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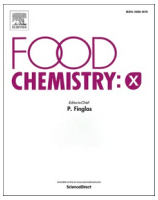

Influence of 10 MeV electron beam irradiation on the lipid stability of oat and barley during storage

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ABSTRACT

This study investigated the effect of electron beam irradiation (EBI) on the lipid stability of oat and barley during long-term storage. Results showed that the initial free fatty acid content in oat was higher than that in barley. This may mean that lipid hydrolysis started under the function of lipase when oat and barley were milled into flours. Both storage and EBI factors influenced lipid-degrading enzyme activity and promoted lipid oxidation in oat and barley. However, it seemed that storage had higher impacts because the DPPH scavenging activity decreased greatly, and the contents of both malondialdehyde and volatile lipid oxidation products increased in all samples. Thus, the antioxidant capacity and level of lipid oxidation after EBI treatment should be considered when producing oat and barley foods. Overall, this study shows the high potential of EBI for use as a non-thermal technique in stabilising the storage quality of oat and barley.

1. Introduction

With the increasing global population, the demand for food is rising; meanwhile, food production and security are being severely challenged. The improvement of yield and quality of agricultural products, such as cereals, can not only solve the problem of food yield, but promote the sustainable development of agriculture and the food production system (Springmann et al., 2018). Oat and barley are important food crops in the kingdom of cereals worldwide. They are widely used in food processing as raw materials due to their good functional properties and nutritional value (Krattinger & Keller, 2022; Sharma, Mokhtari, Jafari, & Sharma, 2021). Oat and barley have a high lipid content, and the flavour compounds resulting from lipids contribute to the unique flavour. However, a degradation in lipids can result in an undesirable off-flavour, leading to a reduction in the edible value, shelf life and economic utilisation value of oat and barley food products (McGorrin, 2019; Yang, Piironen, & Lampi, 2017). A primary reason for the formation of these off-flavours is lipid-degrading enzymes, including lipase, lipoxygenase (LOX) and peroxidase (POD).

Lipase is found in oat and barley, which can act on ester bonds of triglycerides, allowing the stepwise degradation of triglycerides into glycerol and free fatty acids (FFAs) (Decker, Rose, & Stewart, 2014; Wang, Cui, Wang, Li, & Qiu, 2021). The function of lipase in oat has been well studied (Yang et al., 2017). An endogenous lipase will start the hydrolysis cascade of acyl lipids as long as the seed kernels are broken and the enzymes come into contact with the substrates. The liberated FFAs are good substrates for the subsequent chemical and enzymatic reactions (e.g., by LOX). LOX catalyses the lipid oxidation of polyunsaturated fatty acids (PUFAs, e.g., linoleic acid and linolenic acid) containing a 1,4-pentadiene structure under aerobic conditions to form hydroperoxides, which are quite unstable and can further react to form volatile compounds (e.g., hexanal) or non-volatile compounds (e.g., epoxy fatty acids) (Yang et al., 2017). Oat has low LOX activity, whereas its activity in barley is relatively high (Yang et al., 2017; Zheng, Wang, Xiong, & Zhang, 2023). POD activity has been found in oat, which oxidises a series of aromatic compounds, such as phenols, in the presence of H_2O_2 to produce free radicals (Li, Oey, & Kebede, 2022). Therefore, proper processing is needed for the inactivation of these enzymes to inhibit the formation of off-flavours, as well as to extend the shelf-life of cereal foods, including oat and barley.

Currently, the industrial production of cereal foods is mainly performed by thermal processing, which can effectively inhibit the enzyme activity, formation of off-flavours, and prolong the shelf-life of food products. For example, superheated steam can effectively inhibit lipase activity, thus reducing FFAs derived from hydrolysis and decreasing the generation of off-flavours in highland barley (Wang et al., 2021). Bai

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et al. (2021) used three thermal processing methods (steam, microwave and hot-air drying) to treat oat. They found that steam treatment can effectively reduce lipase activity, delay lipid oxidation and improve storage time. However, thermal processing may have adverse effects on bioactive substances and nutrients in cereal grains. Therefore, it is necessary to explore the potential application of non-thermal treatment technology (e.g., cold plasma, ultrasound and irradiation) for the treatment of oat, barley and other cereals to maintain nutritional value and improve the quality of final food products (Yang et al., 2023).

Among non-thermal techniques, the irradiation methods (e.g., γ-ray, electron beam irradiation/EBI and low energy X-ray irradiation) have been widely used in food processing due to their advantages of energy conservation, high efficiency and little effect on the physical properties of materials (Yang et al., 2023). As a novel irradiation technology, a 10 MeV high energy electron accelerator has been efficiently used in many areas, including the food industry, for the purpose of sterilisation and insect control by penetrating a high energy electron beam into substances. An average irradiation dose of less than 10 kGy does not lead to special nutritional or microbiological concerns in foods (Codex General Standard for Irradiated Foods No. 106-1983). However, the irradiation dose is not limited to below 10 kGy in terms of food type, country/region and irradiation use (FAO-IAEA-WHO 1999). For instance, the US allows a maximum irradiation dose of 44 kGy for frozen packaged meats (Pi et al., 2022). Irradiation has been allowed in some cereals in countries such as China and Belgium. Recently, some studies have explored the effect of EBI in grain storage and the food quality of cereals, such as rice, wheat and corn. EBI could effectively reduce lipase activity, decline relative crystallinity, promote digestion and hardly damage the quality of rice (Pan, Xing, Zhang, Luo, & Chen, 2020). It can also decrease the total number of microbial colonies, improve color and reduce odour generation in wheat (Wang et al., 2021). Furthermore, irradiation increases the content of soluble solids in corn, forms a stable colloidal solution and inhibits the gelation of corn flour (Xue, Zhao, Wen, Cheng, & Lin, 2017). These studies showed that an electron beam may have positive effect on food quality and nutritional value. However, the effect of EBI on the flavour quality of cereals, including oat and barley, during storage is still unknown.

Thus, the overall aim of this present study was to investigate the effect of EBI on changes in lipid-degrading enzymes and the formation of lipid-derived off-flavours in oat and barley during storage. Therefore, EBI treatment and different storage times were used to treat the oat and barley samples. In this study, two irradiation doses were selected as the practical choice: a low irradiation dose of 2 kGy and a high irradiation dose of 6 kGy. The storage period was up to 12 weeks. The results of this study may provide more knowledge on the application of EBI as a non-thermal technique in cereal processing and stable food production.

2. Materials and methods

2.1. Oat and barley samples

Dehulled oat was purchased from Qingmai Food Co., Ltd. (Qinghai, China). Dehulled barley samples, including white barley (WB) and bule barley (BB) cultivars, were purchased from Xinning Biotechnology Co., Ltd. (Qinghai, China). The irradiation process was conducted using a 10 MeV electron beam accelerator (CIAE-DZ-10/20, the China Institute of Atomic Energy). About 1 kg of each grain sample was tiled evenly in plastic bags with a thickness of less than 1 cm, and then the samples were irradiated at doses of 2 kGy and 6 kGy, respectively. Thus, the irradiation energy is supposed to be evenly distributed in the same batch of samples during EBI processing. A sample without irradiation was used as a control variable.

The grain samples were ground into powder using a high-speed multifunctional grinder (Huangdai, SUS 304) and then passed through a 60-mesh sieve. After that, the milled flours were placed in dark-brown bottles and subjected to storage at 40 °C for 0 (control), 3, 6, 9 and 12 weeks. After storage in each time set, the samples were stored at −20 °C before analysis. The moisture, protein (dry basis) and ash contents of the oat sample were 10.58 ± 0.04%, 12.59 ± 0.03%, and 1.68 ± 0.04%, respectively. In the BB sample, the values were 10.20 ± 0.02% moisture, 10.58 ± 0.10% protein, and 1.55 ± 0.04% ash, respectively. Finally, in the WB sample, the values were 8.80 ± 0.11% moisture, 9.97 ± 0.03% protein, and 1.45 ± 0.01% ash.

2.2. Chemical and reagents

Linoleic acid (purity > 99%), 4-nitrophenyl butyrate (purity > 98%) and 3,4,5-trihydroxy benzoic acid (purity > 99%) were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Guaiacol (purity > 99%), fatty acids and their methyl esters (C16-C18 and C18-unsaturated, Me esters, purity > 99%) were supplied by Macklin Biochemical Co., Ltd. (Shanghai, China). Folin-Ciocalteu reagent (purity > 98%) was obtained from Sigma Alderich (St. Louis, USA). The 1,1-diphenyl-2-picrylhydrazyl (DPPH) (purity > 98.5%) was purchased from Huaxia Chemical Reagent Co., Ltd. (Chengdu, China). Methyl nonadecanoate (purity > 98%) was purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). A commercial assay kit for malondialdehyde (MDA) measurement was purchased from Suzhou Grace Biotechnology Co., Ltd. (Jiangsu, China). The *n*-Hexane (HPLC Grade) was supplied by Tedia (Ohio, USA). All other reagents were of analytical reagent grade.

2.3. Analysis of enzyme activity in oat and barley

2.3.1. Analysis of lipase activity

Lipase activity was measured using 4-nitrophenyl butyrate as the substrate by a spectrophotometry method (Yang et al., 2017). The 4-nitrophenyl butyrate was dissolved in dimethyl sulfoxide to prepare a concentrated substrate solution of 100 mM and then stored at −20 °C. The buffer solution included 50 mM potassium phosphate buffer containing 0.1% Triton X-100 at pH 8. The 200 μL of concentrated substrate solution was added into a 10 mL buffer to prepare the measurement solution (2 mM substrate).

For the extraction of lipase, oat and barley flour samples (*ca.* 2 g) were mixed with 10 mL of distilled water (dH_2O) and vortexed thoroughly. The slurry was stood at room temperature for 30 min, and then centrifuged at 4 °C and 9000 × g for 10 min. The supernatant was collected as a crude enzyme extract. For a reaction, 100 μL of the enzyme extract was added to 900 μL of the substrate solution. The kinetic change in absorbance during 180 s at 405 nm was measured using a UV–visible spectrophotometer (Unico UV-2802, USA). The molar extinction coefficient value was 16.05 mM^{-1} cm^{-1}. Lipase activity was finally expressed as μmol min^{-1} g^{-1} flour. Each sample was measured in triplicate.

2.3.2. Analysis of LOX activity

LOX activity was determined using linoleic acid as a substrate (Yang et al., 2017). To prepare the stock solution, 70 mg of Tween 20 and equivalent linoleic acid were mixed with 4 mL of dH_2O using a vortex, and 275 μL of 1 M NaOH was added to the mixture and then volumed to 25 mL in a volumetric flask. Finally, a 10 mM substrate solution for LOX activity measurement was prepared by dilute the stock solution using dH_2O.

Extraction of the crude enzyme was the same as described in *Section 2.3.1*, except that 4 g of each flour sample was used for extraction. In the following experiment, for the oat LOX reaction, 800 μL of oat crude extract was added to the mixture containing 2 mL of buffer (0.1 M potassium phosphate buffer at pH 6) and 200 μL substrate solution. For the BB LOX reaction, 50 μL of BB crude extract was mixed with 2.75 mL of buffer and 200 μL of substrate solution. For the WB LOX analysis, 30 μL of enzyme extract was added to 2.77 mL of buffer and 200 μL of substrate solution. After the addition of the crude enzyme extract, the mixture was

vortexed, placed in a 30 °C water bath and reacted for 3 min. Then, the reaction was terminated by adding 1 mL of 0.3 M KOH. A UV visible spectrophotometer (Unico UV-2802, USA) was used to detect the absorbance value of the solution at 234 nm, and the molar extinction coefficient was 26,000 L/mol cm^{-1}. Finally, LOX activity was given as μmol min^{-1} g^{-1} flour. Each sample was measured in triplicate.

2.3.3. Analysis of POD activity

The POD activity of oat and barley was analysed using a UV–visible spectrophotometer (Unico UV-2802, USA) following the method described by Li et al. (2022) with some modifications. About 0.5 g of each flour sample was mixed with 10 mL of dH_2O using a stir bar. The mixture was allowed to stand for 30 min at room temperature and centrifuged at 9000 × *g* for 10 min at 4 °C. The supernatant was collected and used as a crude POD extract.

To determine enzyme activity, 250 μL of 1% (w/v) guaiacol, 250 μL of 1.5% (w/v) H_2O_2, 900 μL of 50 mM potassium phosphate buffer (pH 6) and 100 μL of crude enzyme solution were mixed. After that, the mixture was immediately placed into a UV–visible spectrophotometer (Unico UV-2802, USA) with a wavelength of 470 nm and the absorbance change was recorded within 3 min. POD activity was described as an increase of 0.01 (1 U) in absorbance per gram of samples within 1 min (U min^{-1} g^{-1} flour). Each sample was measured in triplicate.

2.4. Analysis of free fatty acid (FFA) content in oat and barley

The FFA content of oat and barley was measured according to Yang, Huang, Xing, Guo, and Zhu (2021) with slight modifications. About 2.5 g of each flour sample was added to 15 mL 95% ethanol, stirred and then shaken in a shaker at 250 rpm and 25 °C for 1 h. After that, the mixture was centrifuged at 8000 × *g* and 20 °C for 10 min. The supernatant was collected and titrated using 0.05 mM NaOH-95% ethanol as the titrant. Phenolphthalein was used as the indicator, and the titration was continued until solution turned pink and lasted for at least three seconds. The FFA content was calculated as shown in Equation (1) by mg NaOH/100 g.

$$FFA = \frac{8415 \times (V_1 - V_0) \times c}{m} \times \frac{100}{100 - \omega} \quad (1)$$

The V_1 and V_0 are volumes of the sample and blank titration (mL), respectively; c is the concentration of titrant (mol/L); m is the sample mass (g); and ω is the moisture content of the sample.

2.5. Analysis of extractable total phenolic content (TPC) in oat and barley

The extractable TPC in the samples was determined using a Folin-Ciocalteu spectrophotometric method described by Jogihalli, Singh, Kumar, and Sharanagat (2017). Each of the oat and barley flour samples (0.2 g) was dispersed in 4 mL HCl/methanol/water solution (1:80:10, vol:vol:vol) and then vibrated at 250 rpm and 25 °C for 2 h. After that, the mixture was centrifuged at 2934 × *g* and 20 °C for 15 min to collect the supernatant. Then, 200 μL of the supernatant was mixed with 2.5 mL of Folin-Ciocalteu reagent (0.2 mol/L) and reacted for 10 min. After that, 2 mL of 7.5% (w/v) sodium carbonate solution was added and reacted in the dark at room temperature for 1 h. For the quantification of TPC, gallic acid was used as the external standard with concentrations of 0, 10, 20, 30, 40, 50 and 60 mg/L to make the standard curves. The absorbance of the reactants was measured using a UV visible spectrophotometer (Unico UV-2802, USA) at 760 nm. The final extractable TPC content was expressed as gallic acid equivalent (mg GAE/g). Each sample was measured in triplicate.

2.6. Analysis of DPPH scavenging activity in oat and barley

The DPPH scavenging activity in oat and barley samples was analysed according the method described by Sharma and Gujral (2011). The flour sample (100 mg) in each group was mixed with 1 mL methanol and shaken in an oscillator at 250 rpm and 25 °C for 2 h, and then the mixture was centrifuged at 1650 × g and 20 °C for 15 min. Then, 100 μL of the supernatant was mixed to 6×10^{-5} M of 3.9 mL DPPH-methanol reagent and reacted in the dark at 25 °C for 1 h. The absorbance of the solution was measured at 515 nm using a UV–visible spectrophotometer (Unico UV-2802, USA). Finally, the final value of DPPH scavenging activity was expressed as shown in Equation (2).

$$\%Antioxidant\ activity = (1 - \frac{A\ of\ sample}{A\ of\ control}) \times 100 \quad (2)$$

A of sample is the absorbance of the sample; *A of control* is the absorbance of the blank sample.

2.7. Analysis of malondialdehyde (MDA) in oat and barley

The contents of MDA in oat and barley samples were measured using the assay kits following the manufacturer's instructions (Jia et al., 2022). Each sample was measured in triplicate.

2.8. Analysis of fatty acid composition in oat and barley

Lipids in the oat and barley samples were extracted using the method of Wang, Wang, Qiu, and Li (2020). About 0.5 g of the samples were mixed with 1 mL toluene (containing 5 mg methyl nonadecanoate) and 2 mL of sulfate methanol (1:99, vol:vol), and then the mixture was heated in a water bath at 50 °C for 12 h. After cooling to room temperature, 5 mL of saturated sodium chloride solution and equivalent *n*-hexane were subsequently added to the mixture and centrifuged for 5 min at 2934 × *g*. This process was repeated twice. After that, the *n*-hexane layer was collected and added to 4 mL of potassium bicarbonate solution (2%, w/v), and then the mixture was centrifuged for 5 min at 2934 × *g*. The upper supernatant was collected and dried with nitrogen to remove the solvents. The dried residue was redissolved by 1 mL *n*-hexane and filtered through a Teflon membrane (0.13 × 0.22 μm).

The fatty acid composition was analyzed using the method reported by Yang, Zhou, Xing, Guo, and Zhu (2022). Gas chromatography with a flame ionisation detector (GC-FID) (GC System-7890A, Agilent Technologies, USA) equipped with a DB-WAX (30 m × 250 μm × 0.25 μm) column (Agilent Technologies, California, USA) was used for detection. For lipid analysis, the injection volume was 1 μL, and nitrogen was used as a carrier gas with a purge flow of 3 mL/min and a split ratio of 80:1. The temperature of the front detector and back inlet were 250 °C. The initial temperature of the oven was set to 150 °C and the oven temperature was changed from 150 °C to 240 °C by an increase of 5 °C per minute, and maintained for 2 min after increasing to 190 °C. Then, the temperature was increased continuously and maintained for 10 min after increasing to 240 °C. Fatty acids, C16-C18 and C18-unsaturated methyl esters were used as standards for qualitative analysis, and methyl nonadecanoate was used as an internal standard for quantification. The fatty acid composition was calculated as mg/g flour. Each sample was measured in triplicate.

2.9. Analysis of volatile compounds in oat and barley

Lipid-derived volatile compounds in oat and barley after EBI treatment during storage was measured according to the method described by Heiniö, Lehtinen, Oksman-Caldentey, and Poutanen (2002). The headspace solid-phase microextraction gas chromatography-mass spectrometer (HS-SPME-GC–MS) (GC System 7890A-5975C, Agilent Technologies, USA) equipped with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 30/50 μm) SPME fibre (Sigma Aldrich, USA) and a DB-WAX (30 m × 250 μm × 0.25 μm) column (Agilent Technologies, California, USA) were used for analysis. About 0.5 g of oat and barley flour samples were placed in a sealed vial and

waited for 10 min, then the flour sample was stirred at 250 rpm for 30 min in a 60 °C headspace injector oven. Helium was used as the carrier gas; the flow rate was 0.8 mL/min and the temperature at the injection was 250 °C. The initial oven temperature was 60 °C, maintained for 4 min, and then rose to 90 °C at an increase of 5 °C per minute; after that, the temperature was increased to 240 °C at 10 °C per minute and held for 8 min before mass spectrometry analysis. The identification of volatile compounds was performed by matching their total ion mass spectra with the NIST (Version 2.0, Gaithersburg, MD, USA) and by calculating the linear retention index (LRI). The amounts of volatile compounds were quantitatively given by peak areas (counts * s * 10^6). Each sample was analysed in triplicate.

2.10. Statistical analysis

All the results were carried out by mean values ± standard deviations in triplicates. The data were performed using analysis of variance (ANOVA), followed by Tukey's honestly significant difference test via SPSS (IBM SPSS Statistics 26, USA). Differences between the averages were considered significant at a level of $p < 0.05$ for data analysis. Figures were drawn using Origin 2021 (Origin Lab Corporation, USA).

3. Results and discussion

3.1. Influence of electron beam irradiation (EBI) on lipid-degrading enzyme activity in oat and barley

As shown in Fig. 1, oat contained higher lipase activity than the BB and WB samples. Similarly, Decker et al. (2014) found that oat had higher lipase activity compared to other cereals. Lipase activity decreased in both oat and barley samples with prolonged storage, which decreased from 0.56 ± 0.03 to 0.07 ± 0.01 μmol min^{-1} g^{-1} flour, 0.09 ± 0.02 to 0.04 ± 0.01 μmol min^{-1} g^{-1} flour and 0.12 ± 0.00 to 0.05 ± 0.01 μmol min^{-1} g^{-1} flour in oat, BB and WB, respectively (Fig. 1). A reduction in lipase activity was also found after several weeks of storage in black bean noodles and oat noodles (Yang, Zhou, Xing, Guo, and Zhu, 2021; Yang et al., 2022). The decrease of enzyme activity could be due to the denaturation of enzymes during long-term storage. A significant difference ($p < 0.05$) was observed for oat lipase activity at different irradiation doses. Compared to the control sample, lipase activity was reduced by 6.65% and 23.33% in oat after EBI treatment at 2 kGy and 6 kGy, respectively (Fig. 1a). Luo et al. (2019) found that lipase activity decreased with EBI irradiation levels in brown and milled rice. Similarly, Zhai et al. (2022) found a slight increase of lipase activity in high-moisture rice after 1 kGy treatment by EBI, but the values decreased and fluctuated when the samples were irradiated at doses of 2–4 kGy. The reason may be that EBI produces more free radicals which can destroy the lipase structure of rice. However, EBI treatment did not greatly influence lipase activity in the BB and WB samples, as the activity remained constant compared to the unirradiated sample in each storage time set (Fig. 1b and Fig. 1c). The low inactivation ratios may indicate the little effect of EBI to lipase activity of barley, due to the irradiation dose was set up at utmost of 6 kGy and may be did not influence greatly of enzyme structure. Luo et al. (2021) also reported that EBI treatment up to 8 kGy did not greatly change the initial value of lipase activity in quinoa, but a dynamic change is found during storage, indicating that storage plays more important role than EBI treatment.

LOX activity was not detected in oat samples, the same as that reported by Yang et al. (2017), but the activity was measured in both the BB and WB samples (Fig. 2). A recent study also reported the presence of LOX activity in highland barley (Zheng et al., 2023). In that study, LOX activity (3 U) was detected, which is consistent with our research results.

Storage is a key factor that negatively influenced LOX activity in barley. For instance, the LOX activity in BB decreased from *ca.* 1.5 μmol min^{-1} g^{-1} flour to 0.3 μmol min^{-1} g^{-1} flour (Fig. 2a). The reduction in LOX activity was 60.01% from 0 to 3 weeks of BB, but it declined only

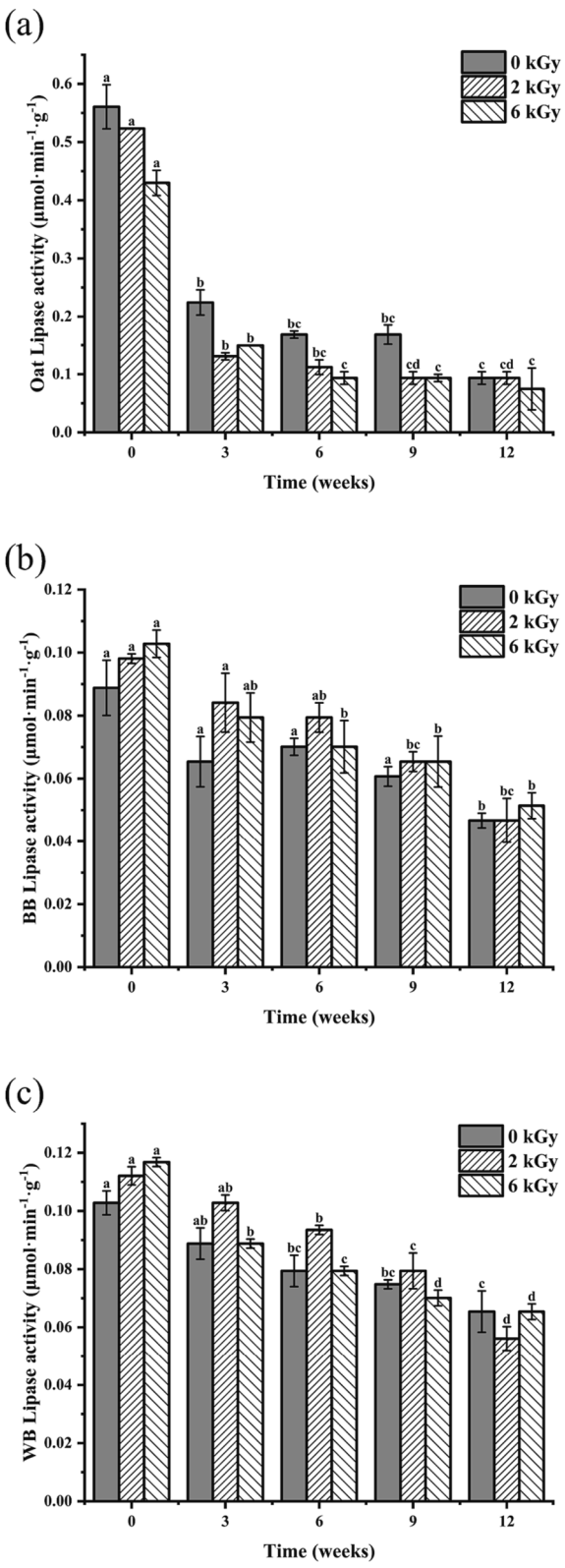

Fig. 1. Lipase activity in (a) oat, (b) blue barley (BB) and (c) white barley (WB) samples treated by electron beam irradiation (EBI) at different doses during storage at 40 °C. [a-d] Different letters indicate significant differences within each treatment condition during 12 weeks of storage as estimated by Tukey's test ($p < 0.05$).

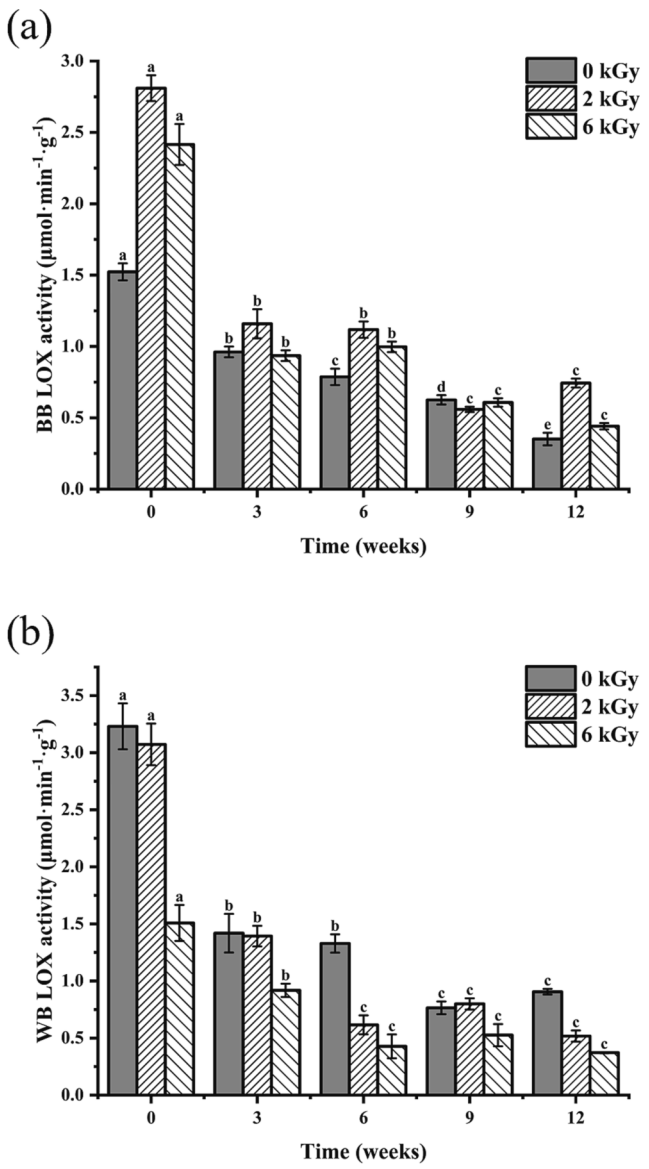

Fig. 2. Lipoxygenase (LOX) activity in (a) blue barley (BB) and (b) white barley (WB) samples treated by electron beam irradiation (EBI) at different doses during storage at 40 °C. [a-e] Different letters indicate significant differences within each treatment condition during 12 weeks of storage as estimated by Tukey's test ($p < 0.05$).

39.36% between 3 and 12 weeks. Like BB, LOX activity decreased rapidly during the initial storage period in WB, and the activity decreased by 76.83% after 12 weeks of storage. EBI also influenced the LOX activity of barley differently. The initial values of LOX activity in EBI-treated BB samples ranged around 2.4–2.8 μmol min^{-1} g^{-1} flour, higher than that of the control sample (Fig. 2a). However, it seemed that a high dose of EBI inhibited LOX activity in WB, as seen when compared with the non-irradiated sample, the activity decreased by 4.89% and 53.31% at doses of 2 kGy and 6 kGy, respectively (Fig. 2b). The reason for this difference might be due to the genotypes of BB and WB. According to Kumar et al. (2017), EBI can significantly reduce LOX isozymes' content in soy flour, which was attributed to the changes of LOX's protein structure. However, the same study also found that in one soy genotype of NRC107, the LOX-3 enzyme activity (443 ± 16 units/g) was higher after 4.8 kGy irradiation than the non-irradiated group (430 ± 16 units/g). Thus, the genotype and isozymes should be further investigated when studying the influence of EBI on LOX activity in cereals including barley.

POD activity was detected in both oat and barley (Fig. 3). Oat possessed much higher POD activity (*ca.* 4-fold) than barley of the BB and WB samples. The initial value of POD activity in oat was *ca.* 7.87 × 10^3 U·min^{-1} g^{-1} flour (Fig. 3a); however, a great decrease was found during storage and after EBI treatment. The activity of the control group decreased by 34.35% after 12 weeks of storage. After treatment with 2 kGy and 6 kGy, the activity declined by 23.05% and 18.64%, respectively. For the barley samples, storage seemed to play more important role than EBI in influencing POD activity. In both the BB and WB samples, after 12 weeks of storage, the POD activity in BB decreased by over 80% of its initial value (Fig. 3b). For WB, the POD activity accounted for only 76.22%, 61.77%, 51.26% and 47.95% of the original activity with prolonged storage (Fig. 3c). EBI treatment only slightly influenced POD activity in the initial stage of barley, but the reduction ratios were higher in the EBI groups than in the non-irradiated samples during storage. Thus, both storage and EBI factors should be considered when studying the activity of lipid-modifying enzymes in cereals.

3.2. Influence of EBI on free fatty acid (FFA) content of oat and barley flour during storage

The release of FFA is an important indicator for evaluating the storage quality of cereals, including oat and barley. As shown in Fig. 4, it was obvious that the contents of FFAs increased greatly in all oat and barley samples. The continuous accumulation of FFAs could be ascribed to the hydrolysis of triglycerides (TAGs) by lipase activity (Yang et al., 2017). Although lipase activity is reduced with prolonged storage, the residue activity should be sufficient to hydrolyse TAGs.

Oat had an initial FFA content of 169.15 ± 0.94 mg NaOH/100 g (Fig. 4a), but the value increased rapidly during the first 3 weeks of storage (reaching 357.14% of their original levels) due to high lipase activity, but the increase levelled off between 3 and 12 weeks. EBI treatment also had a significant influence on FFA in oat, where the highest accumulation of FFA was observed at 2 kGy but slightly reduced after 6 kGy irradiation. This might be because lipase activity was not greatly inhibited when irradiated at 2 kGy (Fig. 1a) but promoted the lipolysis of TAGs. However, 6 kGy irradiation condition not only reduce lipase activity but should have been enough to sterilise the microorganisms that were also responsible for the rise of fatty acid values (Zhai et al., 2022). Thus, research on the effect of EBI on microorganisms, as well as their impacts to lipids and off-flavours in cereals should be further studied.

Compared to oat, the initial contents of FFAs in the BB and WB samples were much smaller, with values of only *ca.* 53 mg NaOH/100 g and 45 mg NaOH/100 g (Fig. 4b and Fig. 4c), respectively. This was because both the lipase activity and lipid content in barley were less than those in oat (Fig. 1, Table S1a, Table S1b and Table S1c). The content of FFAs also increased during storage in barley cultivars. The increasing trend was slower compared to that of oat, with values of only 50% and 59% for BB and WB at 12 weeks, respectively. The effect after irradiation was less pronounced in BB, but the differences among irradiation doses varied for the WB group. FFAs in non-irradiated WB samples did not differ from those irradiated at 2 kGy, but a significant change occurred when the dose was increased to 6 kGy (Fig. 4c). It might be that irradiation at 6 kGy may reform lipid hydrolysis by generating free radicals to interact with lipids and even cause chain reactions that may amplify the radiation effect (Kiang, Fukumoto, & Gorbunov, 2012). Furthermore, irradiation with 6 kGy significantly reduced the level of FFAs in the BB group, while the values increased in WB group. This could be related to the influence of EBI to LOX activity in barley. It was seen that after 6 kGy irradiation, the initial value of LOX activity in WB group (*ca.* 1.5 μmol min^{-1} g^{-1}) was greatly lower than that of BB LOX (*ca.* 2.4 μmol min^{-1} g^{-1}), which may result in a higher oxidation ratio of polyunsaturated FFAs by BB LOX into hydroperoxides that were further degraded into other compounds.

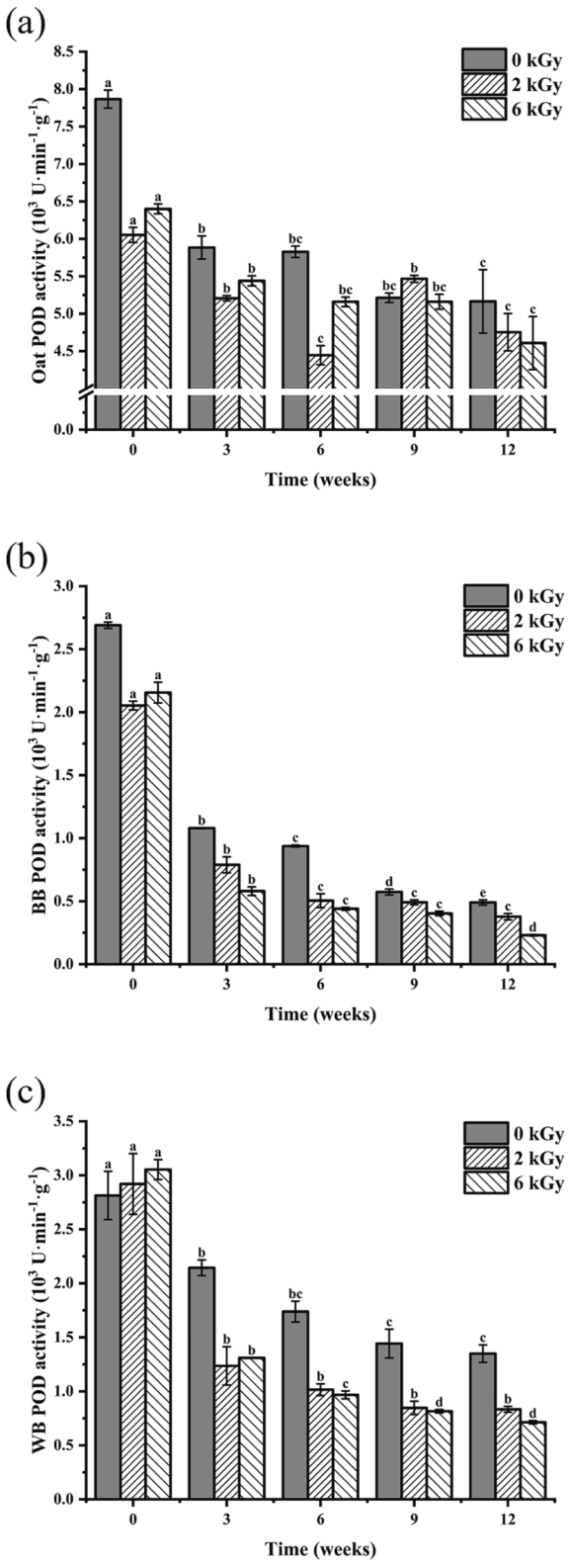

Fig. 3. Peroxidase (POD) activity in (a) oat, (b) blue barley (BB) and (c) white barley (WB) samples treated by electron beam irradiation (EBI) at different doses during storage at 40 °C. [a-e] Different letters indicate significant differences within each treatment condition during 12 weeks of storage as estimated by Tukey's test ($p < 0.05$).

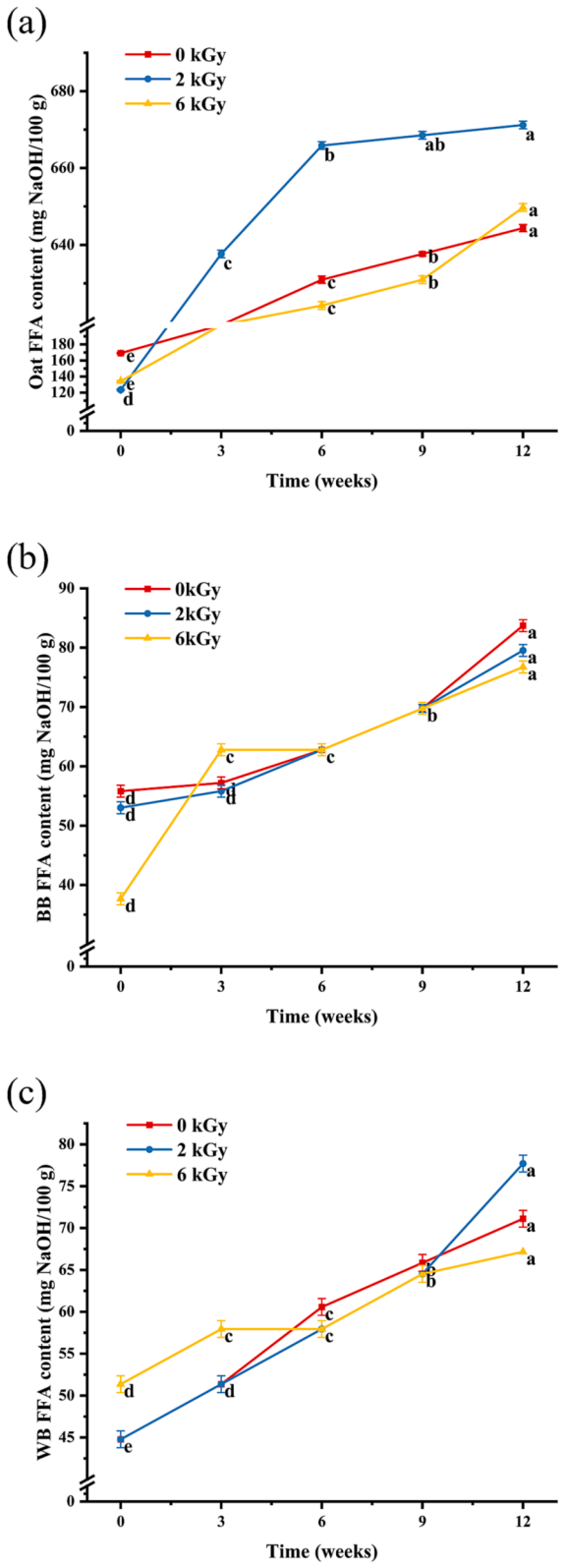

Fig. 4. Contents of free fatty acid (FFA) in (a) oat, (b) blue barley (BB) and (c) white barley (WB) samples treated by electron beam irradiation (EBI) at different doses during storage at 40 °C. [a-e] Different letters indicate significant differences within each treatment condition during 12 weeks of storage as estimated by Tukey's test ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Influence of EBI on lipid oxidation and antioxidant capacity in oat and barley during storage

Phenolics can clean up free radicals and retain antioxidant activity, which has a huge impact on grain storage. The extractable total phenolic content (TPCs) in the native oat and barley samples were similar, between 4.73 and 5.17 mg GAE/g (Fig. 5). Some studies also reported that the TPC content in barley was higher than that in oat (Sharma & Gujral, 2011; Soong, Tan, Leong, & Henry, 2014).

Storage seemed did not influence the TPCs in either the oat or barley samples, as it was seen that with the prolonging of storage, the change in total phenolics fluctuated but remained stable (Fig. 5). However, the influence of EBI on TPC in oat and barley was quite different. Compared to the initial value of TPC in oat (4.74 mg GAE/g), a significant loss was found after 2 kGy and 6 kGy irradiation, with values of 2.41% and 12.04%, respectively (Fig. 5a). However, a great increase in TPC was observed in the BB and WB samples after EBI treatment (Fig. 5b and Fig. 5c). A possible reason might be that a high dose of irradiation may release the phenolics from bound forms, but some complex food matrices (e.g., oat) could also influence their release and may even degrade during irradiation. Bhat, Wani, and Sultan (2023) also found a significant decrease in TPC at 2.5 kGy, but an increase at 5 kGy in brown rice flours by γ-ray irradiation. Zhu, Cai, Bao, and Corke (2010) reported that the effect of irradiation on total phenolic acids was irregular after irradiating rice at different doses.

The initial values of DPPH scavenging activity of barley were higher than that of oat, with the ranking of BB (38.91%) > WB (37.61%) > oat (21.50%) (Fig. 6). Choi, Jeong, and Lee (2007) found that the antioxidant activity in barley was between 60% and 70%, which was higher than that measured in this present study. Furthermore, storage was the main factor that decreased the DPPH scavenging activity in all oat and barley samples. Compared to the initial antioxidant capacity, the values of DPPH scavenging activity decreased by 24.45%, 22.51% and 7.35% after 12 weeks of storage in the control samples of oat, BB and WB, respectively. The possible reason could be due to the degradation of some antioxidant compounds (e.g., tocols, flavonoids, anthocyanins and carotenoids) during storage. For instance, the total amount of tocols decreased from 9.51 μg/g to 4.08 μg/g in dried oat noodles during 12 weeks of storage (Yang et al., 2021). Although EBI treatment did not greatly influence TPC during storage (Fig. 5), it influenced the contents of DPPH scavenging activity differently in oat and barley cultivars, as the initial values of DPPH scavenging activity decreased after irradiation in oat and BB but increased in WB. An increase in the DPPH-scavenging rate was observed in *Tartary* buckwheat flour (Huang et al., 2022) and brown whole rice flour (Bhat et al., 2023) after irradiation, which may be due to EBI treatment liberating the antioxidants from their bound forms and, thus, improving the DPPH-scavenging activity (Huang et al., 2022). However, our study further showed that the DPPH scavenging activity decreased during the long-term storage of oat and barley. This was in line with the results of malondialdehyde (MDA) contents, which were used to indicate lipid oxidation in oat and barley during storage. As seen in Fig. S1., the built up of MDA increased greatly after EBI treatment in both oat and barley samples, indicating the promotion of lipid oxidation by EBI, and the values raised up mildly during storage. According to Pi et al. (2022), free radicals generated by the water radiolysis may induce the crosslink, oxidation and degradation in food components such as proteins and lipids. Thus, lipid oxidation and antioxidant capacity after the EBI treatment should be considered when producing oat and barley foods during storage. Proper irradiation doses should be also used in terms of the food materials and purposes of EBI application.

3.4. Influence of EBI on fatty acid compositions of oat and barley during storage

Changes in lipids in oat and barley by EBI treatment during storage are shown in Table S1a, Table S1b and Table S1c. In general, oat

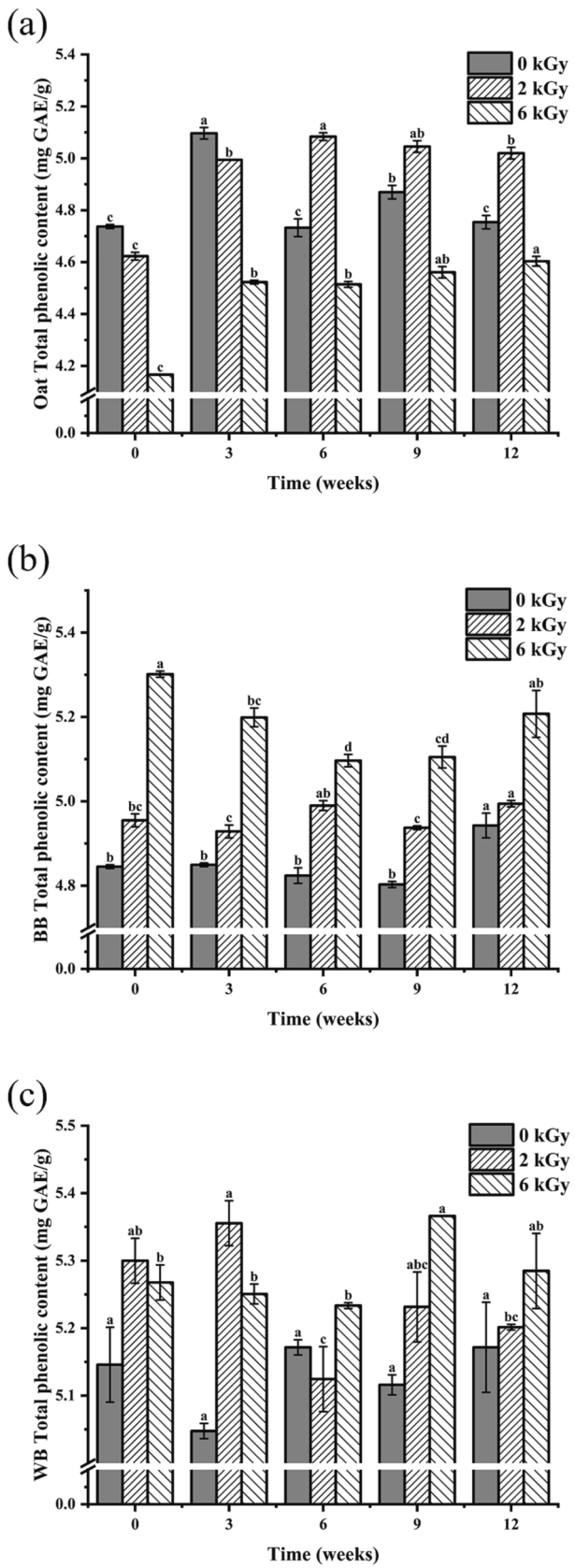

Fig. 5. Extractable total phenolic content (TPC) in (a) oat, (b) blue barley (BB) and (c) white barley (WB) samples treated by electron beam irradiation (EBI) at different doses during storage at 40 °C. [a-d] Different letters indicate significant differences within each treatment condition during 12 weeks of storage as estimated by Tukey's test ($p < 0.05$).

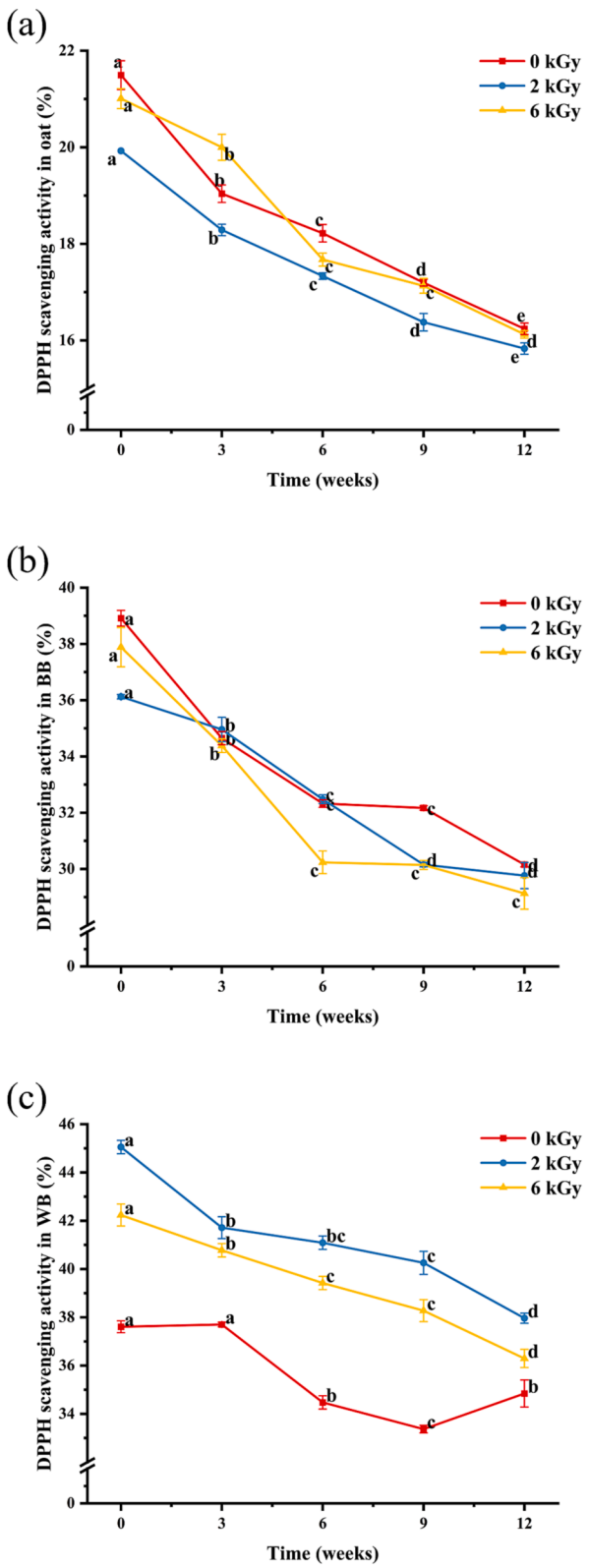

Fig. 6. DPPH scavenging activity in (a) oat, (b) blue barley (BB) and (c) white barley (WB) samples treated by electron beam irradiation (EBI) at different doses during storage at 40 °C. $^{a\text{-}e}$ Different letters indicate significant differences within each treatment condition during 12 weeks of storage as estimated by Tukey's test ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

contained higher lipids than barley, with palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) as the main fatty acids (Table S1a). The fatty acid content in oat ranged from 53.91 ± 0.04 to 59.27 ± 0.24 mg/g, in which the unsaturated fatty acids (UFAs) accounted for 80.34% of total fatty acids. Similarly, Lampi et al. (2015) reported that oat contains *ca.* 50–57 mg/g fatty acids measured by different extraction methods, with the majority being oleic acid and linoleic acid (*ca.* 20 mg/g flour each). The BB and WB samples had similar fatty acid contents, with the highest values being 19.84 ± 0.05 mg/g flour and 19.75 ± 0.04 mg/g flour, respectively (Table S1b and Table S1c). Wang et al. (2020) found that palmitic acid, oleic acid and linoleic acid were the main components of fatty acids in buckwheat, accounting for about 90% of the total fatty acids.

The content of total UFAs, especially linoleic acid, decreased after 12 weeks of storage in both oat and barley (Table S1a, Table S1b and Table S1c), which may be due to lipid oxidation by chemical and enzymatic reactions such as LOX (Liburdi et al., 2021). The amount of saturated fatty acids (SFAs) remained stable. Although LOX activity was not detected in oat in our present study, its activity has been found in other studies that should be able to oxidise the PUFAs and result in off-flavours (Yang et al., 2023). A reduction in lipids was also found in native oat flours, especially when the amount of TAGs decreased dramatically over 15 weeks of storage (Lampi et al., 2015).

When comparing the influence of EBI treatment on lipid profiles in oat and barley during storage, it seemed that EBI had no great influence. A study also reported that the lipid content did not significantly change after EBI treatment at doses of 1.79–14.97 kGy in yam flour (Wang et al., 2022). Thus, our study may also show that a relatively high irradiation dose of up to 6 kGy EBI treatment had little effect on changing the lipids in oat and barley. However, the effect of EBI on the content of FFAs and antioxidant capacity should be considered (Fig. 4 and Fig. 6), which are also essential factors that influence the process of lipid degradation and the formation of off-flavours.

3.5. *Influence of EBI on the formation of lipid-derived volatile compounds in oat and barley during storage*

Finally, the influence of EBI treatment in oat and barley on the formation of lipid-derived volatile compounds was studied. As shown in Table S2, 29 lipid-derived volatile compounds in the oat and barley samples were measured. The major volatile products were categorised as aldehydes, alkanes, furans and alcohols, which were mainly derived from UFAs that are considered as precursors for the production of these compounds through lipid oxidation.

The initial volatiles in BB were higher than in oat (Table S3a and Table S3b). With prolonged storage, the total amount of volatile compounds in non-irradiated oat increased over eight-folds than the initial value (from 500.87 counts * s * 10^6 to 4202.70 counts * s * 10^6) after 12 weeks of storage (Table S3a). However, the increase was only moderate in BB, with an increased ratio of less than two-folds (Table S3b) and even decreased in the WB group (Table S3c). The great increase in volatile compounds in oat could be attributed to its high lipid content, especially the UFAs (Table S1a). Similarly, a huge increase in volatile lipid oxidation products was found in non-heat-treated oat flour during storage (Yang, Piironen, & Lampi, 2019). Although LOX activity was not detected in our study, it was found in another study when preparing oat noodles by extrusion, indicating the important role of enzymatic reactions (Yang et al., 2021). It has been reported that faba bean LOX prefers to use FFAs as substrates over TAGs to promote formation of volatile compounds (Lampi, Yang, Mustonen, & Piironen, 2020), while the role of oat and barley LOX is still unclear. Thus, it may be speculated that the great release of FFAs enhanced the LOX-catalyzed lipid oxidation in oat, and the less significant production of volatiles in barley could be due to the lower lipid content and higher antioxidant capacity than that of oat. EBI treatment at a high dose (6 kGy) seemed to enhance the production of volatile lipid oxidation products in oat and barley during

the initial stage of storage. As seen in Table S3a, Table S3b and Table S3c, an irradiation dose of 2 kGy influenced little to the total volatile compounds in oat and barley, but the value doubled when treated at 6 kGy in oat. However, when comparing the production of volatile compounds by EBI treatment during storage, interestingly, the increase in these compounds was not as dramatic as that of the non-irradiated oat and barley samples. This may indicate that EBI did not enhance the lipid oxidation process in terms of lipid-derived volatiles during long-term storage.

Aldehydes were the most abundantly formed compounds in the control samples of oat and barley, with percentages of 27.14%, 42.33% and 42.60% in oat, BB and WB, respectively (Table S3a, Table S3b and Table S3c). Among the volatile compounds, aldehydes contributed more to the special odour of oat and had a low odor threshold (McGorrin, 2019). Among the aldehydes, hexanal was the major compound that increased dramatically during storage, especially in oat and BB (Table S3a and Table S3b). Hexanal has been also reported as the main volatile product in oat and barley (Yang et al., 2019; Zheng et al., 2023), which is derived from autooxidation or LOX-catalysed oxidation of linoleic acid that is usually used to indicate lipid oxidation (Feussner & Wasternack, 2002). Furthermore, the content of 1-nonanal also increased in oat by both EBI treatment and during storage, which may indicate the degradation of oleic acid, although its degradation was slower than PUFAs. Yang et al. (2021) also reported a great increase of 1-nonanal in oat noodles during storage. However, the build-up of 1-nonanal in barley was mild, which may be due to the low content of oleic acid.

The 2-pentylfuran is another major volatile product found in oat and barley that possesses a beany and green-leaf flavour (Hu, Lu, Guo, & Zhu, 2020). As shown in Table S3a, the build-up of 2-pentylfuran was extremely large in both the control and EBI-treated oat samples, with final values of 1987.57–2185.30 counts * s * 10^6, which were *ca.* 27–52 folds higher than the initial values. The formation of 2-pentylfuran is suggested to be related to the LOX-pathway in oat (Lampi et al., 2015). Although the activity of LOX was low, its activity should have been enough to catalyse the oxidation of PUFAs (e.g., linoleic acid). A dramatic increase in 2-pentylfuran was also observed in the barley samples, but the amount was not as large because of barley's lower lipid content compared to oat.

Other compounds were also formed during the storage of oat and barley, including alcohols, ketones and hydrocarbons (Table S3a, Table S3b and Table S3c). Although the contents of these compounds were not huge compared to aldehydes and furans, they may also contribute to off-flavours in oat and barley since some compounds possess quite low thresholds, such as 1-octen-3-ol, 1-hexanol and 1-nonanol (Hu et al., 2020). Alcohols are secondary lipid oxidation products that are usually formed from aldehydes by alcohol dehydrogenase. In line with the formation of 1-hexanal, 1-hexanol was the major product among the alcohols in oat and barley. Storage seemed to influence the formation of volatile compounds more than that of EBI since most of the contents of these products did not dramatically change after EBI treatment during storage compared to the control groups. Furthermore, it is worth noting that the built up of undesirable off-flavour is a synthetical combination of the volatile compounds rather than one or two (Hu et al., 2020; McGorrin, 2019). Thus, sensory evaluation is necessary to investigate the influence of EBI to oat and barley foods in future.

4. Conclusions

High activity of lipid-degrading enzymes was found in oat and barley, which played crucial roles in lipid degradation and the formation of undesirable off-flavours due to high lipid content, especially UFAs. Oat possessed higher lipase and POD activity than barley, but LOX activity was not found in oat, indicating that the degradation of lipids may occur via different pathways. FFAs were present at the initial stage of storage in both oat and barley, and the values in oat were high. This may show that lipid hydrolysis started under the function of lipase when oat and barley were milled into flours. Although LOX activity in oat was low, its potential activity should be enough to produce lipid oxidation products. Storage was a key factor that greatly influenced changes of lipids and lipid oxidation because the total UFAs decreased and volatile compounds were dramatically formed during storage in all the oat and barley sampling groups. Although the contents of extractable TPCs fluctuated, a great decrease of DPPH scavenging activity and raising of MDA values was found in oat and barley during storage, showing a dramatic reduction of antioxidant capacity in these food products. Furthermore, it seemed that EBI treatment could inactivate some of the enzyme activities, such as oat lipase and POD, and antioxidant capacity was also affected. However, EBI treatment changed little of lipase activity but more of LOX activity during initial stage of storage in barley. The genotype and isozyme factors should be further considered when studying the influence of EBI on LOX in barley. EBI did not greatly change the lipid profile and volatile compounds after storage, although it may have promoted lipid oxidation in oat and barley as the MDA values increased after EBI treatment. Overall, this study showed that as a non-thermal technique, EBI has high potential for use as an alternative method in stabilising the cereals' storage quality.

CRediT authorship contribution statement

Yue Tang: Writing – original draft, Writing – review & editing. **Lei Xu:** Writing – review & editing. **Zhiyang Yu:** Writing – review & editing. **Sufen Zhang:** Writing – review & editing. **Enguang Nie:** Writing – review & editing. **Haiyan Wang:** Writing – review & editing. **Zhen Yang:** Conceptualization, Writing – original draft, Writing – review & editing, Project administration.

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 data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100904.

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