and Pt in the wine, with X0.5 showing the highest levels ($p < 0.05$). Although an increase in Pt and Mv content was also observed in X2, the levels of Cy and Dp in X2 decreased compared to X0.5. This may be due to the fact that Cy and Dp, which have catechol structures, are more prone to oxidation. Similar changes have been observed in studies using γ-rays (Enaru et al., 2021; Mihaljević Žulj et al., 2019). Acylated anthocyanins are more stable and contribute to maintaining the color characteristics of wine. Both types of irradiation increased Pn-ac, Mv-ac, and Mv-co content, consistent with results observed by Morata et al. (2015) when treating Tempranillo grapes with high-energy electron beams. This may be due to the increased stability provided by the acyl

group, facilitating more extraction (Fei et al., 2021). Pectinase treatment was less effective than irradiation in promoting the extraction of acylated anthocyanins. Comparing the changes in monomeric anthocyanins suggests that irradiation may promote the acylation of anthocyanins, though further research is needed.

Previous studies have also reported the enhancement of anthocyanin extraction using various physical methods. For instance, Guler (2023) found that ultrasound treatment alone reduced the anthocyanin content in grape juice to 1.94 mg/100 g, while the combination of ultrasound and microwave treatment increased the anthocyanin content to 31.04 mg/100 g, more than doubling the content obtained with pectinase treatment alone (14.57 mg/100 g) (Xie et al., 2023). When comparing the two irradiation treatments at doses of 0.5 and 2 kGy, X-rays demonstrated a more effective enhancement in the extraction of non-acylated anthocyanins compared to electron beams. Specifically, the X0.5 treatment surpassed the traditional pectinase treatment (EN) in extraction efficiency. However, at 7 kGy, X-rays caused more significant degradation of non-acylated anthocyanins, indicating a higher sensitivity of anthocyanins to X-ray irradiation. Overall, 0.5 kGy irradiation was the most effective in promoting the extraction of monomeric anthocyanins. Conversely, previous studies on Merlot grapes found that higher doses (2.7 kGy) of gamma radiation enhanced anthocyanin extraction, possibly due to varietal differences (Mihaljević Žulj et al., 2019).

3.3.3. Monomeric phenols

Monomeric phenols can impart diverse flavors to wine, influence the expression of copigmentation in red wine, thereby enhancing its color, and also exhibit antioxidant and health benefits (Zhang et al., 2018). The 17 non-anthocyanin monomeric phenols were detected in the wine using LC-MS, including flavonoids, flavanols, phenolic acids, and other compounds (Table S2). For flavonoids, all treatments significantly increased kaempferol content, with the highest observed in the E7 (27.53 mg/L), nearly three times higher than the control (CK, 10.96 mg/L) ($p < 0.05$). Previous studies confirmed that electron beam treatment can increase kaempferol extraction in *Bauhinia variegata* L. var. candida alba Buch.-Ham flowers from 1.02 mg/g to 2.17 mg/g at 0.5 kGy, consistent with our findings (Villavicencio et al., 2018). However, the dosage differences may be due to variations in plant species and the glycosylation of kaempferol. Quercetin content significantly increased only in the X0.5 and X7 treatments, a trend also reported by Gupta et al. (2015) in irradiated Cabernet Sauvignon and Shiraz grapes ($p < 0.05$). Contrarily, earlier studies, including gamma irradiation of grapes, did not show a significant increase in quercetin content (Mihaljević Žulj et al., 2019), and quercetin in cranberry syrup exhibited high resistance to gamma irradiation ($p < 0.05$) (Rodríguez-Pérez et al., 2015). For flavanols, catechin, epicatechin, procyanidin B1, and procyanidin B2 were measured. None of the treatments negatively impacted these compounds. Apart from procyanidin B1 in X7, where no significant difference was observed compared to the control, all flavanols showed a significant increase at 0.5 kGy and 7 kGy. Additionally, epicatechin and procyanidin B2 increased in X2, consistent with previous studies, possibly due to their higher stability under irradiation compared to their isomers (Mihaljević Žulj et al., 2019).

Like flavanols, no significant reduction in hydroxybenzoic acids was observed due to irradiation. X-ray irradiation significantly increased the content of gallic acid and protocatechuic acid by 42.38 % and 83.93 %, respectively, under X7 treatment ($p < 0.05$). Both acids exhibit strong antioxidant capabilities, with protocatechuic acid showing superior extraction efficiency due to its higher hydroxyl group content compared to gallic acid (Farhoosh et al., 2016). Previous studies have also confirmed that pectinex treatment leads to increased phenolic acid levels due to cell wall polysaccharide degradation (Arnous & Meyer, 2010). Guler (2023) observed similar results under ultrasonic and microwave treatments. Syringic acid and vanillic acid levels increased only under low-dose irradiation. Overall, electron beam irradiation was less effective than X-ray in enhancing phenolic acid extraction. However, E2 treatment resulted in syringic acid levels nearly tripling compared to CK, an effect not seen with X-ray treatment. Among hydroxycinnamic acids, irradiation increased caffeic acid content, while ferulic acid and p-coumaric acid showed varying degrees of reduction, indicating sensitivity to irradiation. Contrarily, Mihaljević Žulj et al. (2019) reported different trends, likely due to varietal differences. Post-irradiation, hydroxybenzoic acid levels increased more significantly than hydroxycinnamic acids, possibly because the propylene double bonds in hydroxycinnamic acids are more effective in scavenging free radicals, leading to greater oxidation during irradiation (Rice-Evans et al., 1996). Additionally, all irradiation treatments significantly increased trans-resveratrol content, from 0.32 mg/L to 0.54 mg/L, consistent with previously reported ranges (Guler, 2023). Similar to other monomeric phenols, X0.5, X7, and E0.5 treatments enhanced polydatin extraction. Overall, X-rays demonstrated superior efficacy in promoting monomeric phenol extraction. X0.5 significantly increased the content of 15 monomeric phenols, while X2 and X7 also elevated levels of highly antioxidant phenols ($p < 0.05$). Due to the strong oxidative nature of electron beams, E0.5 only increased the content of 11 monomeric phenols, and E2 and E7 showed more degradation effects. Interestingly, isoquercitrin, vanillic acid, and polydatin achieved optimal extraction in the EN treatment, suggesting these phenolic compounds are more easily released with the mild cell structure disruption caused by traditional pectinase maceration. While irradiation increased disruption, it also heightened degradation rates. Similar to monomeric anthocyanin changes, differences in hydroxyl group content, spatial distribution, conjugated structures, and methoxy donor groups are primary factors influencing varied extraction efficiencies of different monomeric phenols in this study.

Cluster analysis of the phenolic compound content under different treatments was performed, and the results are presented in a heatmap (Fig. 2F). Wine samples were categorized into four clusters: CK as a standalone category (Cluster 1), E2, X2, and E0.5 as Cluster 2, X7 and E7 as Cluster 3, and EN and X0.5 as Cluster 4. The results indicated that irradiation treatments significantly differentiated from the control (CK), with X0.5 showing better extraction efficiency than EN. Phenolic compounds were grouped into five clusters. Cluster 4 included TP, TF, TT, TFO, and three flavan-3-ol monomers, with higher concentrations in X7, E7, and X0.5 treatments, showing the highest levels at 7 kGy. Clusters 5 and 2 had the highest extraction levels under X0.5 treatment, but the phenolic compounds in Cluster 2 were significantly lower in X7 treatment. This is because Cluster 2 contains five non-acylated anthocyanin monomers and two hydroxycinnamic acids, which are relatively unstable compounds. Cluster analysis effectively distinguished compounds with varying stability under different irradiation treatments, with stability in the order of Cluster 4 > Cluster 5 > Cluster 1. However, compounds in Cluster 3 had higher levels in E2 and E7 treatments, while those in Cluster 1 had the highest levels in EN treatment, indicating irradiation had minimal effect on increasing their content.

3.3.4. Antioxidant activity

The antioxidant activity is highly correlated with the content of phenolic compounds (Xie et al., 2023). To assess the potential antioxidant activity of wine treated with two irradiation technologies, DPPH and ABTS radical scavenging activities were measured. The ranges of DPPH and ABTS radical scavenging activities were 27.09–37.65 mM TE/L and 19.56–27.13 mM TE/L, respectively (Fig. 2G–H). The results demonstrated that all irradiation treatments significantly enhanced the antioxidant activity of the wine ($p < 0.05$). Both radical scavenging activities increased initially with the dose and then decreased, with the highest antioxidant activity observed at X7, where DPPH and ABTS scavenging activities increased by 38.98 % and 38.70 %, respectively, compared to the control. This trend is similar to the changes in phenolic content. Similar results have been reported in previous studies (Shen et al., 2022). Wang et al. (2023) found a dose-dependent increase in total

flavonoid content and antioxidant activity in extracts from Citri Sarcodactylis Fructus when using gamma-ray-assisted extraction, with increases ranging from 9.5 %–21.62 % and 6.6 %–62.29 %, respectively. Our study indicates that irradiation treatments can effectively enhance the phenolic content and antioxidant activity in wine, positively affecting the basic chemical composition of wine. To explore the relationship between antioxidant capacity and phenolic compounds, correlation analysis was conducted for ABTS, DPPH with TP, TA, TT, TF, TFO, and all phenolic compounds (Fig. 2I). The results, as shown in the figure, indicated that ABTS and DPPH were significantly positively correlated with TP and TFO (ABTS: $R_{TP} = 0.79$, $R_{TFO} = 0.85$; DPPH: $R_{TP} = 0.87$, $R_{TFO} = 0.94$) ($p < 0.05$). Additionally, DPPH was significantly positively correlated with TT and TF ($R_{TT} = 0.78$, $R_{TF} = 0.80$, $p < 0.05$), suggesting that TP, TT, TF, and TFO are the main contributors to DPPH antioxidant capacity, similar to previous reports (Guler, 2023). Among the monomeric phenols, ABTS and DPPH were significantly positively correlated with kaempferol, epicatechin, and procyanidin B2 (ABTS: $R_{kaempferol} = 0.71$, $R_{epicatechin} = 0.88$, $R_{procyanidin\ B2} = 0.78$; DPPH: $R_{kaempferol} = 0.77$, $R_{epicatechin} = 0.83$, $R_{procyanidin\ B2} = 0.77$) ($p < 0.05$), indicating that these three monomeric phenols are major contributors to antioxidant capacity.

3.4. The analysis of CIELab parameters of the wine

The color of wine is influenced by various factors, and as expected, the color parameters were significantly affected by irradiation treatments (Table 1). In the CIELAB parameters, L^* represents lightness, a^* the red-green axis, and b^* the yellow-blue axis. All X-ray treatments, as well as E7 and EN, significantly reduced the lightness of the wine, consistent with previous reports ($p < 0.05$) (Błaszak et al., 2019; Mihaljević Žulj et al., 2019). The L^* value for both X-ray and electron beam treatments initially increased and then decreased with higher irradiation doses, with X0.5 showing the lowest L^* value, indicating the deepest color. This is likely because these treatments allow more phenolic compounds to enter the wine, thereby increasing color depth. The reduction in TF and TA content in E2 explains the increase in L^* value. All treatments, except X7, increased the red hue of the wine. The highest a^* values were observed in X0.5 and E7 for the two irradiation methods. For X-ray treatments, the a^* value showed a negative dose-dependence, while for electron beam treatments, the red hue intensified with increasing doses. Anthocyanins are the primary contributors to the red hue, and similar findings were reported by Gupta et al. (2015), showing that the a^* value of wine correlates with total anthocyanin content. Lower L^* values and higher a^* values are associated with the aging potential of wine. X0.5, X2, E0.5, and E7 all increased the red hue while reducing brightness, which is considered beneficial for the potential quality of the wine. All treatments increased the yellow hue of the wine to varying degrees. The EN treatment resulted in the most significant increase in the yellow hue, while X0.5 and E7 were the treatments that most significantly increased the b^* value among the irradiation treatments. This is attributed to the increase in flavonoid compounds in the wine. Like the a* values trend, all irradiation treatments, except X7, increased the chroma (C^*) of the wine, making the color more vivid. Similar results were reported in a study using gamma irradiation on mango juice (Naresh et al., 2015). Furthermore, all treatments, except E2, increased the hue angle (h^*) of the wine, which contrasts with the findings of Morata et al. (2015), who found that electron beam treatments decreased the h^* of the wine. A ΔE greater than 3 indicates that the color difference can be easily perceived by the human eye. The color differences between all treatments and CK were noticeable to the naked eye, with X0.5 and EN showing the most significant differences from CK, while E0.5 and E2 had the smallest ΔE. Additionally, X0.5 and EN exhibited similar color trends, indicating that wine produced using X-ray irradiation has a color profile close to that of traditional pectinase maceration.

3.5. The analysis of volatile compounds of the wine

Volatile compounds in wine are critical factors influencing its quality. Using GC–MS, 55 volatile compounds were identified, including alcohols (6 C6 alcohols), esters (5 acetate esters), acids, terpenes, and aldehydes/ketones. Alcohols and esters accounted for over 90 % of the

Table 1
The effect of irradiation treatments on the CIElab chromatic parameters of wine.

Sample	Simulated Color	L^*	a^*	b^*	C^*	h^*	ΔE
CK		41.69 ± 0.16b	51.82 ± 0.1f	14.28 ± 0.12e	53.75 ± 0.09f	15.41 ± 0.13f	79.3 ± 0.09f
EN		37.43 ± 0.14e	55.32 ± 0.1a	17.71 ± 0.06a	58.08 ± 0.08a	17.75 ± 0.09a	85.37 ± 0.06b
X0.5		34.71 ± 0.03f	53.29 ± 0.09d	16.95 ± 0.09b	55.92 ± 0.11c	17.65 ± 0.07ab	85.97 ± 0.08a
X2		38.66 ± 0.23d	52.52 ± 0.24e	15.72 ± 0.12c	54.82 ± 0.24e	16.67 ± 0.12d	82.27 ± 0.03d
X7		37.53 ± 0.28e	51.25 ± 0.2 g	15.9 ± 0.28c	53.66 ± 0.17f	17.23 ± 0.32bc	82.35 ± 0.19d
E0.5		42.03 ± 0.09ab	53.26 ± 0.14d	15.23 ± 0.09d	55.4 ± 0.16d	15.96 ± 0.07e	80.18 ± 0.15e
E2		42.3 ± 0.06a	53.92 ± 0.05c	15.11 ± 0.05d	56.00 ± 0.04c	15.65 ± 0.06ef	80.41 ± 0.02e
E7		39.61 ± 0.04c	54.67 ± 0.01b	16.73 ± 0.17b	57.17 ± 0.06b	17.02 ± 0.16 cd	83.16 ± 0.02c

Different letters indicate significant differences among wine samples ($p < 0.05$). The color difference (ΔE) was calculated using distilled water as the reference. Sample coding: CK, the control; EN, The pectinase addition; X0.5, X2 X7: grape irradiated by X-radiation at 0.5, 2,7 kGy, respectively; E0.5, E2, E7: grape irradiated by electron beam at 0.5, 2,7 kGy, respectively.

volatile compounds, consistent with previously reported data (Table S3) (Wei et al., 2023). Fig. 3A–H illustrates the total amounts of several major volatile compounds. Specifically, E0.5 and E7 treatments most significantly enhanced the extraction of total volatile compounds, with E0.5 treatment having the highest total volatile compound content at 354.85 mg/L, a 24.8 % increase compared to CK (284.38 mg/L) ($p < 0.05$). Ester compounds, typically associated with floral and fruity aromas in wine, are primarily produced during alcoholic fermentation by yeast (Chen et al., 2023). Fig. 3C shows that E0.5 treatment significantly increased the ester content, with acetate ester content increasing by 61.77 % compared to CK. This increase might be due to irradiation enhancing the extraction of esters and their precursors ($p < 0.05$). Additionally, irradiation may improve the availability of nitrogen sources such as amino acids (Mihaljević Žulj et al., 2019), boosting yeast metabolism and resulting in more ester production. Furthermore, Ling et al. (2022) confirmed that the addition of phenolic compounds synergistically promotes ester formation. However, excessively high doses can lead to the cleavage of ester bonds, reducing their content (Morata et al., 2015). Higher alcohols are associated with fruity, spicy, and vegetal notes in wine. The E0.5 treatment significantly increased the content of higher alcohols ($p < 0.05$). Although high-dose X-ray treatment reduced the content of ester compounds, the X7 treatment significantly decreased the levels of C6 alcohols, including 1-Hexanol and 3-methyl-1-pe ntanol, which are known to impart undesirable green aromas to the wine($p < 0.05$) (Chen et al., 2023). Aldehydes and ketones are derived from yeast metabolism, alcohol oxidation, and acid decarboxylation. In this study, all irradiation treatments significantly increased the total content of aldehydes and ketones, with the highest increase observed in the X7 treatment, which showed a 57.9 % increase compared to CK ($p < 0.05$). The lowest alcohol content was found in the X7 treatment, indicating that irradiation promotes the oxidation of alcohols, thereby increasing the content of aldehydes and ketones. 2,3-Butanedione and acetoin, both oxidation products of 2,3-Butanediol, increased in all irradiation treatments. Notably, the X7 treatment resulted in an acetoin content of 4947.70 μg/L, an increase of 189.89 % compared to CK, enhancing the creamy and strawberry aroma characteristics of the wine (He et al., 2022). Isoprenoids originate from berries and contribute to the complex floral aroma of wine. They exist in free form or are bound to sugars via glycosidic bonds. During maceration and fermentation, a small amount of bound glycosides release free terpenes. The three doses of electron beam treatment and X2 significantly increased the total amount of terpenes and norisoprenoids, attributed to irradiation releasing more glycosides and inducing glycoside hydrolysis ($p < 0.05$) (Gupta et al., 2015). Compared to CK, none of the treatments showed a significant increase in the content of acid compounds ($p < 0.05$).

To further interpret the results, cluster analysis and principal component analysis (PCA) were performed on the aroma volatile compounds data (Fig. 4). The cluster heatmap shows the relative abundance of volatile compounds and the grouping patterns among different treatments. Notably, the E0.5 treatment significantly increased the volatile compound content, forming a distinct cluster separate from the other treatments ($p < 0.05$). PCA identified the principal components that explained most of the variance in the data. The first two principal components (PCs) accounted for 62.3 % of the total variance, with PC1 and PC2 explaining 44.9 % and 17.4 %, respectively. The distribution of samples in the PC1 and PC2 plane showed that PC1 provided better discrimination. The scores plot indicated that treatments E2, E7, and CK were on the right side of PC1, suggesting the dose levels of electron beam treatment could be distinguished by PC1 values. In contrast, X-ray treatments and the EN control were on the left side of PC1. Like the electron beam, the dose levels of X-ray treatment could be differentiated by PC2 values, with higher doses resulting in higher PC2 values. The non-irradiated CK and EN control groups had negative values in the PC2 region and were closely positioned, indicating that traditional pectinase treatment had less impact on wine aroma compared to irradiation treatments. In the first two principal components, the control group and E0.5 were the most distant, while EN and X0.5 were closer, suggesting that E0.5 significantly altered the aroma compound profile. An orthogonal partial least squares discriminant analysis (OPLS-DA) model

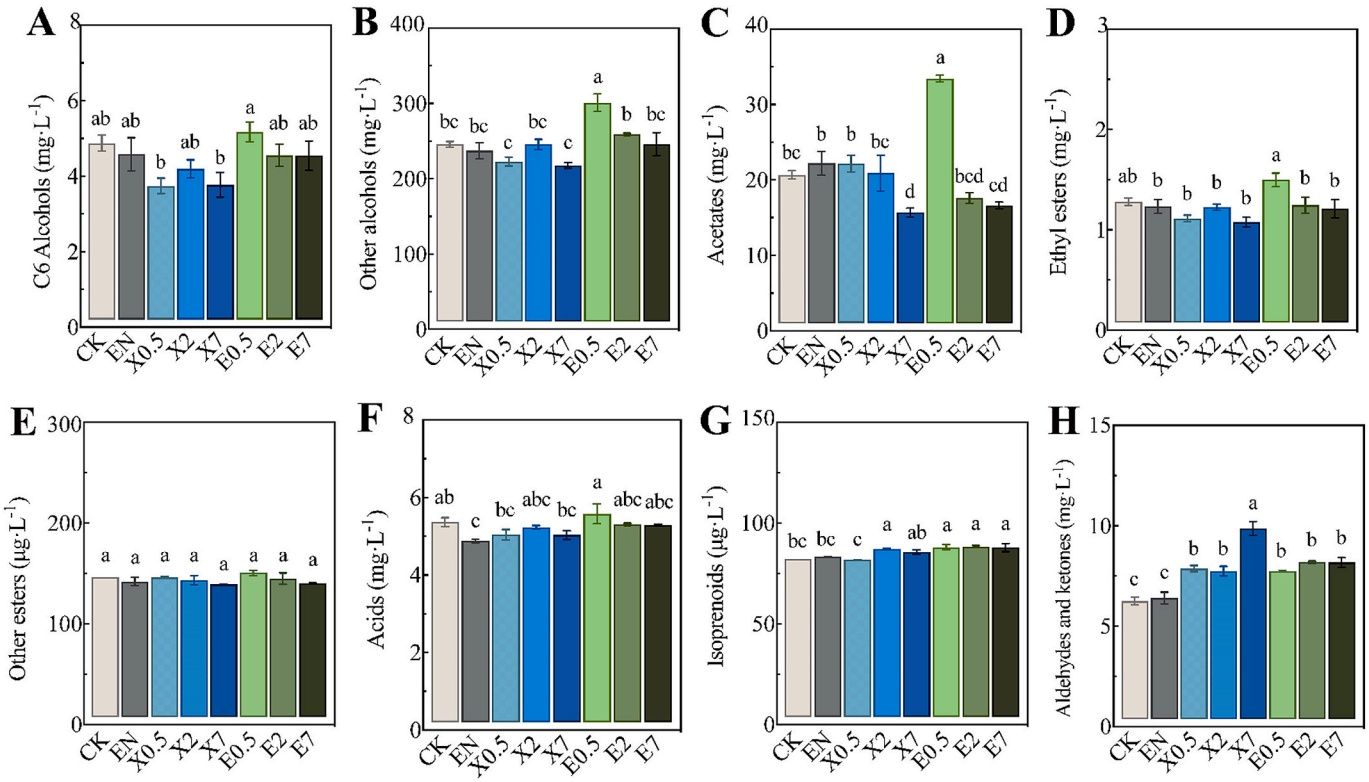

Fig. 3. Content of various aroma compounds in wines under different irradiation pretreatments.

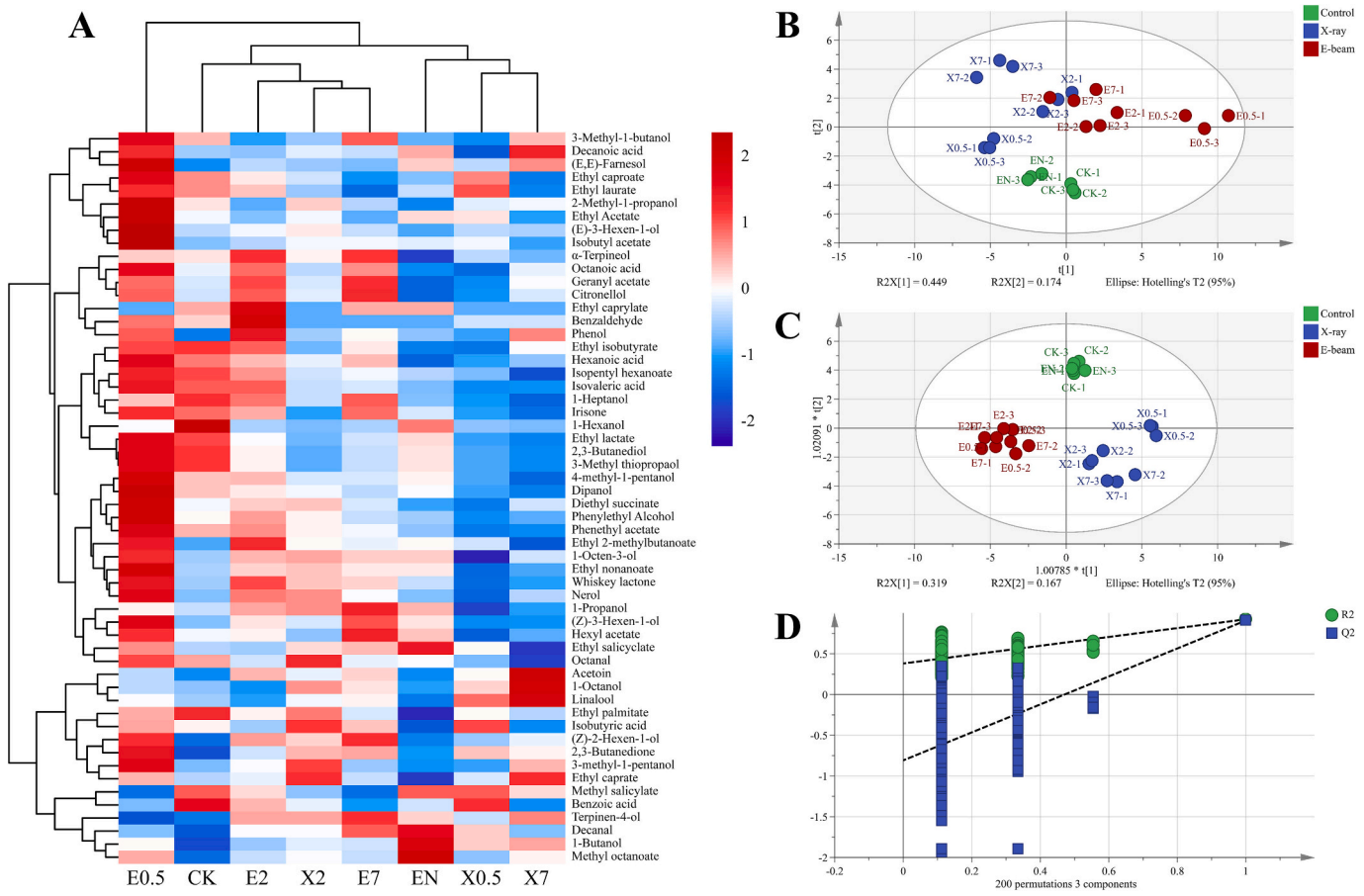

Fig. 4. Analysis of variations in volatile compounds in wines under different irradiation pretreatments.

was used to identify differential compounds in wines treated with different irradiation methods. The model showed high explanatory and predictive power, with R^2X of 0.755, R^2Y of 0.919, and Q^2 of 0.896 (Fig. 4C). OPLS-DA indicated that wines treated with the two irradiation methods clustered separately from non-irradiated wines, highlighting the presence of differential compounds between the different irradiation treatments. Permutation tests with 200 iterations confirmed that the Q^2 values were all lower than the R^2 values, indicating no overfitting in the model (Fig. 4D). Based on VIP values >1, 22 differential compounds were identified (Table S4), of which 8 had OAV values greater than 0.1, indicating that they were the main compounds contributing to the differences in wine aroma characteristics.

3.6. Sensory analysis

Fig. 5 shows the sensory evaluation results of 17 experts for the control group and eight irradiated wine samples. No significant differences in limpidity were observed across different irradiation treatments. However, irradiation improved the color density of the wines, attributed to the extraction of more anthocyanins and flavonoid pigments,

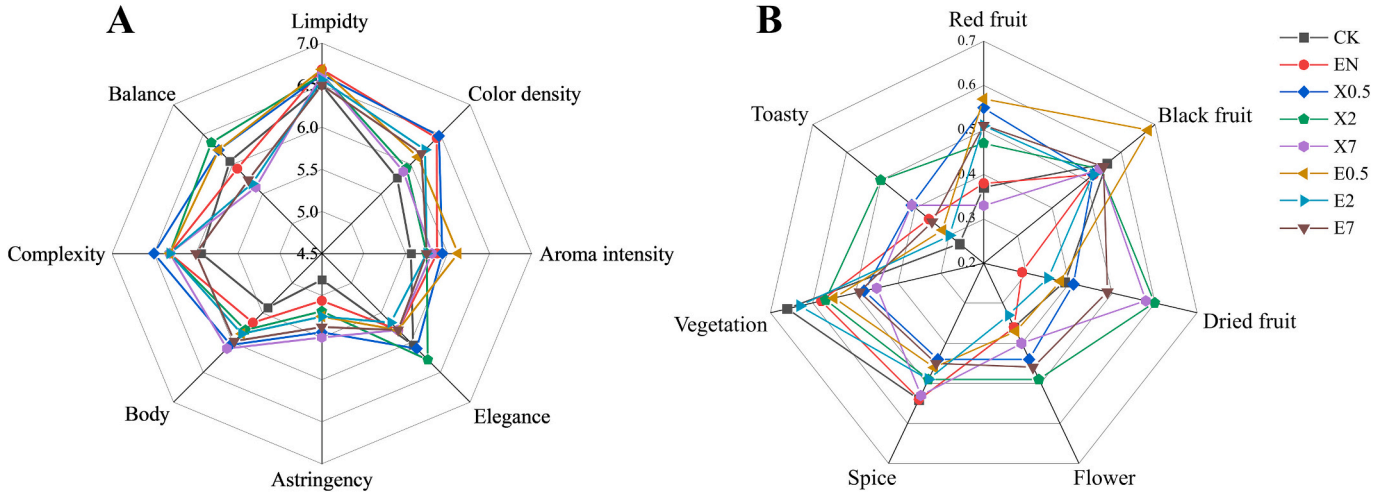

Fig. 5. Sensory evaluation of wines under different irradiation pretreatments.

consistent with the color parameter results. Additionally, irradiation enhanced the aroma intensity scores, with E0.5 receiving the highest score due to the increased volatile compound content in the wine. Higher scores in body and complexity indicate stronger "fullness" and "complexity" of the wine, which are attributes associated with consumer preference. X0.5 and X7 treatments received the highest scores in these two indicators. Different doses also improved the intensity of astringency, related to the increased phenolic content, which adds structure and astringency to the wine. Overall, X2, X0.5, and E0.5 treatments resulted in better balance ratings, suggesting that low-dose irradiation is more beneficial for overall wine quality improvement. In terms of aroma, E0.5 had the strongest black fruit and red fruit aromas, attributed to the higher content of esters and terpenes that contribute to fruity aromas. Irradiation treatments increased the toasty aromas and reduced the green, vegetal notes, which are considered undesirable, due to the reduction of C6 alcohols. The sensory panel did not detect any off-flavors in the irradiated samples. However, high-dose treatments weakened the floral and fruity characteristics, reducing the balance ratings. The results indicate that low to medium doses of irradiation (0.5 kGy, 2 kGy) are beneficial in enhancing the sensory characteristics of wine, particularly in terms of aroma and taste attributes.

4. Conclusion

This study employed X-ray and electron beam technologies to investigate the effects of different pretreatment methods and doses on wine quality. The results demonstrated that irradiation enhanced maceration by disrupting the grape skin structure, thereby increasing the extraction of phenolic compounds, improving antioxidant activity, and enhancing wine color. The highest phenolic extraction was observed at 7 kGy, followed by 0.5 kGy. However, higher doses reduced the stability of anthocyanins and other phenolics, negatively impacting taste, which makes 0.5 kGy a more suitable dose for phenolic extraction. Additionally, irradiation significantly increased the content of volatile compounds, particularly enhancing the extraction and synthesis of esters, terpenes, and their glycosides, which boosted fruity aromas. Principal component analysis (PCA) and cluster analysis effectively differentiated the effects of various irradiation treatments, with E0.5 showing a 24.8 % increase in volatile compounds. In contrast, higher doses oxidized higher alcohols into aldehydes and ketones. Due to its high penetration and low oxidation properties, X-ray irradiation was better suited for phenolic extraction, whereas electron beam irradiation was more effective in increasing volatile compounds. In conclusion, irradiation technology is not only an effective alternative to sulfur dioxide in the wine industry but also enhances the phenolic content and aroma of Cabernet Sauvignon, providing unique advantages. Future research should explore a wider range of grape varieties, doses, and their effects on aging to further optimize winemaking processes.

CRediT authorship contribution statement

Weikang Ding: Writing – review & editing, Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Qian Tu:** Writing – review & editing, Software, Methodology, Formal analysis. **Xuexue Xi:** Methodology. **Xiaojie Wu:** Methodology. **Junqing Bai:** Resources, Methodology. **Shuang Liu:** Data curation. **Junjun Li:** Supervision, Project administration. **Chunlong Yuan:** Writing – review & editing, Validation, Supervision, Funding acquisition.

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.

Acknowledgments

This study was funded by the Key Technology Integration and Demonstration of Organic Specialty Wine in the Hoxud Region program (No. TG20240591) and the Technology and Process Integration of Specialty Wine in the Hoxud Region program (No. TG20240330). We thank the Life Science Research Core Services, NWAFU (Dr. Liu Yao, Dr. Huang Ke-rang) for their technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.102124.

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