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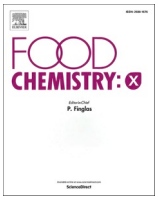

Biochemistry behind firmness retention of jujube fruit by combined treatment of acidic electrolyzed water and high-voltage electrostatic field

Xiaojie Chang [a,c,d], Yueguang Liang [b], Fei Shi [b], Tianjing Guo [b], Yu Wang [a,b,*]

[a] College of Horticulture, Shanxi Agricultural University, Taigu 030800, China
[b] College of Food Science and Engineering, Shanxi Agricultural University, Taigu 030800, China
[c] Life Sciences Department, Yuncheng University, Yuncheng 044000, China
[d] Shanxi Center of Technology Innovation for High Value Added echelon Utilization of Premium Agro-Products, Yuncheng University, Yuncheng 044000, China

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Glutathione (PubChem CID: 124886)
Trolox (PubChem CID: 40634)
Gallic acid (PubChem CID: 370)
Rutin (PubChem CID: 5280805)
Galacturonic acid (PubChem CID: 439215)
p-Nitrophenol (PubChem CID: 980)
Glucose (PubChem CID:107526)

ABSTRACT

Harvested jujube (*Zizyphus jujuba* Mill) is prone to softening due to active metabolism. This study investigated the effects of acidic electrolyzed water (AEW), high-voltage electrostatic field (HVEF) and their combination (AEW + HVEF) on softening and associated cell wall degrading enzymes (CWDEs), cell membrane integrity and antioxidant system of 'Huping' jujube during storage at 0 ± 1 °C. The results indicated that fruit subjected to AEW + HVEF, AEW or HVEF treatments maintained firmness 15.7%, 10.7%, and 5.3% higher than that of untreated control fruit at the end of 90 days cool storage. Fruit treated with AEW + HVEF could better maintain cell membrane integrity and exhibit lower activities of CWDEs and higher antioxidant capacity than that treated with either AEW or HVEF. Correlation analysis suggested that inhibition of softening was associated with reduction of CWDEs activities, and maintenance of membrane integrity and antioxidant system.

Introduction

Huping jujube (*Zizyphus jujuba* Mill. cv. Huping), growing as the primary cultivar in Jinzhong city, Shanxi province of China, is very popular for its thin peel, crisp texture, sweet and juicy taste and abundant nutrients (Zhang et al., 2023). However, due to various metabolic activities and physiological disorders after harvest, fresh jujube is prone to deteriorating quickly and showing undesirable symptoms such as softening, peel reddening and shrinkage, which seriously destroy the sensory quality and nutritional value, leading to food waste and environmental pollution (Jia, Li, Liu, & He, 2023; Zhang et al., 2023). Numerous approaches have been applied to maintain fruit quality and prolong the storage life. Nevertheless, due to concerns about potential environmental pollution and safety issues associated with chemical preservation, physical and biological preservation technologies with higher safety margins have gained more attention for fruit storage recently (Islam, Acıkalın, Ozturk, Aglar, & Kaiser, 2022; Jia, Li, Liu, & He, 2022; Lv et al., 2022; Zhang et al., 2023). The reported physical preservation methods include ultraviolet (UV-C) (Jia et al., 2023), low temperature (Sang, Yang, et al., 2022), hydrogen sulfide (Lv et al., 2022) and nitric oxide fumigations (Zhao, Zhu, Hou, Wang, & Li, 2019). The biological methods include glycine betaine (GB) (Zhang et al., 2023), salicylic acid (Yang, Kang, Liu, Guo, & Chen, 2022), melatonin (Tang et al., 2020) and so on. However, the above-mentioned methods have considerable drawbacks such as poor application effect, low degree of commercialization, high cost and inconvenient operation (Jia et al., 2022; Sang, Yang, et al., 2022; Yang et al., 2022). Therefore, it's urgently required to develop simple, eco-friendly, low-cost, and highly effective prevention methods for improving storability of jujube.

Acidic electrolyzed water (AEW) can be conveniently produced by

* Corresponding author at: College of Horticulture, Shanxi Agricultural University, Taigu 030800, China.
E-mail address: sxnydxwy@163.com (Y. Wang).

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electrolysis of dilute sodium chloride or hydrochloric acid solution in an electrolytic device with a diaphragm (Tang, Chen, Lin, Hung, Xie, & Chen, 2021; Wu et al., 2022; Wanli Zhang, Cao, & Jiang, 2021). It has unique physical and chemical properties, such as an oxidation–reduction potential (ORP) greater than 1100 mV, a pH range of 2–3.5, and an available chlorine concentration (ACC) exceeding 5 mg/L (Jia et al., 2022). The high ORP, low pH and high ACC of AEW play a synergistic effect on its antimicrobial efficiency by altering the cell membrane structure of pathogens (Li, Yue, Xu, Tian, Zhao, & Xu, 2020). Additionally, AEW could suppress the disease development, preserve the structural integrity of cellular membrane, improve quality properties and increase commercial acceptability in longan (Li et al., 2023; Tang et al., 2021), blueberry (Chen, Hung, Chen, & Lin, 2017; Chen, Hung, Chen, Lin, & Lin, 2019) and 'Lingwu long' jujube (Jia et al., 2022). Furthermore, AEW can be easily converted back to ordinary water when it comes into contact with organic matters or is diluted with tap water, posing no threat to the environment or human health (Wanli Zhang et al., 2021). Thus, the AEW treatment is considered an environment-friendly, highly efficient, convenient and low-cost approach for preserving postharvest fruit by improving their storability and quality properties (Jia et al., 2022; Li et al., 2023).

HVEF is a non-thermal, highly efficient, low energy consumption, no vestiges and low-cost physical preservation technology (Huang et al., 2022; Liu, Chen, Chang, Li, Lu, & Hsieh, 2017). It's considered that the external HVEF can affect the inherent electric field inside fruits and vegetables (Zhang, Zhang, Law, & Guo, 2022), and ionize the air to produce unstable ozone, negative air ions and other active substances, which can eliminate ethylene, reduce the stomatal opening on the epidermis, impede the normal sugar metabolism of fruits and vegetables, and have excellent antibacterial or bactericidal effects (Liu et al., 2017; Lotfi, Hamdami, Dalvi-Isfahan, & Fallah-Joshaqani, 2022; Yan et al., 2020; Zhang et al., 2022). In addition, HVEF treatment could regulate the antioxidant system and other metabolism pathways to maintain better storage quality in A. *bisporus* (Yan et al., 2020), fresh-cut broccoli (Kao, Tu, Sridhar, & Tsai, 2019), pomegranate (Lotfi et al., 2022), persimmon (Liu et al., 2017), pakchoi (Zhang et al., 2022), fresh-cut cabbage and baby corn (Huang, Yang, Sridhar, & Tsai, 2021).

The aforementioned findings indicated that postharvest treatments with AEW and HVEF can help to actively regulate physiological processes and maintain postharvest quality of fruits and vegetables. To our knowledge, there have been no studies on the effects of single or combined treatment with AEW and HVEF on the firmness-related properties and potential mechanism of Huping jujube. Preliminary experiments showed that the white mature Huping jujube treated with AEW (pH of 2.8, ORP of 1550 ± 5 mV, ACC of 90 mg/L, 10 min) and HVEF (-2kV/cm, 3 h) maintained good sensory quality. Accordingly, AEW (pH of 2.8, ORP of 1550 ± 5 mV, ACC of 90 mg/L, 10 min) and HVEF (-2kV/cm, 3 h) were selected as the final treatment conditions. We hypothesized that the applications of AEW, HVEF and AEW + HVEF could maintain the firmness of Huping jujube fruit and enhance the antioxidant capacity, thereby retarding senescence and quality deterioration. This study aimed to explore the impacts of AEW, HVEF and AEW + HVEF treatments on firmness, the related indices of cell membrane integrity, CWDEs activities and antioxidant metabolism to provide evidence for extending the storage life of Huping jujube fruit at 0 ± 1 °C. Results were expected to pave the way for AEW + HVEF treatment to improve storage quality of Huping jujube, and provide a theoretical foundation for further investigation into its mechanism.

Materials and methods

HVEF treatment system

The HVEF experimental system adopted in this study was designed by the laboratory team, which consisted of a high voltage generator (DW-N303-1ACF 7, Dongwen High Voltage Power, Tianjin, China) with output −30–0 kV and output current 1 mA, two parallel rectangular stainless electrode plates used as cathode or anode, treatment chamber, one voltmeter and one amperometer. Different electric field strengths can be generated by adjusting the distance between the cathode and the anode as well as the output voltage. In this experiment, the output voltage was set to − 30 kV and the distance between the cathode and the anode was adjusted to 15 cm, so that the field intensity of − 2 kV/cm could be generated. The jujubes were placed horizontally, without overlapping. The schematic diagram of HVEF system was shown in Fig. 1.

AEW preparation

AEW was produced by electrolyzing 1‰ NaCl solution in an electrolyzed water generator (XYS-C-12, Xin-yu Optical Electromechanical Co., Ltd., Baoji, China). According to the preliminary experiment results, appropriate AEW treatment conditions were selected: pH of 2.8, ORP of 1550 ± 5 mV, ACC of 90 mg/L. The pH and ORP were determined using the pH meter (pH-208, Fu-an-pu-he Electronics Co., ltd., Fujian, China) and ORP meter (ORP986, Fu-an-pu-he Electronics Co., ltd., Fujian, China), respectively. The ACC of SAEW was quantified by iodometry.

Materials and treatments

Huping jujubes were hand-harvested at the white mature stage (picking date: 2022.8.17; maturity stage: 80 days after flowering) from an orchard in Xiaobai Township, Taigu District, Jinzhong City, Shanxi Province of China and immediately transported to the Fruits and Vegetables Storage and Preservation Laboratory located in Shanxi Agricultural University within 3 h. Fruits were pre-cooled at 4 °C for 24 h, then selected with uniform appearance and without flaws and injuries, finally stored at 0 ± 1 °C (relative humidity: 85–95 %) for 90 days. All jujubes were randomly separated into five groups, with three biological replicates per group and 350 fruits per replicate.

Five groups were treated and tagged as follows: (1) CK group (fruits were untreated), (2) DW group (fruits were soaked in 15L distilled water for 10 min while stirring constantly, and then air- dried for 2 h at room temperature), (3) AEW group (fruits were immersed in 15L AEW for 10 min while stirring constantly, and then air-dried for 2 h at room temperature.), (4) HVEF group (fruits were treated with −2kV/cm HVEF for 3 h), (5) AEW + HVEF group (fruits were soaked in 15L AEW for 10 min while stirring constantly, air-dried for 2 h at room temperature and then treated with −2kV/cm HVEF for 3 h). Subsequently, they were packed into perforated polyethylene bags (50 jujubes per bag) and stored at 0 ± 1 °C (relative humidity: 85–95 %). Ninety jujubes (30 jujubes × 3 replicates) were randomly chosen from different groups at 15 d interval for the analysis of physiochemical quality attributes (firmness, electrolyte leakage rate), and the remaining sixty samples (20 jujubes × 3 replicates) were pitted, frozen by liquid nitrogen, and then placed at − 80 °C for subsequent determination of indicators related to antioxidant system, lipoxygenase (LOX) and cell wall degrading enzymes (CWDEs) activities, as well as the contents of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2). All analyses were performed in triplicate.

Measurement of fruit firmness

Fruit firmness was measured using a texture analyzer (Serial number 07–1063-08, Food Technology Corporation, USA) with a 2.0 mm diameter stainless probe and insertion depth of 5 mm following the procedure described by Ge et al. (2020) with slight modifications. Pre-test, test and post-test speeds were 1.5 mm/s, 1.5 mm/s, and 2.5 mm/s, respectively. Ten peeled jujubes were randomly selected from each replication at each sampling date, and the firmness was measured at two relative points on the equatorial position of each fruit. The maximum force was recorded as the firmness (N).

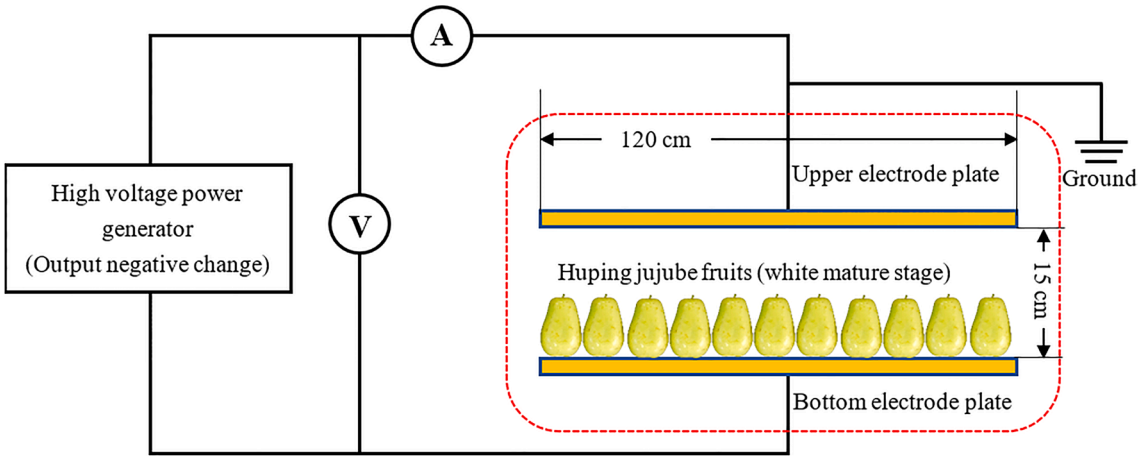

Fig. 1. Schematic diagram of high voltage electrostatic field (HVEF) treatment.

The activities of CWDEs

Ten grams of frozen pulp tissue from 20 jujubes were used as samples to determine the activities of CWDEs, including polygalacturonase (PG), cellulase (Cel), β-glucosidase (β-Glu) and β-galactosidase (β-gal). PG, Cel, β-Glu and β-gal were extracted and measured according to the methods by Zhao et al. (2019), Zhang et al. (2023) and Ge et al. (2020). The results were described as U. The amount that catalyzes the formation of 1 mg galacturonic acid, reducing sugar, glucose and 1 mmol p-nitrophenol per hour per g of fresh weight was a unit of PG, Cel, β-Glu and β-gal activities, respectively.

Electrolyte leakage (EL) rate, LOX, MDA and H_2O_2

EL rate was measured by a DDS-307A conductivity meter (Yidian Scientific Instruments Co., Ltd., Shanghai, China) following the method of Wu et al. (2017). LOX activity in 10 g of frozen jujube tissue from 20 jujubes were determined following the method of Wang et al. (2022). One LOX activity unit (U) was defined as a change of 0.1 in absorbance per min per 100 mg of fresh weight at 234 nm. MDA and H_2O_2 contents in 5 g of frozen pulp tissue from 20 jujubes were measured according to the methods of Zhang et al. (2023) and expressed as μmol·Kg^{-1} as well as μmol·g^{-1}, respectively.

Antioxidant enzyme activities

Ten grams of frozen pulp tissue from 20 jujubes were homogenized in different buffers to extract superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD), respectively. Specifically, SOD, CAT, APX and POD were extracted and determined according to previous studies (Tang et al., 2020; Wang, Chen, Zhao, Wu, Kou, & Xue, 2022).

One U of SOD activity was defined as the amount of enzyme that would inhibit 50% photoreduction of nitroblue tetrazolium per minute per g of fresh weight. One U of CAT, APX and POD activities was defined as a change of 0.01 in absorbance per min per g of fresh weight at 240, 290 and 470 nm, respectively.

The contents of non-enzyme antioxidants

As mentioned by Jia et al. (2023), ascorbic acid (AsA) content was determined by 2, 6-dichlor-ophenolindophenol titration and expressed as mg·100 g^{-1}.

The extraction and determination of glutathione (GSH) were conducted following the method of Tang et al. (2020) and the content was represented as μmol·g^{-1}.

The total phenolics (TP) and total flavonoids (TF) were investigated following the methods of a previous research (Lv et al., 2022) using 5 g of frozen pulp tissue from 20 jujubes. The results were stated as mg gallic acid equivalents (GAE)·100 g^{-1} and mg rutin equivalents (RE)·100 g^{-1}, respectively.

Antioxidant activity

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging capacity and Ferric Ions (Fe^{+3}) Reducing Antioxidant Power (FRAP) were assessed employed the method described by Islam et al. (2022) and the results were expressed as mmol Trolox equivalent (TE)·100 g^{-1}.

Statistical analysis

All measurements were conducted in triplicate. The data were processed using SPSS v. 24.0 (IBM Corp., Armonk, NY, USA) software following one-way analysis of variance and the results were presented as mean ± standard deviation (n = 3). Significant differences were detected using Duncan's test ($p < 0.05$) with a confidence interval of 95 % and correlations among various indicators were determined using Pearson's correlation test. Origin 2021 software (OriginLab Corporation, Northampton, MA, USA) was used to generate plots.

Results and discussions

Changes in the firmness

Firmness reduction is a characteristic of harvested fruit during ripening, which affects the postharvest life and market value (Ge, Zhang, Li, Xue, Zhang, & Lv, 2020). Thus, firmness is used as an important indicator to assess the storage quality of fruit (Zhang, Kang, Yang, Guo, Guo, & Chen, 2022). As depicted in Fig. 2, the firmness of all specimens displayed a downward trend during storage. The firmness of CK and DW groups showed little difference at the same sampling time point but was markedly lower than that of other three groups. Meanwhile, the firmness of AEW + HVEF group was notably higher than that of other groups. From day 60, the firmness of AEW group exhibited a significant increase compared to that of HVEF group. By the end of storage, the firmness of AEW + HVEF group was 4.6% and 9.9% higher than that of AEW and HVEF groups, respectively, while that of AEW group was 9.4% greater than that of DW group and that of HVEF was 5.3% higher than that of CK group. Similarly, the 'Lingwu Long' jujube treated with AEW (ACC of 60 mg/L, pH of 2.2 and ORP of 1177 ± 5 mV) presented prominently higher firmness and lower disease index than the control group (Jia et al.,

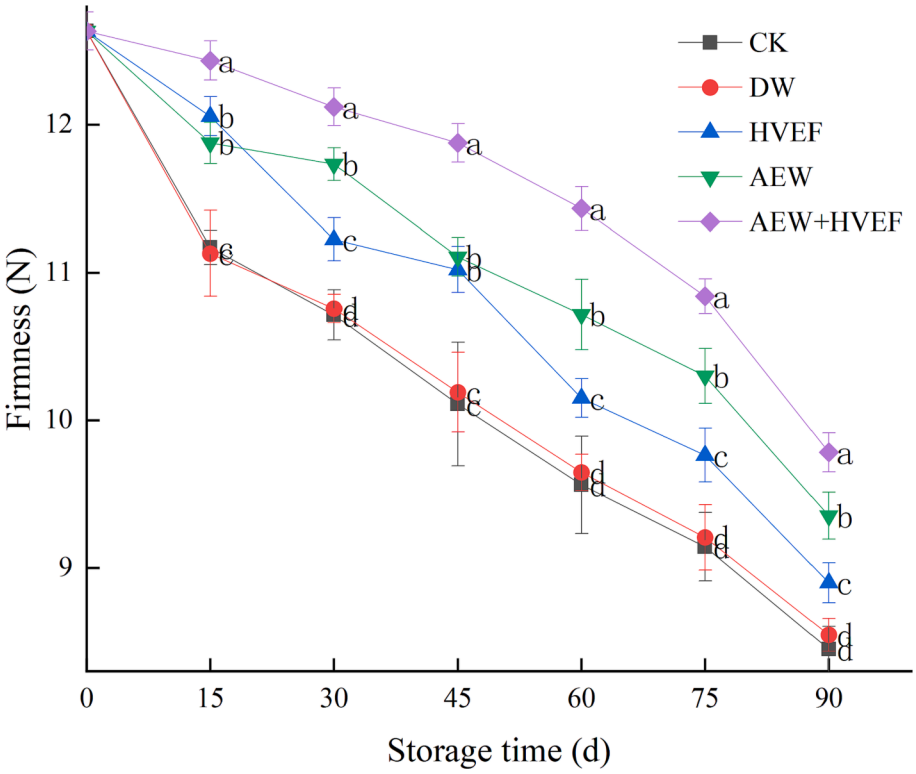

Fig. 2. Effects of HVEF, AEW, and AEW + HVEF treatments on the firmness of Huping jujube during storage at 0 ± 1 °C for 90 days. The data presented are the mean values of three replicates; vertical bars represent the standard deviation of the mean values; values followed by different superscripts (a-d) are significantly different ($P < 0.05$) on the same sampling date.

2022). Additionally, cherry tomatoes treated with HVEF (150 kV/m, 2 h) also showed significantly higher hardness than the control samples (Zhao, Li, Gao, Wang, Li, & Xiong, 2023). It also has been reported that fresh-cut cabbage and baby corn treated with modified atmosphere packaging (MAP) + HVEF maintained better hardness (Huang et al., 2021). The aforementioned findings collectively demonstrated that AEW and HVEF treatments were beneficial for improving the firmness of fruits and vegetables. Moreover, our results suggested that compared to individual applications of AEW and HVEF, AEW + HVEF treatment could more effectively delay the declining of fruit firmness.

Changes in the activities of CWDEs

The maintenance of fruit firmness has been found to correlate with the inhibition of CWDEs (Tang et al., 2020). CWDEs, including PG, Cel, β-Glu and β-gal, can break down pectin molecules in the cell wall, leading to tissue softening (Chen et al., 2017). In Fig. 3, the activities of PG, β-gal, Cel and β-Glu exhibited an overall upward trend. However, the activities of Cel and β-Glu slightly decreased only on day 45, while the activities of PG and β-gal decreased only on day 60. The four enzyme activities between CK and DW groups were almost identical but remarkably higher than those of other groups at the same storage period. There were no discernible differences in the activities of PG, Cel and β-Glu between AEW and HVEF groups during the early stages of storage. However, the activities of these four enzymes were observably higher in HVEF group than in AEW group during later stages. Additionally, the activities of CWDEs in AEW + HVEF group remained consistently lower than those in other groups throughout the storage. It has been proven that the ethylene content in fruit is associated with the activities of CWDEs, while the chlorine in AEW could reduce ethylene production by directly inhibiting ethylene synthesis genes and HVEF is also conducive to the removal of ethylene (Weiqing, Jie, Jianfeng, & Weishuo, 2011; Wanli Zhang et al., 2021). This may explain our findings that the AEW, HVEF and AEW + HVEF treatments can inhibit the activities of CWDEs.

PG participates in the degradation of pectin by catalyzing the hydrolysis of glycoside bonds of polygalacturonic acid, which affects the integrity of the middle lamella of cell wall, damages the cell structure and finally leads to fruit softening and senescence (Fan et al., 2019; Ge et al., 2020). In Fig. 3A, on day 90, the PG activity of AEW + HVEF group was 11.3% and 14.5% lower than that of AEW and HVEF groups, respectively. The PG activity of AEW group was 8.9% lower than that of DW group, while that of HVEF group exhibited a reduction of 4.8% compared to CK group. Similar results were noted in low voltage electrostatic field (LVEF) application on strawberries, which could impede protopectin degradation into water-soluble pectin by maintaining the low levels of PG and Cel activities throughout storage (Xu, Zhang, Liang, Fu, Wang, & Jiang, 2022).

β-Gal, as a glycosyl hydrolase, hydrolyzes β-1, 4-galactose bond to destroy cell wall structure by removing galactosyl residues from pectin side chains, thus accelerating fruit softening (Jia et al., 2022). Fig. 3B demonstrated that AEW + HVEF treatment decreased the β-gal activities significantly, which was 9.6% and 13.8% lower than that in AEW and HVEF groups at the end of storage, respectively. The β-gal activity was 11.0% lower in AEW group than in DW group while HVEF group exhibited 7.3% lower compared to CK group on day 90.

Cel, as a multi-enzyme system composed of endoglucanase, *exo*-glucanase and glucosidase, can degrade cellulose and xyloglucan and damage cell wall structure, thereby leading to fruit softening. Additionally, β-Glu, as a member of the cellulase system, plays an important role in cell wall degradation (Chen et al., 2017; Ge et al., 2020). On day 90, the Cel activity in AEW + HVEF group was 9.7% and 17.7% lower than that in AEW and HVEF groups, respectively, while Cel activity in AEW group was 14.7% lower than that in DW group and that in HVEF group was 7.4% lower than that in CK group (Fig. 3C). Furthermore, β-Glu activity in AEW + HVEF group was 4.4% and 8.4% lower than that in AEW and HVEF groups at the end of storage, respectively. Meanwhile,

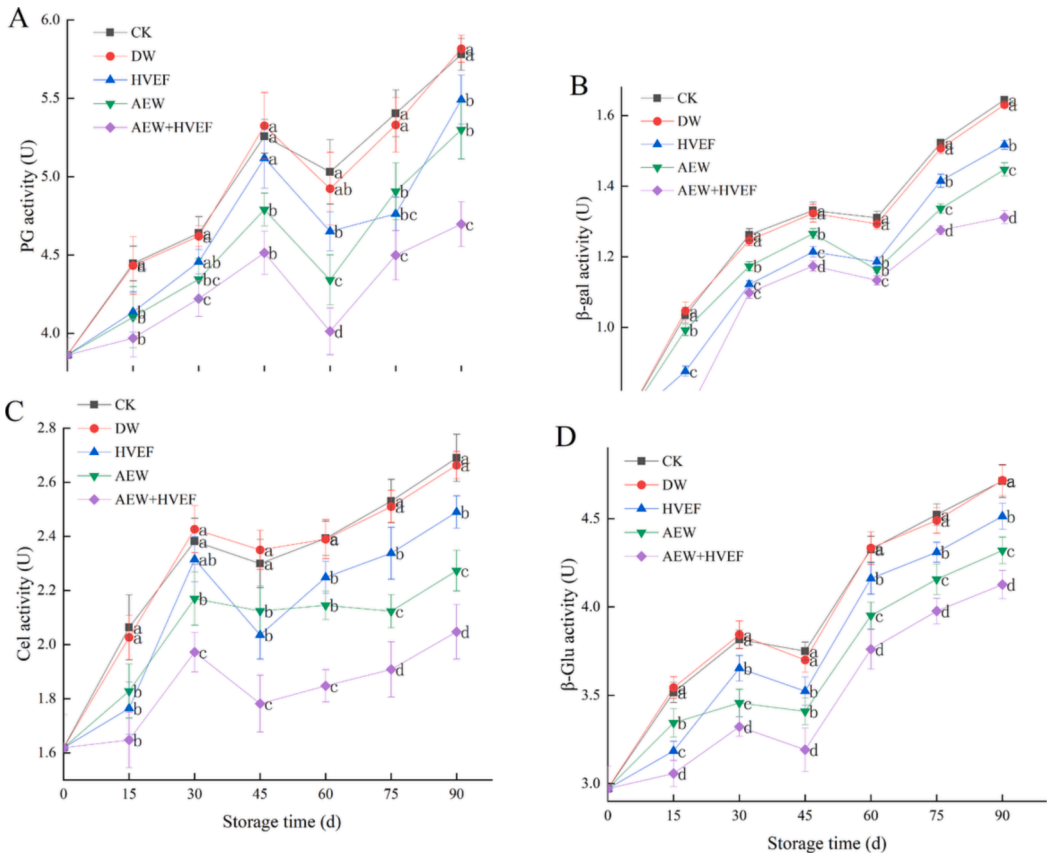

Fig. 3. Effects of HVEF, AEW, and AEW + HVEF treatments on PG (A), β-gal (B), Cel (C) and β-Glu (D) activities of Huping jujube during storage at 0 ± 1 °C for 90 days. The data presented are the mean values of three replicates; vertical bars represent the standard deviation of the mean values; values followed by different superscripts (a-d) are significantly different ($P < 0.05$) on the same sampling date.

the β-Glu activity in AEW group was 8.5% lower than that in DW group and that in HVEF group was 4.2% lower than in CK group on day 90 (Fig. 3D). A previous study reported that AEW (ACC of 48 mg/L, pH of 2.8) could retard the softening process of blueberry via deactivating the activities of CWDEs (such as PG, Cel and β-gal) (Chen et al., 2017).The results in 'Lingwu Long' jujube illustrated that AEW treatment could keep lower activities of PG, β-gal, and Cel than the control group at the end of storage (Jia et al., 2022). Additionally, other treatments, such as GB and UV-C, have been used to delay the softening of postharvest jujube by restraining the activities of PG, β-gal, Cel and β-Glu and related gene expressions (Jia et al., 2023; Zhang et al., 2023). These findings were consistent with our results.

Taken together, it could be considered that the AEW + HVEF treatment exerted a more pronounced inhibitory effect on CWDEs than either treatment alone, thereby retarding fruit softening, which was consistent with the finding presented in Fig. 2.

Changes in EL rate, LOX activity and the contents of MDA and H_2O_2

The cell membrane integrity is related to lipid peroxidation metabolism and reactive oxygen species (ROS) accumulation (Wang et al., 2022). LOX specifically catalyzes the conversion of membrane lipid unsaturated fatty acids to saturated fatty acids, causing lipid peroxidation on cell membranes and producing a cytotoxic metabolite known as MDA (Zhang et al., 2018). ROS plays a pivotal role in oxidative damage of plants, while H_2O_2 is a crucial ROS in higher plants. (Ji et al., 2020; Lv et al., 2022). Excessive H_2O_2 accumulation can cause membrane lipid peroxidation. The disruption of cell membrane integrity caused by lipid peroxidation can disturb the dynamic equilibrium of membrane permeability, leading to an elevation in electrolyte leakage rate and ultimately resulting in fruit softening and aging (Wu et al., 2017; Zhang et al., 2022). Therefore, EL rate, LOX activity, MDA and H_2O_2 contents are commonly used as crucial indicators to reflect the cell membrane integrity as well as fruit softening and senescence.

In Fig. 4, the EL rate, LOX activity, MDA and H_2O_2 contents of all groups exhibited an overall upward trend throughout storage. The four indices of CK and DW groups showed almost no difference but were significantly higher than other groups at the same sampling time during storage. At the later stages of storage, the four indices in AEW + HVEF group exhibited significantly lower than those in AEW and HVEF groups. Meanwhile, the EL rate, MDA, and H_2O_2 contents were remarkably higher in the HVEF group than in the AEW group, while LOX activity showed an opposite trend.

At the end of storage, the EL rate of AEW + HVEF group was 4.7% and 16.7% lower than that of AEW and HVEF groups, respectively, while the EL rate of AEW group was 18.9% lower than that of DW group and that of HVEF group was 5.1% lower than CK group (Fig. 4A). It was reported that the MAP + HVEF treatment could reduce the EL rate and prolong the shelf life of fresh-cut cabbage and baby corn (Huang et al., 2021). The fresh-cut broccoli treated with HVEF (3000 kV/m and 2250 kV/cm) exhibited lower levels of EL rate (6.03% and 14.68%, respectively) during a storage period of 40 days, in contrast to the untreated samples which showed a much higher level of EL rate at 36.12% (Kao et al., 2019). However, it should be noted that higher electric field intensity may result in greater damage to tissue and cell structure. For example, the EL rate of pomegranates treated with HVEF (3 kV/cm and 1.5 kV/cm) increased up to 14.80% and 3.82% after 60 days, respectively, compared to the control group (Lotfi et al., 2022). Therefore, it can be inferred that the impact of electric field on EL rate may vary depending on the strength and duration of the applied electric field as

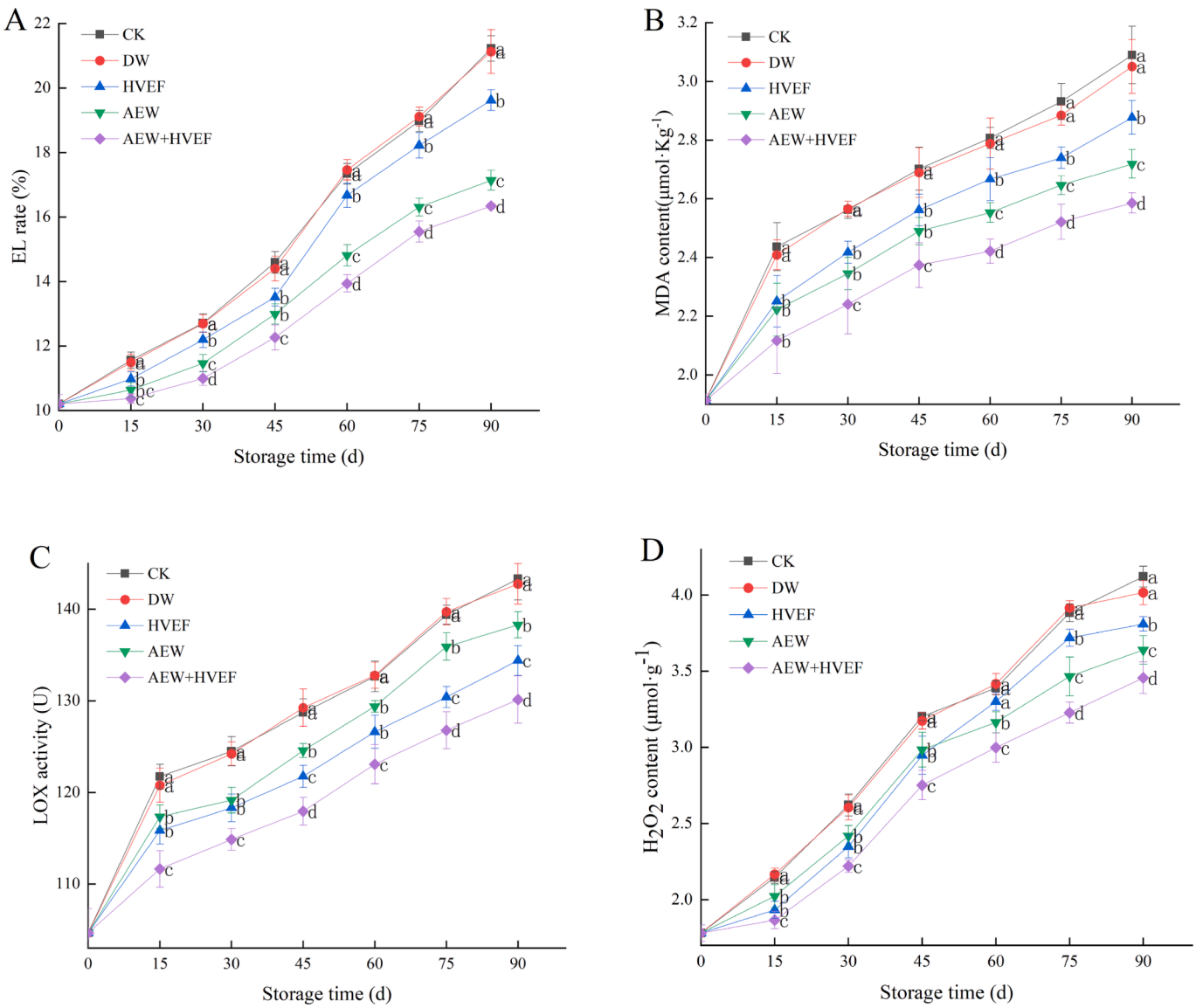

Fig. 4. Effects of HVEF, AEW, and AEW + HVEF treatments on EL rate (A), MDA content (B), LOX activity (C) and H_2O_2 content (D) of Huping jujube during storage at 0 ± 1 °C for 90 days. The data presented are the mean values of three replicates; vertical bars represent the standard deviation of the mean values; values followed by different superscripts (a-d) are significantly different ($P < 0.05$) on the same sampling date.

well as the type of samples. Additionally, Wu et al. (2017) found that the combined treatment of low-concentration acidic electrolyzed water (LcEW) and ultrasound (US) was more effective in decreasing the EL rate of mushroom slices than LcEW (Wu et al., 2017).

In Fig. 4B, the MDA content in AEW + HVEF group was 4.8% and 10.1% lower than that in AEW and HVEF groups on day 90, respectively. Meanwhile, the MDA content of AEW group was 10.8% lower than that of DW group and that of HVEF group was 6.8% lower than that of CK group. Pang et al. (2021) reported that sweet potato roots treated with high-voltage alternating electric field (HVAEF, 4 kV/m) exhibited significantly lower EL rate and MDA content during storage. Similarly, 'Lingwu Long' jujube fruit treated with AEW (ACC of 60 mg/L, pH of 2.2, ORP of 1177 ± 5 mV) maintained 20% lower MDA content than the control on day 30. (Jia et al., 2022).

In Fig. 4C, on day 90, the LOX activity in AEW + HVEF group was 5.9% and 3.2% lower than that in AEW and HVEF groups, respectively. However, the LOX activity in AEW group was 3.1% lower than that in DW group and that of HVEF group was 6.2% lower than that of CK group at the end of storage. Hsieh et al. (2020) reported that oyster mushrooms (*Pleurotus ostreatus,* P. ostreatus) treated with alternating current electric field (ACEF, 600 k V/m, 50 Hz, 2 h) exhibited approximately 40% less EL rate and 30% lower MDA content by inhibiting LOX activity compared with the control group during storage, therefore finally delaying the decay of *P. ostreatus* mushroom (Hsieh et al, 2020).

In Fig. 4D, the H_2O_2 content of AEW + HVEF group was 4.9% and 9.2% lower than that of AEW and HVEF groups at the end of storage, respectively. The H_2O_2 content of AEW group was 9.5% lower than that of DW group, while that of HVEF group was7.5% lower than that of CK group on day 90. A previous study showed that the H_2O_2 content of pomegranates treated with HVEF (1.5 kV/cm and 3 kV/cm) were 61% and 37.5% lower than the control group at day 60, respectively (Lotfi et al., 2022). However, AEW (ACC of 80 mg/L, pH of 2.5, ORP of 4907 ± 5 mV) treatment boosted the amount of H_2O_2 but decreased MDA content in 'Fuyan' longan fruit. (Tang et al., 2021), which was not in line with our findings. This discrepancy may be attributed to the differences in fruit species, harvest maturity and AEW parameters employed. Additionally, another study demonstrated that the combined treatment of slightly acidic electrolyzed water (SAEW, pH of 6.7; ACC of 30 mg/L) and LVEF (150 V, 1000 Hz) exhibited superior efficacy in reducing H_2O_2 and MDA levels and inhibiting LOX activity of fresh-cut pineapples

during cold storage compared to the single LVEF and SAEW treatments (Cheng, Li, Wang, Cheng, Wu, & Sun, 2022), which was consistent with our results.

Therefore, it was suggested that AEW, HVEF, and AEW + HVEF treatments significantly inhibited the increase of EL rate, LOX activity, MDA and H_2O_2 contents, which was conducive to improving the cell structural integrity, as well as delaying softening and senescence. Furthermore, the AEW + HVEF treatment exhibited obvious synergistic effect.

Changes in antioxidant enzyme activities

The process of adversity and tissue aging can result in the excessive accumulation of ROS in plants, which disrupts the dynamic balance between ROS production and clearance systems, damages the membrane integrity and accelerates fruit senescence (Lv et al., 2022; Zhang et al., 2022). Interestingly, plants possess both enzymatic and nonenzymatic ROS scavenging systems, which is conducive to maintaining an equilibrium of ROS levels, reducing the damage and improving the storage quality of fruit (Jia et al., 2023). CAT, SOD, POD and APX are

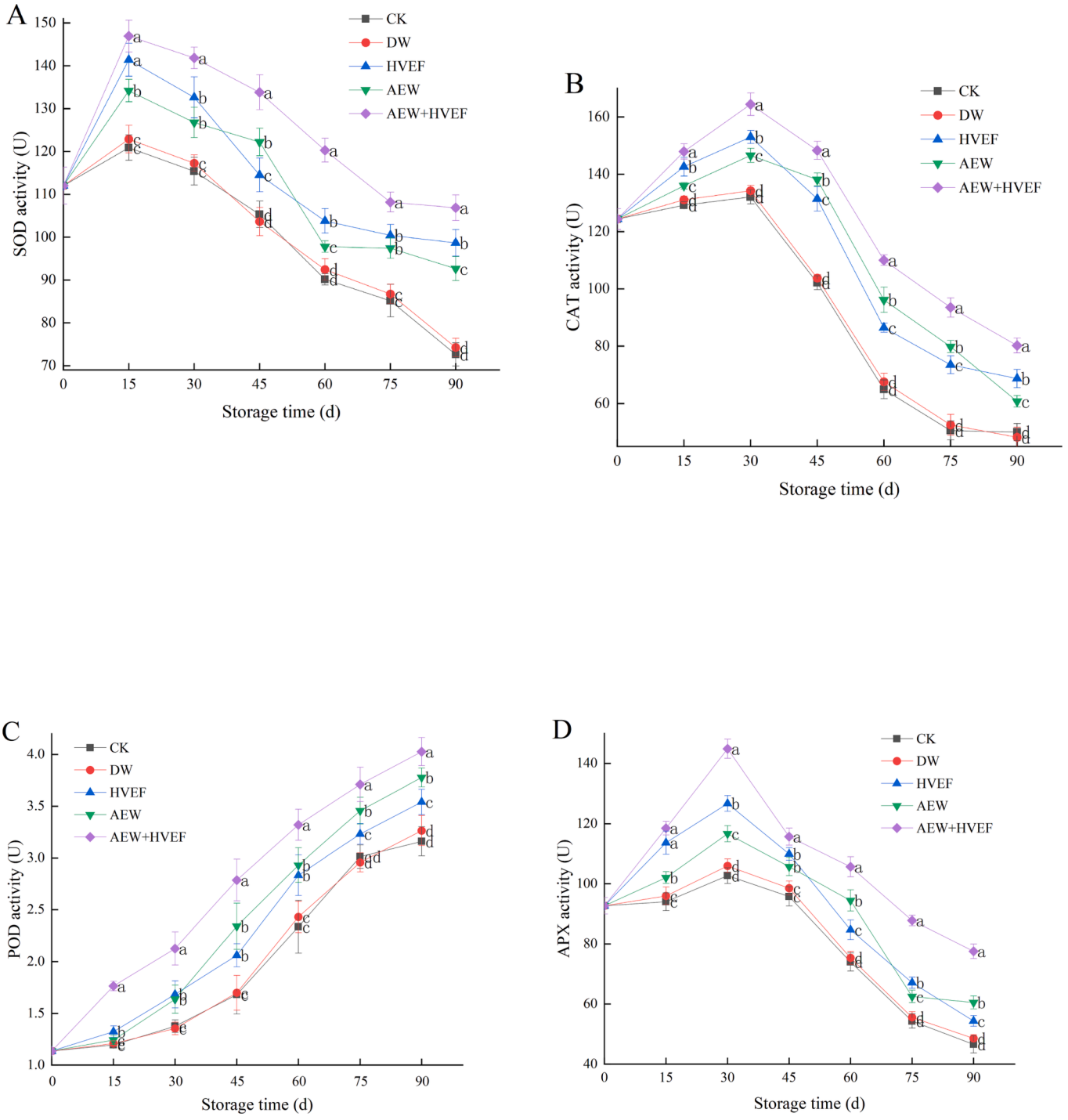

Fig. 5. Effects of HVEF, AEW, and AEW + HVEF treatments on the activities of SOD (A), CAT (B), POD (C) and APX (D) of Huping jujube during storage at 0 ± 1 °C for 90 days. The data presented are the mean values of three replicates; vertical bars represent the standard deviation of the mean values; values followed by different superscripts (a-d) are significantly different ($P < 0.05$) on the same sampling date.

regarded as crucial antioxidant enzymes in the ROS scavenging system (Sang, Yang, et al., 2022; Zhang et al., 2023), which can effectively eliminate ROS generated during metabolism, mitigate oxidative damage to cells caused by ROS and retard fruit senescence (Zhang et al., 2022). Fig. 5 exhibited that the activities of SOD, CAT and APX presented an initial increase followed by a subsequent decline, while the activities of POD continuously rose throughout the entire storage. However, SOD activity peaked on day 15, whereas the activities of CAT and APX peaked on day 30. Moreover, except for day 15, the four enzyme activities in the CK and DW groups were the lowest ($p < 0.05$) during the same storage period, while those in AEW + HVEF group were the highest ($p < 0.05$).

SOD is the first response to the cell's natural defense against oxidative stress, which is an enzyme containing metal ions and can disproportionate superoxide anion to form H_2O_2 and O_2 (Huang et al., 2021; Sang, Yang, et al., 2022; Zhang et al., 2022). Fig. 5A demonstrated that SOD activities in HVEF group were significantly higher than that in AEW group on days 15, 60 and 90, but notably lower than that in AEW group on day 45. At the end of storage, SOD activity of AEW + HVEF group was 15.4% and 8.3% higher than that of AEW and HVEF groups, respectively. Meanwhile, the SOD activity of AEW group was 24.8% higher than that of DW group while that of HVEF group was 35.9% higher than that of CK group.

CAT can promote the decomposition of H_2O_2 into H_2O and O_2, and protect plants from the toxicity of H_2O_2, thereby delaying fruit senescence (Tang et al., 2021). The CAT activities in HVEF group were prominently lower than that in AEW group on days 45, 60 and 75, but were reversed at other sampling points. At the peak time, the CAT activity of AEW + HVEF group was 12.2% and 7.5% higher than that of AEW and HVEF groups, respectively. At day 90, the CAT activity of AEW + HVEF group was 32.1% and 16.8% higher than that of AEW and HVEF groups, respectively. Compared to the DW group, CAT activity in AEW group was 26.0% higher while it was 37.4% higher in HVEF group compared to CK group (Fig. 5B).

POD, as an oxidoreductase, not only catalyzes the cross-bonding of proteins, pectin and cellulose and stabilizes the structure of the cell wall, but also decomposes H_2O_2 into H_2O (Zhang et al., 2022). The POD activities in HVEF group were higher on day 15 and lower on days 75 and 90 than that in AEW group ($p < 0.05$). At the end of storage, the POD activity of AEW + HVEF group was 6.6% and 13.8% higher than that of AEW and HVEF groups, respectively (Fig. 5C).

APX plays a synergistic role with CAT to convert H_2O_2 into H_2O (Zhang et al., 2022), but unlike CAT, APX may be responsible for the fine regulation of ROS signal (Wang et al., 2022). The APX activities in the HVEF group were memorably higher than those in the AEW group on days 15, 30 and 75 but lower on days 60 and 90 ($P < 0.05$). On day 90, the APX activity of the AEW + HVEF group exhibited a significant increase of 28.1% and 42.6% compared to that of the AEW and HVEF groups, respectively. The APX activity in the AEW group was elevated by 24.6% when compared with that in the DW group, while an increase of 16.8% was observed in the HVEF group as compared to that in the CK group on day 90 (Fig. 5D).

A previous study reported that HVEF can affect the electron transfer in the respiratory chain, metal ion valence and enzyme conformation (Zhang et al., 2022). The electric field treatment puts the fruit in a state of stress which stimulates antioxidant systems in order to maintain free radical scavenging balance and delays fruit senescence (Zhao et al., 2023). HVEF (10 kV/m) delayed the cell senescence of postharvest *Agaricus bisporus* by enhancing the SOD and CAT activities (Yan et al., 2020). Meanwhile, HVEF (1.5 kV/m) reinforced SOD, CAT and APX activities in pomegranate, thereby preventing the overproduction of ROS and reducing the oxidative damage (Lotfi et al., 2022). Likewise, green mature tomatoes treated with HVEF (±2kV/m) showed higher ability for scavenging free radical by increasing the activities of SOD, APX, CAT and POD (Zhao, Hao, Xue, Liu, & Li, 2011). AEW (ACC of 60 mg/L, pH of 2.2, and ORP of 1177 ± 5 mV) treatment alleviated oxidative injury of 'Lingwu Long' jujube fruit by enhancing CAT and SOD activities (Jia et al., 2022). Additionally, AEW (pH of 2.5 and ACC of 80 mg/L) treatment reduced cellular membrane damage of 'Fuyan' longan by enhancing the activities of SOD, CAT and APX (Tang et al., 2021). Also, blueberries treated with AEW (pH of 2.8, ORP of 1125 mV and ACC of 48 mg/L) exhibited higher activities of SOD, CAT and APX while a slower increase in oxidative stress (Chen et al., 2019). These findings provided support for our work.

To summarize, in conjunction with our findings, it can be inferred that the AEW, HVEF and AEW + HVEF treatments can enhance SOD, CAT, POD and APX activities, reduce oxidative damage and improve the storage quality of jujube. Notably, greater attention should be paid to the AEW + HVEF treatment due to its pronounced synergistic effect.

Changes in the contents of non-enzyme antioxidants and antioxidant capacity

As important nutrients and non-enzymatic antioxidants, AsA, GSH, flavonoids, and phenolics play a crucial role in plant resistance against excessive ROS accumulation. This contributes to maintaining better defense capability of fruit and delaying ripening and senescence (Sang, Yang, et al., 2022; Zhang et al., 2022; Zhang et al., 2022). In Fig. 6, the AsA content displayed a rapid increase initially, peaked at day 15, then decreased gradually. At the same storage period, the AsA contents of the CK and DW groups were very similar, but were markedly lower than those of other groups (except for day 30, $p < 0.05$). Except for day 15, the AsA contents showed a descending order of AEW + HVEF > HVEF > AEW ($p < 0.05$). The GSH and AsA contents presented the similar trend. Except for day 45, the AEW + HVEF group consistently exhibited the highest levels of GSH content among all groups ($p < 0.05$). Furthermore, the GSH contents in HVEF and AEW groups were significantly higher than that in CK and DW groups (excluding day 30, $p < 0.05$) during storage. Additionally, the TP content increased rapidly and peaked on day 30, then dropped rapidly from day 30 to 60, and finally increased slightly. The TP contents of CK and DW groups showed no obvious difference but were notably lower than other groups. After day 15, the TP content was in the decreasing order of AEW + HVEF > HVEF > AEW (except for day 60, $p < 0.05$). The trend of TF content change was similar to that of TP, which may be attributed to the fact that phenolic substances are precursors for flavonoid synthesis and the increase of TP content is often accompanied by the acceleration of flavonoid synthesis (Yang et al., 2022). However, after day 15, TF contents of AEW and HVEF groups had no obvious difference on day 45 instead of day 60.

AsA, as an endogenous small molecule antioxidant, can directly quench distinct ROS and serve as a substrate for APX to catalyze H_2O_2 into H_2O and O_2 (Yao et al., 2021). Thus, AsA can contribute to enhancing fruit resistance against active oxygen damage and protecting flavonoids and phenolics from degradation (Yu et al., 2021). In Fig. 6A, at the end of storage, AsA content in AEW + HVEF group was 9.7% and 5.0% higher than that in the AEW and HVEF groups, respectively. At the same time, the AsA content was 5.4% higher in the AEW group than in the DW group, and 11.3% higher in the HVEF group than in the CK group. GSH acts as a dedicated electronic donor for monodehydroascorbate reductase and dehydroascorbate reductase to regenerate AsA and eventually is oxidized to glutathione disulfide in the AsA-GSH cycle (Jia et al., 2023; Yao et al., 2021). Furthermore, GSH can directly scavenge free radicals and eliminate the toxic effect of ROS on fruit cells, thereby enhancing the antioxidant capacity of plant cells (Jia et al., 2023). In Fig. 6B, at the peak period, the GSH content of the AEW + HVEF group was 9.2% and 5.7% higher than that in the AEW and HVEF groups, respectively. On day 90, the GSH content in the AEW + HVEF group was 4.2% and 2.4% higher than that in the AEW and HVEF groups, respectively, and the GSH content in AEW group was 5.4% higher than that in DW group while that in the HVEF group was 7.5% higher than that in CK group.

Polyphenols and flavonoids, as key plant secondary metabolites, are not only essential for improving the nutritional qualities of fruit such as

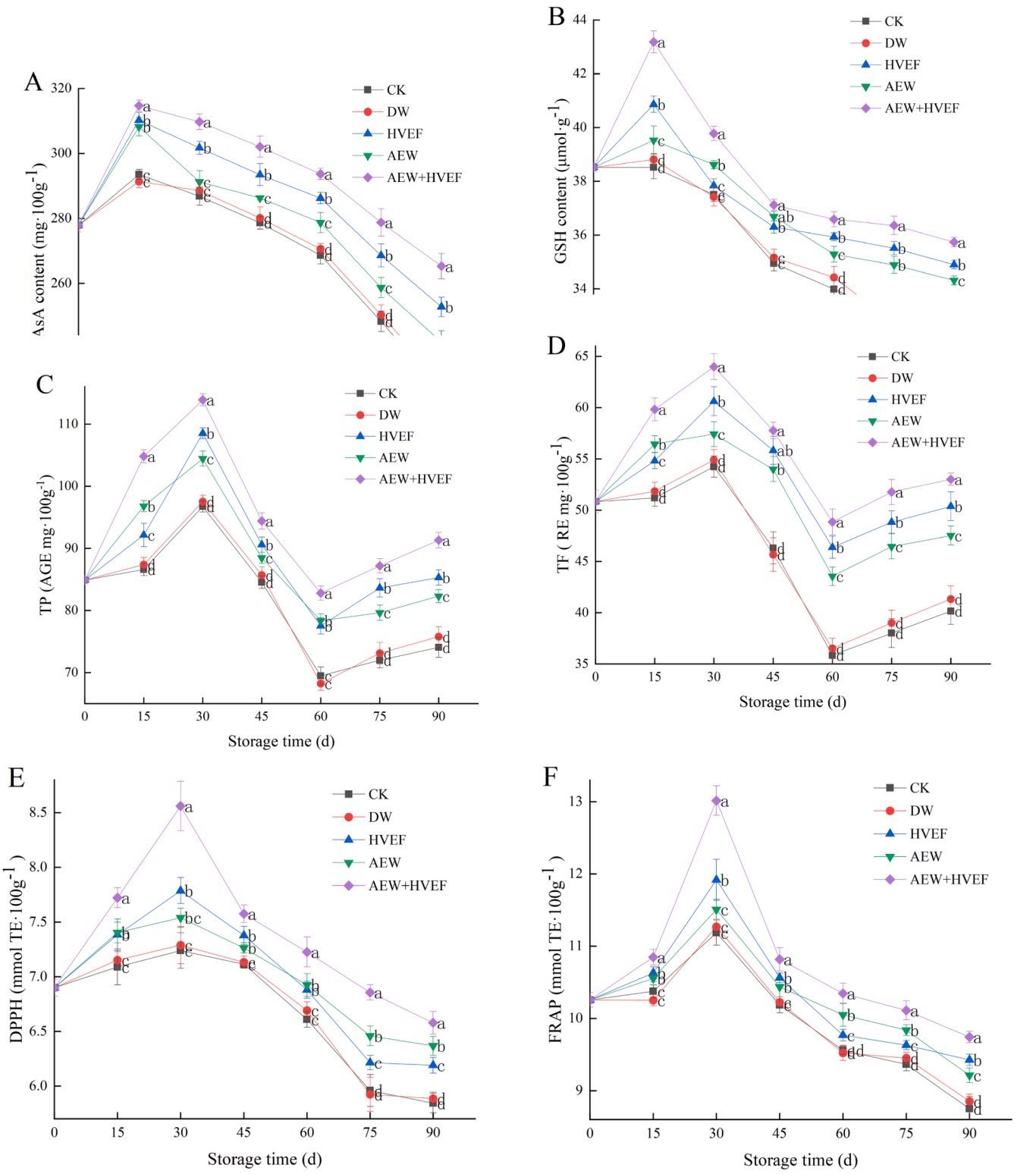

Fig. 6. Effects of HVEF, AEW, and AEW + HVEF treatments on the contents of AsA (A), GSH (B), TP (C) and TF (D) as well as the DPPH free radical scavenging ability (E) and FRAP (F) of Huping jujube during storage at 0 ± 1 °C for 90 days. The data presented are the mean values of three replicates; vertical bars represent the standard deviation of the mean values; values followed by different superscripts (a-d) are significantly different ($P < 0.05$) on the same sampling date.

firmness, color, and flavor, but also contribute to the antioxidant properties of fruit by acting as substrates for various antioxidant enzymes, scavenging free radicals and quenching singlet oxygen (Sang, Liu, Tang, Yang, Guo, & Chen, 2022; Yang et al., 2022; Yu et al., 2021). In Fig. 6C, at its peak, the TP content of AEW + HVEF group was 9.1% and 5.0% higher than that of AEW and HVEF groups, respectively. On day 90, the AEW + HVEF group exhibited a 10.9% and 7.0% increase in TP content compared to the AEW and HVEF groups, respectively. Also, the TP content of the AEW group was 8.6% higher than that of the DW group while that of the HVEF group was 15.1% higher than that of the CK group. Likewise, at the peak time, the TF content of AEW + HVEF group was 11.4% and 5.6% higher than that of the AEW and HVEF

groups, respectively. On day 90, the TF content in the AEW + HVEF group was 11.5% and 5.2% higher than that in the AEW and HVEF groups, respectively. Additionally, the TF content in the AEW group was 15% higher than that in the DW group, while that in the HVEF group was 25.4% higher than that in the CK group (Fig. 6D).

The antioxidant properties of fruit are closely associated with effective free-radical scavengers, which can be quantified by DPPH free radical scavenging capacity and FRAP (Islam et al., 2022; Yu et al., 2021). In Fig. 6E and F, the trend of DPPH free radical scavenging ability in jujube is similar to that of FRAP, both of which initially rapidly increased, peaked on day 30, and then gradually dropped as storage proceeded. Throughout the storage, the AEW + HVEF group consistently maintained the highest DPPH and FRAP values ($p < 0.05$), while the CK and DW groups remained the lowest values ($p < 0.05$). During the last 30 days, the DPPH values of the AEW group were significantly higher than those of the HVEF group ($p < 0.05$), which is different with the alterations in the contents of the four non-enzymatic antioxidants mentioned above. This phenomenon can be explained that antioxidant capacity is not only associated with non-enzymatic antioxidants, but also with the activities of antioxidant enzymes (Sang, Liu, et al., 2022; Yang et al., 2022). The DPPH value of AEW + HVEF group was 13.5% and 10.0% higher than that of AEW and HVEF groups during the peak period, respectively. At the end of storage, the DPPH value of AEW + HVEF group was 3.3% and 6.3% higher than that of AEW and HVEF groups and that of the AEW group was 8.3% higher than that of the DW group, while that was 6.0% higher in the HVEF group than in the CK group. Similarly, the FRAP value of AEW + HVEF group was 13.3% and 9.1% higher than that of AEW and HVEF groups on day 30, respectively. On day 90, the FRAP value of AEW + HVEF group was 5.8% and 3.3% higher than that of AEW and HVEF groups. Meanwhile, the FRAP value of AEW group was 4.1% higher than that of DW group and that was 7.8% higher in the HVEF group than in the CK group.

Thus, it could be inferred that AEW + HVEF, AEW and HVEF treatments were helpful to achieve higher contents of non-enzymatic antioxidants, greater DPPH scavenging capacity and FRAP compared to the DW and CK groups. However, AEW + HVEF treatment was more recommendable based on the above results. Many previous studies have demonstrated similar results with our findings. Tang et al. (2021) found AEW (pH of 2.5 and ACC of 80 mg/L) could increase ROS scavenging capacity of 'Fuyan' longan by maintaining high levels of AsA, GSH and DPPH radical scavenging ability. Likewise, AEW (pH of 2.2, ORP of 1177 ± 5 mV, and ACC of 60 mg/L) treatment could improve the storage quality of 'Lingwu Long' jujube via enhancing DPPH scavenging ability and keeping higher contents of AsA, GSH, TP and TF (Jia et al., 2022). In addition, Zhao et al. (2011) showed that HVEF (2 kV/cm) treatment could increase the contents of GSH, TP and AsA in green mature tomatoes. The contents of TP and AsA in pomegranates were better preserved by HVEF (1.5 k V/cm) treatment (Lotfi et al., 2022). Moreover, HVEF (4 kV/cm)-assisted MAP could effectively maintain the chlorophyll and AsA contents of pakchoi (Zhang et al., 2022). Altogether, it is plausible that AEW + HVEF could better preserve the quality and delay the senescence of jujube.

Correlation analysis of firmness, CWDEs, cell membrane integrity-related indices and antioxidant metabolism in Huping jujube under the AEW + HVEF treatment

In this study, Huping jujube treated with the AEW + HVEF demonstrated superior storage quality compared to other treatments. To better investigate the relationships between different indices and their impacts on storage quality, Pearson's correlation analysis was conducted on firmness, CWDEs, cell membrane integrity-related indices (LOX, MDA, EL and H_2O_2) and antioxidant metabolism in jujube treated with the AEW + HVEF during storage.

According to the Pearson's correlation coefficients (Fig. S), fruit firmness was negatively correlated with PG ($r = -0.89$, $p \leq 0.01$), β-gal ($r = -0.83$, $p \leq 0.01$), Cel ($r = -0.80$, $p \leq 0.05$), β-Glu ($r = -0.95$, $p \leq 0.01$), EL ($r = -0.96$, $p \leq 0.01$), MDA ($r = -0.89$, $p \leq 0.01$), LOX ($r = -0.94$, $p \leq 0.01$), H_2O_2 ($r = -0.93$, $p \leq 0.01$), and POD ($r = -0.93$, $p \leq 0.01$), but positively correlated with CAT ($r = 0.82$, $P \leq 0.05$). Additionally, the firmness was also positively correlated with SOD, APX, AsA, GSH, TF and TP. It indicated that the firmness not only could be characterized by cell membrane integrity and CWDEs activities but also be regulated by antioxidant system. However, DPPH free radical scavenging ability was positively correlated with AsA ($r = 0.88$, $p \leq 0.01$), TP ($r = 0.85$, $p \leq 0.05$), TF ($r = 0.86$, $p \leq 0.05$), FRAP ($r = 0.96$, $p \leq 0.01$), SOD ($r = 0.89$, $p \leq 0.01$), CAT ($r = 0.90$, $p \leq 0.01$) and APX ($r = 0.99$, $p \leq 0.01$). Similarly, FRAP was positively correlated with TP ($r = 0.85$, $p \leq 0.05$), TF ($r = 0.83$, $p \leq 0.05$), CAT ($r = 0.82$, $p \leq 0.05$) and APX ($r = 0.93$, $p \leq 0.01$). These results suggested there was a significant interplay between enzyme and non-enzyme antioxidant systems, and they played a key role in scavenging free radicals, maintaining antioxidant capacity and enhancing storage quality during storage, which is identical with the findings of previous studies (Chen et al., 2019; Wanli Zhang et al., 2021).

Conclusions

In summary, this study has confirmed the feasibility and effectiveness of AEW, HVEF and AEW + HVEF treatments in hindering softening and regulating the antioxidant system of Huping jujube during postharvest cold storage. Specifically, these treatments exerted significant impacts on cell membrane integrity-related indices such as delaying the increase of EL rate, inhibiting LOX activities and reducing MDA and H_20_2 accumulation. Meanwhile, the activities of CWDEs, including PG, Cel, β-Glu and β-gal, were memorably inhibited. Additionally, jujube treated with the three treatments presented higher DPPH free radical scavenging capability and FRAP by enhancing the antioxidant enzyme activities such as SOD, CAT, POD and APX, as well as inducing the accumulation of non-enzymatic antioxidants including AsA, GSH, TP and TF. Furthermore, AEW + HVEF treatment was more effective than either AEW or HVEF treatment alone in delaying the softening of white mature Huping jujubes. According to Pearson's correlation analysis, firmness was closely related to cell membrane integrity, the activities of CWDEs and CAT. These results inferred that AEW + HVEF treatment had great potential as a viable treatment to improve the quality and extend the storage time of Huping jujube. The HVEF used in this experiment has limited single processing capacity and longer processing time, which limits its large-scale industry application to some extent. The problem of limited single processing capacity can be addressed by improving the HVEF system. For instance, connecting multiple parallel electrode plates in vertical space can generate multiple identical electric fields within a confined space, thereby resolving the problem of limited processing capacity. However, further research and exploration are required to solve the problem of longer processing time.

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.2023.100812.

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