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Effects of electron beam irradiation on microbial contamination and quality of Astragali Radix

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Abstract

In this study, astragali radix (AR) was irradiated at doses of 3, 5, 7, 10 and 18 kGy of electron-beam, to investigate the effects on the microbial content, physicochemical quality, and antioxidant activity. Results showed that AR was decontaminated at 7 kGy irradiation, but its color was altered. The total flavonoid, total phenol content, DPPH and ABTS radical scavenging ability increased with an increase in radiation dose for AR. In conclusion, electron beam irradiation can effectively kill the surface microorganisms of AR, increase the contents of flavonoids and total phenols, and increase its free radical scavenging activity without affecting its main active components.

Keywords: Astragali radix (AR); decontamination by electron beam irradiation; High-Performance Liquid Chromatogram (HPLC); active ingredients; quality ingredients.

Practical Application: Cold sterilization is favored by consumers because of its higher completeness. In this study, the influence of electron beam irradiation sterilization on Astragali Radix was reflected. The results showed that the quality of Astragali Radix was improved after electron beam irradiation. Electron beam irradiation can ensure the sterilization effect while keeping the active ingredients of astragalus unaltered. In addition, irradiation increased the content of total phenols and flavonoids of Astragali Radix, which provided a certain reference for the application of cold sterilization technology in traditional Chinese medicine.

1 Introduction

AR, Bge. a member of the Leguminosae family, is the root of *Astragalus membranaceus (Fisch.) Bge.var.mongholicus (Bge.) Hsiao or Astragalus membranaceus (Fisch.)*. AR is one of the most critical Qi-tonifying adaptogenic herbs and has a long history of medicinal use, which mainly contains *astragaloside IV, calycosin-7-O-β-glucopyranoside*, total flavonoid, and total phenol. It has been incorporated into the Pharmacopoeia by China, the United States, the United Kingdom (Qiu et al., 2020).The AR has been used as food and traditional Chinese medicinal herb and currently, it has become a popular food. Studies indicate the beneficial effects of AR for maintaining human health as immune modulation, protection of the cardiovascular system hepatoprotection, anti-inflammatory activity anti-oxidation, and anti-tumor effect. AR can be marketed in the dried form, the powder is made into decoction pieces or used as raw materials to synthesize other drugs. However, AR was often air-dried after harvesting, a process that is often carried out in an open environment, and also prone to high levels of microbial contamination during transport and storage, which can result in quality deterioration and economic loss (Liu et al., 2017). Therefore, methods to ensure its safety and extend its shelf life are needed.

Sterilization techniques such as sulphur fumigation, ethylene oxide and steam have been used to decontaminate Chinese herbs (Ocloo et al., 2023). This decontamination method has recently been widely prohibited worldwide because of health, environmental, or food safety concerns. Thus, new decontamination treatments are of increasing interest to the herbal industry. As a cold sterilization technology, radiation has the characteristics of low cost, environmental friendliness, easy control, good sterilization effect, and high safety, and has been recognized more and more around the world (Pereira et al., 2017; Wei et al., 2022). X-rays, gamma-rays, and electron-beams, have been approved by the Codex Alimentarius Commission, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), have been widely used in food to produced the ideal effec (Kim et al., 2017; Hwang et al., 2021; Pillai & Pillai, 2021; Luo et al., 2023). Nowadays, irradiation technology is widely used in food and medical treatment and has broad application prospects.

In medicinal plants, irradiation has been shown to be effective in reducing microbial contamination, but other effects vary depending on who is irradiated. The treatment of dried oregano with low-energy electron beam irradiation showed that the dose of 12 kGy ensured microbial safety, but had no significant effect on antioxidant activity (Schottroff et al., 2021). However, irradiation could improve the antioxidant activity of mugwort extracts was reported by Ko-Eun Hwang et al. (2021). In addition, The experiment showed that 10.0 kGy dose had no significant effect on the antioxidant activity of goji-berry, and the electron beam irradiation increased the total antioxidant activity of ORAC

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samples, so did the determination of total flavonoids and phenols (Rodrigues et al., 2021). Previously, it had been confirmed that electron beam irradiation can reduce the microbial contamination of AR, but other effects of irradiation on AR remain to be further studied (Jin et al., 2006). In addition, many literatures had reported the quality control of AR and proposed many improved methods (Liu et al., 2018; Zhang et al., 2019), but electron beam irradiation treatment had not been proposed to improve the quality of AR.

By contrast, electron beam irradiation has higher efficiency in pharmaceuticals irradiation than gamma irradiation, produce no nuclear waste, and has superior dose rates (Jacobs, 2022). Therefore, the aim of this study was to investigate the effects of electron beam irradiation (3, 5, 7, 10 and 18 kGy) on the microbial, physicochemical properties, and main active components of AR. The applicability of electron beam irradiation to AR was verified by analyzing the experimental results.

2 Materials and methods

2.1 Sample preparation

AR are provided by Taiji Group Mianyang Pharmaceutical Co., LTD., made in Sichuan province, and stored at room temperature after harvesting and drying.

2.2 Electron beam irradiation treatment

The crushed samples were placed in a polythene ziplock bag (20 cm × 30 cm, 30 wires) each bag 300 g, with a thickness of about 2 cm, and exposed to five absorbed dose levels i.e., 0 (non-irradiated), 3, 5, 7, 10 and 18 kGy. The irradiation treatments were performed by Sichuan Runxiang Irradiation Technology Co., Ltd using a VF-ProAcc-10/20 electron beam accelerator (10 MeV, 20 kW) at room temperature. The absorbed doses were evaluated with silver dichromate dosimeters and the actual dose was within ± 5% of the target dose.

2.3 Microbial analysis

The total number of aerobic bacteria (TAMC) and the total number of mold and yeast (TYMC) were determined by the microbial counting method in accordance with Chinese Pharmacopoeia. Ten grams of each sample were mixed in 250 mL vials with a screw cap containing 90 mL of sterile saline. Enumeration media for TYMC and TAMC were prepared with Sabouraud Dextrose agar and Trypticase Soy agar, respectively. The TYMC and TAMC were analyzed by preparing serial decimal dilutions with sterile saline. The plates of TYMC were incubated at 25 °C for 5~7 days, while those of TAMC were incubated at 35 °C for 3~5 days. The results are expressed as log CFU/g.

2.4 Determination of color values in AR

The color of non-irradiated and irradiated AR were determined in terms of L* (lightness), a* (redness), and b* (yellowness) values using a portable computerized colorimeter (NH3000, 3NH Technology CO, Ltd, Shenzhen, China). The color difference (△E) was calculated using the Equation 1

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

2.5 Determination of moisture content, ash content and the water-soluble extracts in AR

The moisture content, ash content and the water-soluble extracts were carried out according to the Chinese Pharmacopoeia (Commission, 2020).

2.6 Determination of total flavonoid content

The samples were ultrasonically extracted for 1h in 70% ethanol with a solid-liquid ratio of 1:15 (g/mL) and then rapid filtration. Sodium nitrite-aluminum nitrate colorimetry described previously was used to quantify total flavonoid content with minor modifications (Liu et al., 2022). Briefly, every 5ml of sample extract is mixed with 1 mL of 5% sodium nitrite, followed by 1 mL of 10% aluminum nitrate, 10 mL of 4% sodium hydroxide, and finally distilled water is added to a constant volume of 25 mL. After incubation at room temperature for 15 min, absorbance was recorded at 510 nm. The total flavonoid content was expressed as grams of rutin equivalents per milligram of dry weight using a calibration curve with rutin. The rutin standard for determination of flavonoid content was purchased from Efa Biotechnology Co. (Chengdu, China) and also configured to a concentration of 400 μg/mL with 70% ethanol. The linear regression Equation 2 of flavonoid is as follows

$$Y = 0.0784X + 0.0177 \; r^2 = 0.9994, \text{linear range: } 1.6 - 14.4 \; \mu g/mL \quad (2)$$

Y is the absorbance. X is the solution concentration.

2.7 Determination of total phenol content

Total phenols were determined by Folin-Ciocalteu assay. The samples were ultrasonically extracted at 60 °C for 40 min in 60% ethanol with a solid liquid ratio of 1:10 (g/mL) and then centrifuged at 6000 r/min for 10 min. Each of the supernatant of the sample followed by adding 0.5 mL Folin-Ciocalteu (50%,v/v) and 1.5 mL of 7.5 mg/mL sodium carbonate (Na_2CO_3). The volume of the mixture was constant to 10 mL and then stored at a room temperature for 20 min, and the absorbances read at 760 nm using the SPECORD 200 PLUS (Analytik Jena AG). Gallic acid was used as standards for quantification, and the results were expressed as percent gallic acid equivalents (GAE). Gallic acid and Folin-Ciocalteu were purchased from Chron Chemical (Chengdu, China). In this study, the concentration of gallic acid was 100 μg/mL and the concentration of Folin-Ciocalteu was 50%. The linear regression equation of phenol is Equation 3.

$$Y = 0.0856X - 0.0028 \; r^2 = 0.9993, \text{ linear range : } 0.4 - 2.8 \; \mu g/mL \quad (3)$$

Y is the absorbance. X is the solution concentration.

2.8 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity

The DPPH radical scavenging activity assay was performed by the spectrophotometric monitoring of DPPH disappearance at

517 nm. 0.5 mL sample was mixed with 8 mL fresh DPPH solution (0.1 mmol/L) in a test tube. The antioxidant activity of the samples was expressed as the antioxidant capacity of 1 g astragalus to the required Vc (mg/g). The Vc and DPPH standards for determination of DPPH radical scavenging activity were both purchased from Chron Chemical (Chengdu, China), and also the Vc configured to a concentration of 1 mg/mL with 70% ethanol, while the DPPH configured to a concentration of 39.6 μg/mL with 70% ethanol. The linear regression equation of phenol is Equation 4.

$$Y = -0.025X + 1.0692 r^2 = 0.9991, \text{ linear range: } 0 - 30 \text{ mg/mL} \quad (4)$$

Y is the absorbance. X is the solution concentration.

2.9 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging acti-vity

The ABTS radical scavenging activity assay was performed by the spectrophotometric monitoring of ABTS disappearance at 734 nm. The configuration of ABTS test solution was obtained by mixing 7 mmol/L ABTS with 2.45 mmol/L potassium persulfate evenly and standing for 12-16 hours. 30 μL sample was mixed with 6 mL ABTS solution (Absorbance as 0.7 ± 0.02) in a test tube. The antioxidant activity of the samples was expressed as the antioxidant capacity of one gram AR to the required Vc (mg/g). The linear regression equation is Equation 5.

$$Y = -0.6487X + 0.6889 \; r^2 = 0.9992, \text{ linear range: } 0 - 0.5 \text{ mg/mL} \quad (5)$$

Y is the absorbance. X is the solution concentration.

2.10 Determination of calycosin-7-O-β-glucopyranoside by high-performance liquid chro-matography

The *calycosin-7-O-β-glucopyranoside* in AR after electron beam irradiation were analyzed by HPLC (Ultimate 3000DGLC). Samples were separated on a C_{18} column (5 μm, 250 mm × 4.6 mm, ZORBAX SB-C18). at 30 °C with a flow rate of 0.8 mL/min. The mobile phase consist-ed of 0.1% phosphoric acid in water (solvent A) and acetonitrile (solvent B). The elution gradient was as follows: 0-12 min, 15-27% B; 12-14 min, 27-90% B; 14-20 min, 90% B; 20-22 min, 90-15% B; 22-28 min, 15% B. The detection wavelength was 260 nm. The injection volume was 10 μL. The standard for determination of *calycosin-7-O-β-glucopyranoside* content was purchased from Efa Biotechnology Co. (Chengdu, China) and also configured to a concentration of 108 μg/mL with methanol. The linear regression equation of *calycosin-7-O-β-glucopyranoside* is Equation 6.

$$Y = 0.5327X + 0.215 \; r^2 = 0.9997, \text{linear range: } 27 - 108 \text{ μg/mL} \quad (6)$$

Y is the peak area. X is the solution concentration.

2.11 Determination of astragaloside IV by high performance liquid chromatography-evaporative light scattering detection (HPLC-ELSD)

The *astragaloside IV* in AR after electron beam irradiation were analyzed by HPLC-ELSD. Samples were separated on a C_{18} column (5 μm, 250 mm × 4.6 mm). The mobile phase was acetonitrile (A) and water (B) = 32:68. The flow rate was 1.00 mL/min. The injection volume was 10 μL; the Column temperature was 30 °C. Detector parameters: low-temperature evaporation 40 °C, nitrogen pressure 350 KPa. The standard for determination of *astragaloside IV* content was purchased from Shanghai Aladdin Biochemical Technology Co. Ltd. (Shanghai, China) and also configured to a concentration of 0.5 mg/mL with 80% methanol. The content of *astragaloside IV* in the samples was calculated by logarithmic equation with two points of external standard.

2.12 The effects of different irradiation doses on the composition of AR were analyze-d based on HPLC

The Agilent ZORBAX SB-C18 column (5 μm, 250 mm × 4.6 mm) was used for analysis on the Thermo Fisher Uitimate 3000 high-performance liquid chromatograph. The mobile phase consists of acetonitrile (A) and 0.1% aqueous phosphoric acid (B). The gradient elution mode was as follows: 0-12 min, 15-27% B; 12-44 min, 27-91% B; 44-48 min, 91% B; 48-50 min, 91-15% B; 50-57 min, 15% B.

The fingerprint of AR irradiated with different doses of electron beam were determined by HPLC. The 'Similarity Evaluation System of Traditional Chinese Medicine Fingerprints' (2012 edition) formulated by the National Pharmacopoeia Commission was used to establish the fingerprints of AR samples treated with different irradiation doses.

2.13 Statistical analysis

All experiments were performed in triplicate. Data were analyzed by one-way analysis of variance (ANOVA), followed by SPSS software version 22.0 (IBM, New York, USA). Origin 2021 was used to compile graphs.

3 Results and discussion

3.1 Microbiological parameters

The microbiological result are presented in Table 1. The samples of AR are contaminated with TAMC of 1.30×10^5 CFU/g and TYMC of 7.43×10^4 CFU/g, respectively. Irradiation at 3 kGy significantly decreased TAMC and TYMC ($P < 0.05$) by 2.67×10^2 and 2.0×10^2 CFU/g, respectively. Irradiation at 5 kGy decreased TAMC by 2.33×10^2 CFU/g and TYMC has not detected, which are considered acceptable microbial loads in the herbs. In samples treated with e-beam irradiation at doses above 7.0 kGy, no TAMC and TYMC were detected just after treatment. Felix Schottroff et al. showed that gamma irradiation and low energy electron beam irradiation have good inactivation effect on bacteria in Chinese herbs and spices, which is similar to our findings (Schottroff et al., 2021). A more recent study also found that gamma as well as electron beam irradiation treatment of dried apricots and quince was significantly effective in reducing the number of both yeast and mold and bacterial count in a dose dependent manner (Rather et al., 2019). These findings suggest that irradiation may be effective for sterilization effects of TAMC and TYMC. Indeed, as the radiations hit the DNA molecule, the hydrogen bonds in DNA, which are responsible for double helical structure of DNA are broken. This hydrogen bond

breaking favors the breaking of double helical structure of DNA and renders the DNA unable to replicate, thereby causing death of the cell (Egea et al., 2007). Besides, irradiation kills microorganisms by destroying proteins and enzymes through the indirect action of radiation (Kyung et al., 2019). The main mechanism by which microorganisms are killed by irradiation is the decomposition of water molecules into hydrogen (H^+), hydroxyl (·OH), and oxygen radicals (O_2^-). The radicals can react with microbes' DNA, proteins and cell membranes and damage cell structures to inactivate them (Ocloo et al., 2023). Therefore, it is proved that electron beam irradiation is feasible in reducing TAMC and TYMC.

3.2 Evaluation of color, moisture content, ash content, and water-soluble extracts

To compare the effect of electron-beam irradiation on the color scores of AR, the △E values were calculated from Hunter a, b, and L values. According to Young & Whittle (1985). △E value in range of 0-0.5 indicates and imperceptible difference in color between the samples, 0.5-1.5 indicates a slight difference, 1.5-3.0 indicates a just noticeable difference, 3.0-6.0 a remarkable difference, 6.0-12.0 an extremely remarkable difference in color, and value above 12.0 indicates color of different shade. Effect of radiation treaments on color scores of AR is shown in Table 2. The data analysis indicated that showed a difference among color parameters between unirradiated and irradiated. There is a slight difference in color was observed at 3 and 7 kGy of electron beam irradiation. However, there is a just noticeable difference in color observed 5, 10, and 18 kGy. Darkening of the color of AR was defined as a increase in a and b values and decreased L values after irradiation. Nonetheless, it was not noticeable by visual inspection by the bare eye. A similar decrease in L* values was also reported by Barkaoui et al. (2021) after electron beam irradiation of strawberries. The value of 'b' representing 'yellow' is on the rise, which may mean that non-enzymatic browning may have occurred, but it may also be due to environmental factors. These results demonstrate that color changes due to radiation exposure did not proceed in a predictable manner. Similar results on color changes during radiation processing of dried apricot and quince using gamma and electron beam irradiation are reported by Rather et al. (2019). Jung et al. also

Table 1. Effect of electron beam irradiation treatments on microbiological of AR.

Doses (kGy)	total number of aerobic bacteria (log CFU g^{-1})	the total number of mold and yeast (log CFU g^{-1})
Ck	5.11 ± 0.01^a	3.87 ± 0.03^a
3	2.48 ± 0.12^b	2.30 ± 0.19^b
5	2.36 ± 0.22^b	ND
7	ND	ND
10	ND	ND
18	ND	ND

Values represent mean ± standard deviation. The different lowercase letters (a-b) showed statistically significant differences among the four samples ($P \leq 0.05$), and "ND" meant that No colonies were detected.

Table 2. Effect of electron beam irradiation treatments on color, moisture content, ash content, and water-soluble extracts of AR.

Dose (kGy)	Color values			
	*L**	*a**	*b**	*△E*
0	84.68 ± 0.19^a	3.28 ± 0.06^c	12.54 ± 0.29^c	--
3	84.18 ± 0.16^b	3.42 ± 0.10^c	13.48 ± 0.06^a	1.07 ± 0.24
5	83.40 ± 0.06^c	3.51 ± 0.09^b	13.59 ± 0.15^a	1.67 ± 0.11
7	83.55 ± 0.08^c	3.52 ± 0.06^b	13.01 ± 0.04^b	1.25 ± 0.05
10	83.44 ± 0.29^c	3.55 ± 0.13^b	13.53 ± 0.08^a	1.61 ± 0.22
18	82.60 ± 0.23^d	3.84 ± 0.08^a	13.21 ± 0.12^b	2.25 ± 0.08

Values represent mean ± standard deviation. The superscript letter a in the same column mean present no have statistical difference ($P \leq 0.05$).

Table 3. Effects of different doses of electron beam irradiation on the contents of water, ash and water-soluble extract of AR.

Dose (kGy)	Moisture content (%)	Ash content (%)	Water-soluble extracts (%)
0	3.11 ± 0.01^a	4.54 ± 0.04^a	34.58 ± 0.14^a
3	3.05 ± 0.04^a	4.45 ± 0.23^a	34.35 ± 0.29^a
5	3.08 ± 0.02^a	4.44 ± 0.12^a	34.43 ± 0.37^a
7	3.11 ± 0.04^a	4.49 ± 0.03^a	34.32 ± 0.16^a
10	3.21 ± 0.22^a	4.42 ± 0.03^a	34.32 ± 0.14^a
18	3.20 ± 0.01^a	4.49 ± 0.13^a	34.64 ± 0.07^a

Values represent mean ± standard deviation. The superscript letter a in the same column mean present no have statistical difference ($P \leq 0.05$).

found that the color value change of paprika powder was not linearly related to the irradiation dose (Jung et al., 2015).

Effect of irradiation moisture content, ash content, and water-soluble extracts of AR is shown in Table 3. According to the Chinese Pharmacopoeia, the moisture content of AR should be less than 10.00%, the total ash content should be less than 5.00%, and the water-soluble extract content should not be less than 17.00%. Data analysis indicated that no significant ($P \leq 0.05$) difference existed among moisture content, ash content, and water-soluble extracts between unirradiated and irradiated. This is agreement with a study on electron beam irradiation treatment of *Ophiopogon japonicus*, there were no significant differences in water content, ash content and extract content (He et al., 2021).

Electron beam irradiation is done after the sample is packaged, and the sample has limited contact with water molecules in the air, so it does not react easily with water molecules in the air. In addition, the temperature change caused by electron beam irradiation is small, so it has little effect on the moisture content of the sample. Therefore, slight fluctuations in moisture content may be caused by the overall unevenness of AR samples, slightly different environmental impacts during transportation or storage, as well as errors and uncertainties in measurement (Schottroff et al., 2021). Ash refers to the total amount of inorganic matter and other impurities in the sample. The packaging of the sample is isolated from the environment, and the dust in the environment cannot contaminate the AR, so the ash content fluctuates less (Chen et al., 2021). The extraction time, extraction temperature, sample size and extraction solvent affected the extraction content (Sablania et al., 2019). The results of this experiment showed that the content of extracts fluctuated little, indicating that irradiation had no significant effect on the content of extracts ($P \leq 0.05$).

3.3 Total phenolics and flavonoids

Effect of electron beam irradiation on total flavonids and phenolics of AR is shown in Figure 1A and B. The data analysis indicated that in AR positive correlation (r = 0.93) existed between irradiation dose and total flavonoid. As the irradiation dose increased, the flavonoid increased to approximately 2.93% in AR irradiated at a dose of 3 kGy ($P \leq 0.05$); the flavonoid then reached up 10.35% in the irradiated AR to 18 kGy in comparison with that of the non-irradiated AR samples. The data analysis indicated the maximum irradiated values in relation to the non-irradiated samples (25.89 mg RE/100 g) the dose of 18 kGy having the highest flavonoids content (28.57 mg RE/100 g). Some recent study also demonstrated increase in total phenols and flavonoids during irradiation and the increase occurred in a dose-dependent manner (Krishnan et al., 2018; Rather et al., 2019). The findings of Jia et al.

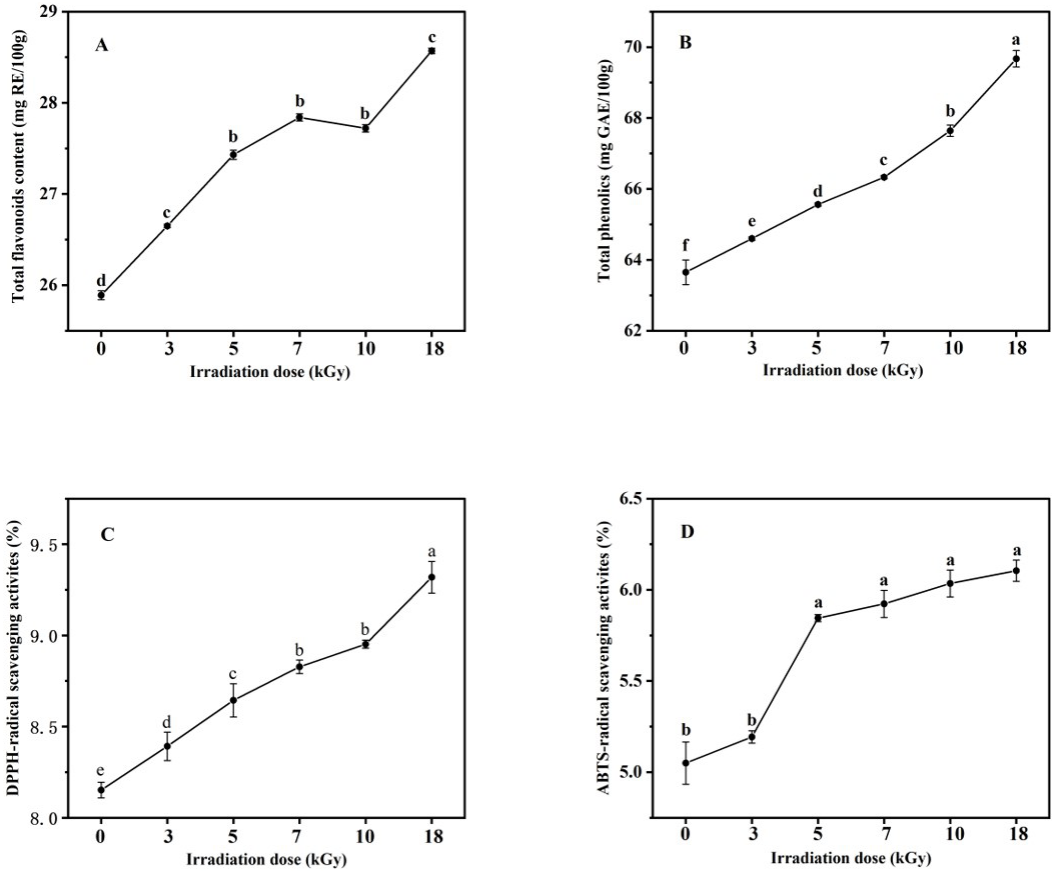

Figure 1. Effect of electron beam irradiation on the content of total flavonoids in AR (A), Effect of electron beam irradiation on total phenol content in AR (B), Effect of electron beam irradiation on DPPH radical scavenging ability of AR (C), Effect of electron beam irradiation on ABTS radical scavenging ability of AR (D). The different lowercase letters showed statistically significant differences among the four samples ($P \leq 0.05$).

(2022) showed that the increase of flavonoids in irradiated star anise was due to the break of glycosidic bonds. As previously reported, irradiation causes the C-O glycoside bond in quercetin-4'-glucoside to break and produce a flavonoid compound called quercetin (Krongrawa et al., 2020). Macromolecular flavonoids compounds are transformed into small molecular flavonoid compounds by irradiation, with a concomitant imporovement in the extraction yield of the flavonoids compounds because of the change in tissue structure by electron beam irradiation (Khattak & Rahman, 2016). A recent study found that X-ray irradiation affected the activities of phenylalanine aminolyase (PAL) and dihydroflavonol reductase (DFR), as well as the expression of genes related to flavonoids (Guo et al., 2022). Therefore, the effect of electron beam irradiation on flavonoids of AR may be related to the expression of flavonoid biosynthetic enzyme genes.

The same was observed in the total phenolic assays, since, according to the results, the total content of phenolic was relatively higher in the irradiated samples, while the non-irradiated samples showed a relatively lower phenolic content. The correlation between the total phenolic and dose was determined to be 0.99. The highest phenolic was observed at a dose of 18 kGy (69.67 mg GAE/100 g) ($P \leq 0.05$), showing an increase in the total phenolic up to 9.46% compared with that of non-irradiated AR samples. Therefore, electron beam irradiation treatment dramatically increases the concentration levels of the phenolic as compared with that of non-irradiated samples ($P \leq 0.05$). The content of phenolic substances in medicinal plants such as ginseng and wormwood was also reported to increase after a certain dose of irradiation. (Esmaeili et al., 2018). In addition, a recent study found a significant increase in phenols in almond skin radiation extract at a dose of 4kGy (Wu et al., 2021). The increase in phenolic compounds can be attributed to their release from glycoside precursors by irradiation and the degradation of large phenolic compounds polymerized during radiation into smaller phenolic substances (Jia et al., 2022). Free radicals formed by radiation, such as hydration electrons and hydrogen peroxide radicals, break some glycosidic bonds and break larger phenolic compounds into smaller ones, leading to a significant increase in phenolic compounds (Cho et al., 2017). For instance, radiation breaks down the glycosidic bonds in flavonols, quercetin, and disaccharide rutin. According to Quan et al. (2020), enhanced interactions between phenolic compounds and proteins, vitamins, carbohydrates, or other compounds lead to oxidative degradation of polyphenols. Therefore, the effect of electron beam irradiation on phenolic compounds of AR may be related to the radiation dose and the chemical composition of AR itself.

3.4 DPPH and ABTS radical scavenging activity

DPPH and ABTS radical scavenging activity of AR was affected by the irradiation dose levels and consequently differed among the tested radiation treatments ($P \leq 0.05$). Figure 1C shows that DPPH radial scavenging activity of AR increased significantly ($P \leq 0.05$) with irradiation dose. However, when the irradiation dose was less than 3 kGy, AR had no significant effect on ABTS radical scavenging activity compared with the control group (Figure 1D). When the irradiation dose was 5-18 kGy, the ABTS radical scavenging activity was significantly different from that of the control group ($P \leq 0.05$). Comparison of the data also showed that AR samples treated with 18 kGy of electron beam irradiation recorded an increase of 14.3% and 10.9% in DPPH and ABTS radical scavenging activity over control. Krishnan et al. also reported an increase of 55% in the DPPH radical scavenging activity of gamma irradiated black soy-bean extract. Similarly, Ko-Eun Hwang et al. (2021) observed that electron-beam irradiation increased the DPPH scavenging activity by 84.27% in mugwort extracts at 10 kGy doses.

In this study, the DPPH scavenging activity and ABTS scavenging activity were well correlated with the content of flavonoid and phenolic compounds. The correlation between the DPPH scavenging activity and the content of flavonoid was determined to be 0.978, and the correlation coefficient between the DPPH scavenging activity and total phenol content was 0.970. Furthermore, strong correlation (r = 0.956) existed between the ABTS scavenging activity and the content of flavonoid whereas moderate correlation (r = 0.858) existed between the ABTS scavenging activity and the content of phenolic. M.I. Elias et al. (2020) observed a tendency to increase the antioxidant activity of raspberries treated with electron beam irradiation, which is consistent with our experimental results. Cho et al. (2017) proposed that irradiation treatment not merely release phenolic compounds but also enhance radical scavenging activity mulberry leaf extract. Positive correlations have been found between antioxidant activity and phenolics or flavonoids, indicating that antioxidant activity is directly related to phenolic or flavonoid profiles. Our finding are consistent with the results of Hwang et al., who observed that DPPH radical scavenging exhibited strong positive correlation with the total phenolic and flavonoid (Hwang et al., 2021). Therefore, due to the hydrogen supply capacity of phenolic and flavonid substances, the content of flavonoids and phenolic compounds increased due to radiation, leading to the increase of free radical scavenging ability. Besides, irradiation can lead to the increase of plant peroxidase and phenylalanine aminolyase enzyme activities, but also can increase the depolymerization and dissociation of cell polysaccharides, so as to increase the antioxidant capacity of active substances (Alothman et al., 2009).

3.5 Calycosin-7-O-β-glucopyranoside and astragaloside IV contents

To verify the stability of the active ingredient in AR with electron beam irradiation, the content of *calycosin-7-O-β-glucopyranoside* and *astragaloside IV* was quantitated by using HPLC. Figure 2A and Figure 2C are chromatograms of AR. The peak of *calycosin-7-O-β-glucopyranoside* and *astragaloside IV* were detected at 10.94 min (Figure 2B) and 10.03 min (Figure 2D), respectively. The chemical structure of *Calycosin-7-glucoside* and *Astragaloside IV* both contain unsaturated bonds. As far as its irradiation mechanism is concerned, electron beam irradiation can easily destroy this kind of chemical structure (Luo et al., 2021). However, the effect of electron beam irradiation on *calycosin-7-O-β-glucopyranoside* and *astragaloside IV* of AR is shown in Table 4. The data analysis indicated no significant statistical difference ($P > 0.05$) among the doses and non-irradiated samples was observed in this study, indicating that electron beam irradiation did not affect the *Calycosin-7-glucoside*

Table 4. Effect of electron beam irradiation treatments on *astragaloside IV* and *calycosin-7-O-β-glucopyranoside* of AR.

Product	Dose(kGy)					
	0	3	5	7	10	18
Astragaloside IV (%)	1.134 ± 0.016[a]	1.149 ± 0.019[a]	1.107 ± 0.090[a]	1.048 ± 0.025[a]	1.091 ± 0.030[a]	1.091 ± 0.024[a]
Calycosin-7-O-β-glucopyranoside (%)	0.057 ± 0.002[a]	0.055 ± 0.001[a]	0.056 ± 0.003[a]	0.056 ± 0.002[a]	0.055 ± 0.001[a]	0.055 ± 0.002[a]

Values represent mean ± standard deviation. The superscript letter a in the same column mean present no have statistical difference ($P \leq 0.05$).

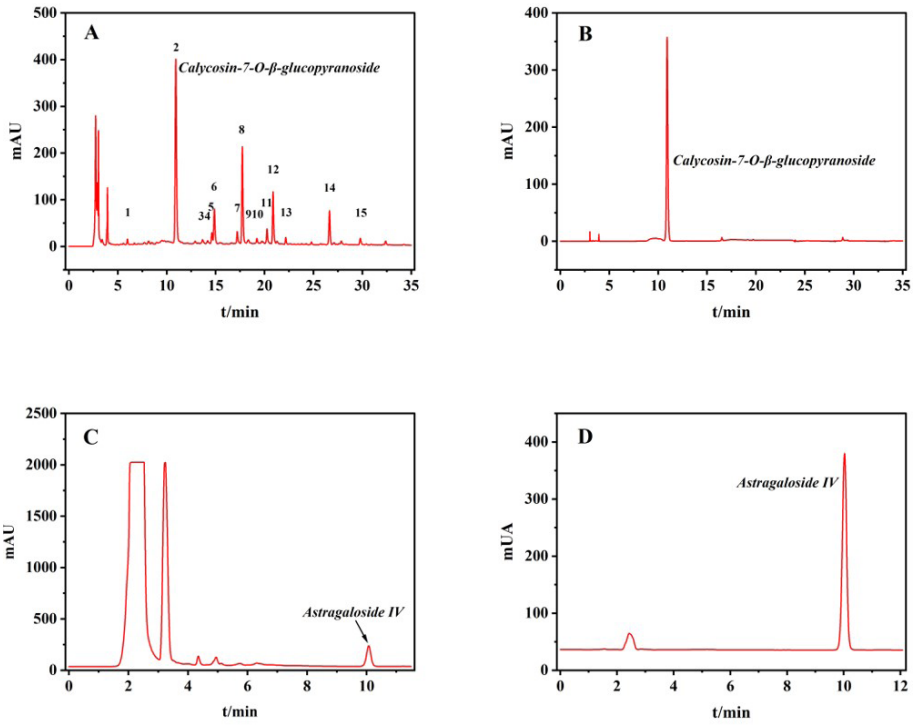

Figure 2. Typical HPLC chromatograms for different doses samples of AR at 260 nm (A), HPLC chromatograms of *calycosin-7-O-β-glucopyranoside* standard solution (B), Typical HPLC chromatograms for different doses samples of AR under evaporative photodetector (C), HPLC chromatograms of *astragaloside IV* standard solution (D).

and *Astragaloside IV* contents of AR from a dose of 3~18 kGy. K. Kishor Kumar et al. (2010) reported that no difference in vanillin or glucovanillin content was noted in cured vanilla beans subjected to different gamma-radiation doses up to 30 kGy, they proposed that the carbon-oxygen linkage in glucovanillin appears to be quite stable. Thus, electron beam irradiation had no significant effect on the content of *calycosin-7-O-β-glucopyra-noside* and *astragaloside IV*, which may also be because the carbon-hydrogen bonds in *calycosin-7-O-β-glucopyranoside* and *astragaloside IV* are very stable. A recent study showed that electron beam irradiation has better degradation effect on zearalenone when it is dissolved in methanol and acetonitrile than when it is distributed in corn flour, which contains limited moisture (Yang et al., 2020). Therefore, *calycosin-7-O-β-glucopyranoside* and *astragaloside IV* become degraded by electron beam irradiation in the matrix of water or organic solvents instead of that in AR with limited water content.

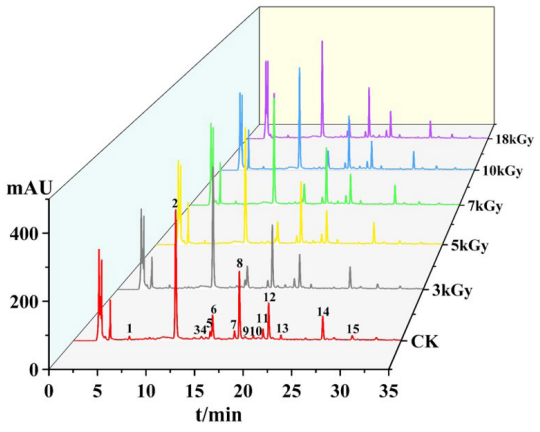

Figure 3. HPLC chromatographic fingerprints of AR by different electron beam irradiation.

3.6 Fingerprint

When the compound is not completely clear, fingerprinting is a feasible method to full control the quality of the sample and reflect its stability (Gong et al., 2021). At present, fingerprint has been widely used in high performance liquid chromatography to detect the complex components of substances (Liu et al.,

Table 5. Calculation table of fingerprint similarity of AR before and after electron beam irradiation.

Sample	CK	3kGy	5kGy	7kGy	10kGy	18kGy	Cross-reference fingerprinting
CK	1	0.999	0.999	0.999	0.999	0.999	0.999
3kGy	0.999	1	1	0.999	0.999	1	1
5kGy	0.999	1	1	1	1	1	1
7kGy	0.999	0.999	1	1	1	1	1
10kGy	0.999	0.999	1	1	1	1	1
18kGy	0.999	1	1	1	1	1	1
Cross-reference fingerprinting	0.999	1	1	1	1	1	1

2021; Wang et al., 2022). The control map R was generated by the median method using multi-point correction and automatic matching (Figure 3). According to the detection results of AR samples treated with different irradiation doses, the chromatographic peak of the main component with good separation (peak 2, *Calycosin-7-O-β-glucopyranoside*) was selected as the characteristic peak, and a total of 15 common peaks were labeled. The median method and multi-point correction method were used to test the similarity of the fingerprints of AR samples treated with different electron-beam irradiation doses. From the results of the similarity comparison, it can be seen that the similarity range of AR samples treated with different electron-beam irradiation doses was relatively small, indicating that the fingerprints of AR samples treated with different electron-beam irradiation doses were very similar. The fingerprint similarity of each irradiation dose could reach 0.999 (Table 5). In conclusion, electron beam irradiation below 18 kGy had no significant effect on the fingerprint of AR.

4 Conclusions

In summary, it could be shown that electron beam irradiation enable an efficient reduction of microbial counts. Simultaneously, electron beam irradiation could retain the product quality to a great extent. Microbial analysis data demonstrated that electron beam irradiation at doses of 5.0 and 7.0 kGy can be used an equally potential alternative to chemical fumigants as gamma irradiation for use as phyto-sanitary treatment. △E data indicated that electron beam irradiation can cause changes in AR color, but this change is not observed by the naked eye. Further, electron beam irradiation increased the total antioxidant activity of DPPH and ABTS samples, which may be due to the increase of flavonoid content and total phenol content caused by irradiation. Finally, the present study recommends that dose of 5.0-7.0 kGy of electron beam irradiation can be used as phytosanitary treatments for AR to achieve microbial inactivation without any detrimental effect on the overall acceptability and antioxidant quality of the product.

Conflict of 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.

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