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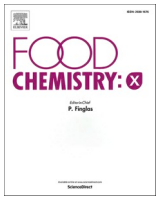

Regulation effect of magnetic field combined with low temperature storage on postharvest quality and cell wall pectic-polysaccharide degradation of *Clausena lansium* (Lour.) Skeels

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ABSTRACT

This study investigated the regulation effect of magnetic field combined with low temperature storage on postharvest quality and cell wall pectic-polysaccharide degradation of wampee stored for 15 d at 4 °C and 15 °C. Results showed that magnetic field combined with low temperature storage reduced browning rate of fruit after 15 d storage, but its effect on weight loss rate and total soluble solids (TSS) did not surpass that of storage temperature. Interestingly, contents of flavonoid, total phenols and malondialdehyde (MDA) were also lowered at varying degrees by combined treatment. Furthermore, molecular weight distribution and monosaccharide compositions of cell wall pectic-polysaccharides were also affected, which resulted from the coordinated action of cell wall pectin-degrading enzymes. The activities of these enzymes during storage, including polygalacturonase (PG), pectin methylesterase (PME) and β-galactosidase (β-Gal) in treated wampee decreased. These findings suggested that magnetic field combined with low temperature storage was an effective technology and had great potential in preservation of postharvest wampee in future.

1. Introduction

Wampee [*Clausena lansium* (Lour.) Skeels], a kind of characteristic fruit distributed in South and Southwest China, is renowned for its rich nutrition and special flavor (Ye et al., 2019). It typically exhibits an oval shape, approximately 2.0 cm in diameter and its edible part includes the pulp and peel. In recent years, the cultivation area and production of wampee continually increase with the development of new cultivars and improvement of cultivation techniques. However, its mature period is very concentrated (mainly in July and August) and it is prone to soften, browning, decay and thus losing the commodity value within three days, making it difficult to transport and sell in distant field thus restraining its industrialization (Zeng et al., 2020).

Softening is a common phenomenon during fruit ripening in most fleshy fruit, involving in a series of physiological and biochemical reaction and closely related to cell wall metabolism (Prasanna et al., 2007). During fruit softening, the degradation of cell wall polysaccharide especially pectin is very obvious, greatly contributing to the destruction of cell wall structure and fruit softening (Pose et al., 2019). According to previous reports, the degree of methyl-esterification of cell wall pectin firstly declined under the effect of pectin methylesterase (PME) and then polygalacturonases (PG) acted on de-esterified pectin (Willats et al., 2001; Prasanna et al., 2007; Yang et al., 2018). Besides, neutral sugar side chains in cell wall pectin were hydrolyzed by β-galactosidases (β-Gal) (Ranwala et al., 1992; Brummel, 2006). In a word, fruit softening came down to the depolymerization, dissolution and rearrangement of cell wall components especially pectin (Goulao & Oliveira, 2008). Therefore, regulating cell wall pectic-polysaccharide metabolism to control fruit softening is a potential approach and of great significance to fruit preservation.

Magnetic field, as an emerging non-thermal preservation technology, has attracted more and more attentions in recent years due to its simplicity, high-efficiency and safety. Yang et al. (2020) reported that magnetic field could delay the ripening of cherry tomato and keep its quality during storage. Lv et al. (2022) found that magnetic field (2 mT, 50 Hz) could maintain better color and firmness of fresh-cut apples during 9 d storage at 4 °C. It was also reported that polyphenol oxidase activity and MDA accumulation of bananas under magnetic field treatment (2 mT, 60 Hz) were lower during 10 d storage (Zhao et al., 2021). Liu et al. (2023) discovered that magnetic field (4 mT, 50 Hz) retard the senescence and decay of harvested strawberries by maintaining energy state and regulating respiratory metabolism. Similarly, studies on

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cucumber and cantaloupe melon also showed magnetic field could extend their storage time (Jia et al., 2015; Zhang et al., 2020). However, current researches are mainly concentrated on effect of magnetic field on storage quality and postharvest physiology of various kinds of fruit, the internal action mechanism of magnetic field on fruit preservation is yet unknown, especially from the perspective of cell wall pectic-polysaccharide degradation.

Considering postharvest fruit is still a life body with strong physiological metabolism, a bold hypothesis is proposed that magnetic field might mediate cell wall pectic-polysaccharide metabolism in fruit, finally contributing to delay of fruit softening and extension of shelf life. Therefore, in this study, experiments were designed to explore the relationship between softening of wampee fruit and its pectic-polysaccharide metabolism under the effect of magnetic field combined with low temperature storage, which integrated different methods to improve storage quality and would produce a synergistic effect. Our study would provide technical support and theoretical basis for resolving its problems of short-time storage and quality deterioration.

2. Materials and methods

2.1. Experimental materials

Wampee was picked from an orchard in Guangdong province and transported to our laboratory within six hours. Uniform fruit (length: 25 ± 5 mm, weight: 10 ± 1 g, maturity: eight mature) with no diseases was randomly divided into three groups. Two groups were stored in incubators with 90 % relative humidity at 15 °C and 4 °C, respectively. Another group was firstly subjected to magnetic field (3 mT, 30 min) at 4 °C and then stored at 4 °C. The magnetic field device was designed by Yangtze University (Jingzhou) and assembled by Helmholtz coil (Fig. S1). For each group, samples were randomly taken out every three days before analysis.

2.2. Appearance and color

When fruit was taken out from incubators, it was observed and photographed firstly. The degree of fruit browning was divided into five grades: grade 1 of fruit (proportion of browning area < 1/4), grade 2 of fruit (1/4 ≤ proportion of browning area < 1/2), grade 3 of fruit (1/2 ≤ proportion of browning area < 3/4), grade 4 of fruit (proportion of browning area ≥ 3/4), grade 5 of fruit (proportion of browning area = 1). Browning index could be calculated by the following equation (Feng et al., 2023).

$$Browningindex = \frac{\sum (G_i \times i)}{G \times N}$$

where G is the total number of fruit, N is the number of supreme browning grade for fruit and equal to 5, G_i is amount of grade i for fruit, i is the number of fruit grade.

Fruit color including L* value (luminance), a* value (red degree), b* value (yellow degree) was determined by CR-10 Plus Colorimeter (Konica Minolta Holdings, Inc., Japan). Five different positions at every fruit were randomly selected and results were averaged based on the measurements.

2.3. Weight loss rate, fruit firmness, total soluble solids (TSS) and titratable acid (TA)

Fruit was randomly selected and conducted penetration test by a texture analyzer (TMS-PRO, USA) equipped with 25 N load cells and a TMS Magness-Taylor probe set (diameter: 3 mm). The penetration speed was 1 mm/s and trigger force was 0.1 N. The firmness was defined as the peak force during the test. TSS of juice obtained from fruit flesh was determined by LB90T hand-held refractometer (Shenzhen Swevy scientific and technical Co., Ltd., China) after calibration by a drop of distilled water. TA was assayed and expressed as a percentage of malic acid. Fruit flesh (2 g) were firstly homogenized with cold distilled water. After centrifugation (12000×*g*, 20 min) at 4 °C, the supernatant liquid (10 mL) was titrated to an end-point pH of 8.2 with NaOH solution (0.02 mol/L). Weight loss rate of fruit was determined according to the following equation (Zhu et al., 2021).

$$Weightlossrate(\%) = \frac{M}{M_0} \times 100$$

where M_0 and M is the weight of fruit at 0 d and stored for a certain time, respectively.

2.4. Flavonoid, total phenols and malondialdehyde (MDA) content

Fruit flesh (2 g) was homogenized with methanol (contained 1 % hydrochloric acid, v/v) in ice bath. After centrifugation (12000 × g, 20 min) at 4 °C, the supernatant was fixed to a certain volume and its absorbance at 325 nm and 280 nm was determined with rutin and gallic acid being the standards. Contents of flavonoid and total phenols were defined as the content of rutin and gallic acid per gram fruit fresh, respectively (Feng et al., 2023).

The extraction of MDA was similar with flavonoid and total phenols except the extractant being trichloroacetic acid solution (10 %, w/v). The supernatant (2 mL) after centrifugation was mixed with 2 mL of thiobarbituric acid solution (0.5 %, w/v), boiled for 20 min and centrifuged again. The absorbance of supernatant at 450 nm, 532 nm and 600 nm was determined, respectively. MDA content could be calculated according to the following equation (Liu et al., 2023):

$$C(\mu mol/L) = 6.45 \times (OD_{532} - OD_{600}) - 0.56 \times OD_{450}$$

2.5. Preparation of cell wall materials

Fruit flesh (50 g) was pulped and mixed with 150 mL ethanol in boiling water bath for 20 min and then filtrated. The residue was firstly washed by trichloromethane (contained 50 % methanol, v/v) and acetone several times until its filtrate was colorless. Subsequently, dimethyl sulfoxide (90 %, v/v) and ethanol (80 %, v/v) were successively used to soak and wash the residue. Final residue was dried at 60 °C to constant weight and called cell wall materials.

2.6. Extraction of cell wall pectin-polysaccharide

According to our previous method (Ren et al., 2020), cell wall materials (0.5 g) were extracted by distilled water (30 mL) with constant stirring for 12 h at room temperature. After centrifugation (4000 × g, 15 min), the supernatant was freeze-dried to obtain water soluble pectin (WSP). The sediment was treated with 30 mL EDTA-2Na solution (0.05 mol/L) and constantly stirred for 12 h at 4 °C. After centrifugation, the supernatant was collected and the precipitate was washed by 30 mL of distilled water and centrifuged again. Both supernatants were merged, neutralized by acetic acid, dialyzed (MW cut off 3500 Da) against distilled water for 48 h and freeze-dried to obtain chelate soluble pectin (CSP). The residual above was treated with 30 mL Na_2CO_3 solution (0.05 mol/L, contained 0.02 mol/L $NaBH_4$) to obtain sodium carbonate soluble pectin (SSP) similar to the extraction steps of CSP.

2.7. Molecular weight distribution of cell wall pectin-polysaccharide

Cell wall pectin-polysaccharide was dissolved in ultrapure water (2 mg/mL) and subjected to HPLC (high performance liquid chromatograph) (Aglient 1200, Agilent Technologies Inc., USA) equipped with differential refractive index detector and TSK gel G4000PWxl chromatographic column (Tosoh Corporation, Japan). The mobile phase was ultrapure water and its flow velocity was 0.6 mL min^{-1}. The

temperatures of detector and column oven were 35 °C and 30 °C, respectively. A series of dextran standard including T-10, T-40, T-70, T-110, T-500 and T-2000 were used for the calibration curve (Ren et al., 2017).

2.8. Monosaccharide compositions of cell wall pectin-polysaccharide

According to the method reported by Zhou et al. (2019) with some modifications, cell wall pectin-polysaccharide (5 mg) was mixed with 2 mL trifluoroacetic acid (2 mol/L) and then hydrolyzed at 110 °C for 6 h. After neutralization by NaOH solution (5 mol/L), the hydrolysate (0.5 mL) was mixed with 0.5 mL NaOH solution (0.3 mol/L) and 0.5 mL methanol containing 0.5 mol/L PMP (1-phenyl-3-methyl-5-pyrazolone). The mixture was reacted at 70 °C for 1 h and neutralized by 0.3 mol/L HCl solution. Then trichloromethane was used to remove PMP in hydrolysate. The hydrolysate remained was analyzed by HPLC (RID-20A, Shimadzu, Japan) equipped UV–vis detector and Kromasil C18 chromatographic column (Akzo Nobel, Holland). Acetonitrile (chromatographic purity) and ultrapure water (contained 1.9 mol/L acetonitrile, 3.3 mmol/L kH_2PO_4, 3.6 mmol/L triethylamine) was used as mobile phases A and mobile phases B respectively and its flow velocity was 1.0 mL min^{-1}. The procedure of gradient elute was 0–10 min: 3 %-6 % mobile phases A, 10–22 min: 6 %-12 % mobile phases A, 22–30 min: 12 % mobile phases A, and 30–35 min: 12 %-3 % mobile phases A. Monosaccharide standards including D-galacturonic acid, D-glucuronic acid, D-galactose, D-glucose, L-arabinose, D-xylose, L-rhamnose and D-mannose were used for the calibration curve.

2.9. Enzyme extraction and analysis of enzyme activity

1) Extraction of crude enzyme: Fruit flesh (1 g) was homogenized with 3 mL of 50 mmol/L acetate buffer (pH 5.5) containing 90 mmol/L polyvingypyrrolidone and 0.2 mol/L NaCl in ice bath. After centrifugation (12000 × g, 25 min), the supernatant was used to assay for enzyme activity (Lin et al., 2019). All steps were performed at 4 °C.

2) PG activity: The measurement of PG activity was referenced with the method of Cheng et al. (2023) with some modifications. 0.2 mL crude enzyme, diluted with 0.6 mL acetate buffer (pH 5.5, 50 mmol/L), was firstly preheated at 40 °C for 5 min. Then 0.05 mL enzyme solution above was taken out and 0.45 mL polygalacturonic acid (10 μg mL^{-1}) was added. The whole solution reacted at 40 °C for 0.5 h, which was stopped by adding 0.5 mL DNS. After boiled for 15 min, the solution was mixed with 5 mL distilled water and its absorbance at 540 nm was determined with galacturonic acid as the standard solution. PG activity (g h^{-1} kg^{-1}) was defined as the amount of galacturonic acid produced by PG hydrolysis within 1 h per kilogram fruit flesh.

3) PME activity: Crude PME solution was obtained by the same method mentioned above except extraction solution (0.2 mol/L NaCl solution containing 90 mmol/L polyvingypyrrolidone) (Cheng et al., 2023). 2 mL pectin solution (5 μg mL^{-1}) was mixed with phosphate buffer (3 mmol/L, pH 7.5, contained 0.16 mmol/L bromothymol blue) and 0.83 mL distilled water. After preheating at 37 °C for 5 min, 50 μL crude enzyme was added and the change of its absorbance at 620 nm within 30 min was recorded. The change of 0.01 per minute was taken as one unit (U). The PME activity (U kg^{-1}) was defined as the amount of enzyme unit per kilogram fruit flesh.

4) β-Gal activity: 0.4 mL PNPG (16 mmol/L, p-nitrophenyl-β-D-galactopyranoside) was firstly mixed with 5 mL acetate buffer (pH 5.5, 50 mmol/L). After preheating at 37 °C for 5 min, 0.1 mL crude enzyme was added and reacted at 37 °C for 0.5 h, which was terminated by 2 mL $NaCO_3$ solution (1 mol/L) (Figueroa et al., 2010). Its absorbance at 400 nm was determined with p-nitrophenol as the standard solution. One unit of β-Gal activity was defined as the amount of p-nitrophenol produced by β-Gal hydrolysis per hour per kilogram fruit flesh.

2.10. Statistical analysis

The data was presented as means ± standard deviation obtained from three times parallel experiments. The software of Origin 2019 (OriginLab Corporation, USA) and SPSS 23.0 (International Business Machines Corporation, USA) was used to draw and significance analysis, respectively. Different letters on the bar indicate significant differences at a significant level of 0.05 ($P < 0.05$).

3. Results

3.1. Change of appearance and color

Changes in appearance, color and browning index of wampee in three groups were depicted in Fig. 1. Fruit was plump in appearance at 0 d and gradually lost water, shrank even decayed after several days' storage (Fig. 1A). This phenomenon was the most obvious when wampee was stored at 15 °C compared with 4 °C and 3 mT + 4 °C groups. Besides, magnetic field treatment combined with low temperature storage effectively improved fruit appearance during storage. As shown in Fig. 1B-D, with the extension of storage time, L and b values declined on the whole while a value gradually increased in three groups. Similar trends were presented in changes of L and b values both with 3 mT + 4 °C groups being the highest at different storage time, which was completely opposite from change of a value. In terms of browning index, a sharp upward trend was observed in 15 °C group and small difference existed between 4 °C and 3 mT + 4 °C groups especially in the late stage of storage (Fig. 1E).

3.2. Change of weight loss rate, firmness, TSS and TA

Fig. 2 exhibited the change of weight loss rate, firmness, TSS and TA of wampee during storage. With the extension of storage time, weight loss rate of fruit had a tendency of straight line growth in three groups and changes in 4 °C and 3 mT + 4 °C groups were almost exactly the same (Fig. 2A). Firmness in 4 °C and 15 °C groups gradually declined during storage with lower firmness values at 15 °C at different storage time. In contrast, firmness of fruit after combined treatment fluctuated even increased in the late storage (Fig. 2B). TSS content in three groups also gradually increased along with the storage time (Fig. 2C). It was worth noting that TSS at 15 d in 15 °C group was unmeasurable on account of severe dehydration and no juice obtained.

TA content presented an irregular change in 4 °C and 15 °C groups while it was maintained by magnetic field treatment combined with low temperature throughout the storage (Fig. 2D).

3.3. Change of flavonoid, total phenols and MDA content

During storage, flavonoid content of wampee gradually increased on the whole in 4 °C and 15 °C groups both with some degree of decline at 6 d, while it always maintained at a relatively low level when fruit was treated by magnetic field combined with low temperature storage (Fig. 3A). The variation tendency of total phenols content in all three groups was almost exactly same as that of flavonoid (Fig. 3B). As far as MDA content, no significant difference existed in 4 °C and 3 mT + 4 °C groups during the whole storage except 12 d (Fig. 3C). It was worth noting that MDA content at 15 d in 15 °C group was far more than other days and another two groups.

3.4. Molecular weight distribution of cell wall pectic-polysaccharides

Molecular weight distribution of cell wall pectic-polysaccharides in wampee during storage was depicted in Fig. 4. Based on the calibration with standard dextrans derived from linear regression ($\log Mw = -0.3423\ t + 9.421$, $R^2 = 0.9956$), the average molecular weight of WSP, CSP and SSP could be estimated. There was no obvious difference in

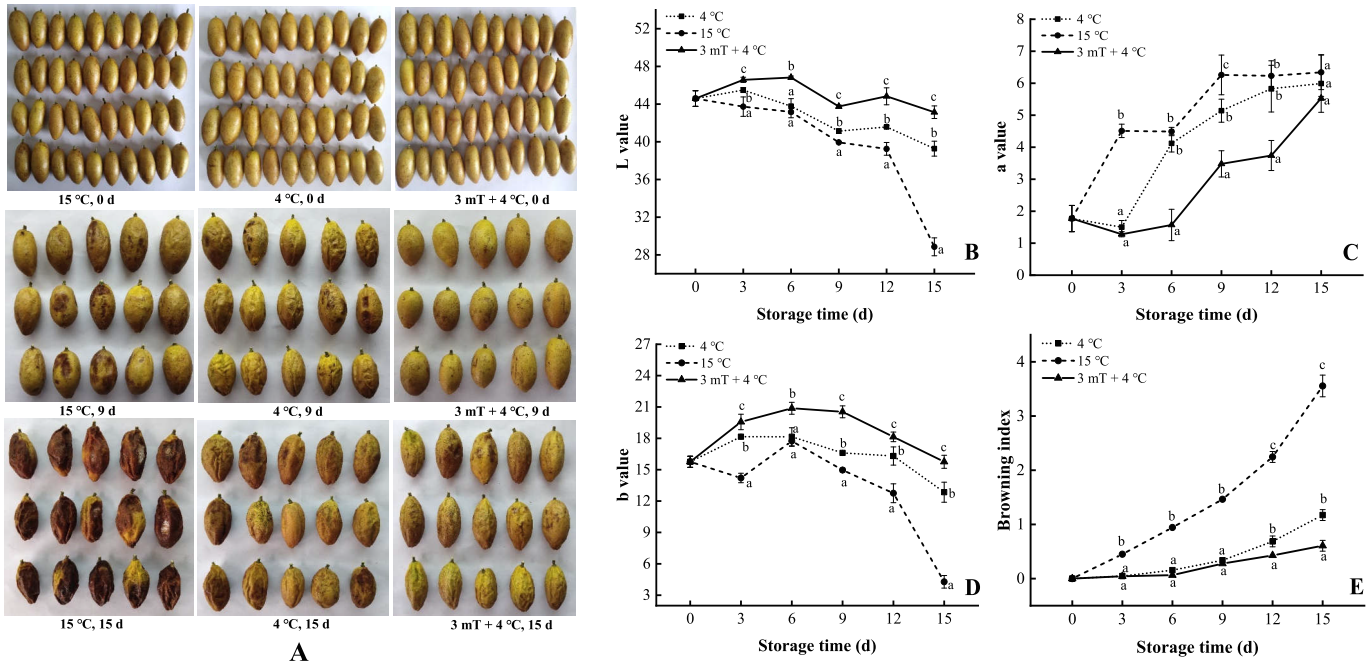

Fig. 1. Effects of magnetic field on appearance (A), L value (B), a value (C), b value (D) and browning index (E) of wampee during storage.

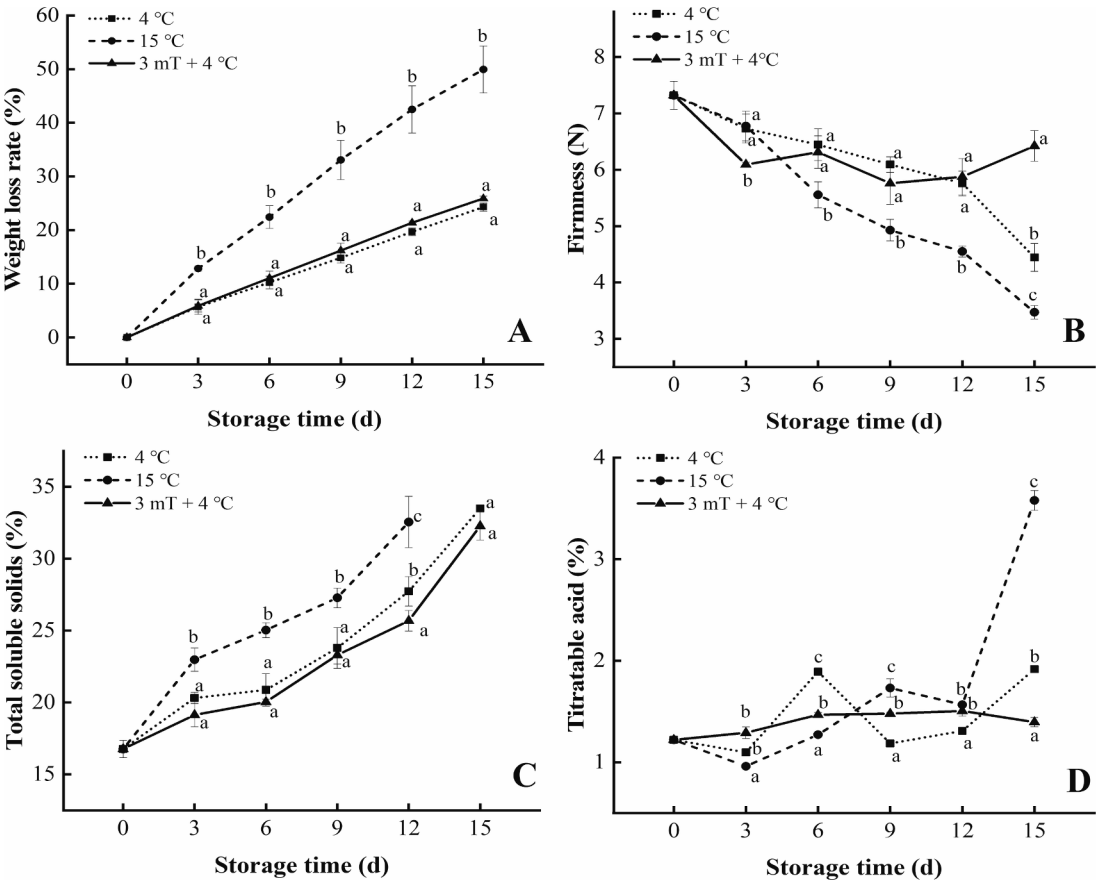

Fig. 2. Effects of magnetic field on weight loss rate (A), firmness (B), total soluble solids (C) and titratable acid (D) of wampee during storage.

total peak numbers and retention time of each peak in WSP in three groups, which corresponded to varieties and molecular weight of carbohydrate in WSP, respectively (Fig. 4A-C). However, with the extension of storage time, the signal intensity of each peak greatly decreased, especially at approximately 7.5 min, which represented the variation of carbohydrate content in WSP. Thereinto, compared with 4 °C and 3 mT + 4 °C groups, this phenomenon was the most obvious in 15 °C group. Different from WSP, there were mainly two peaks close together in CSP

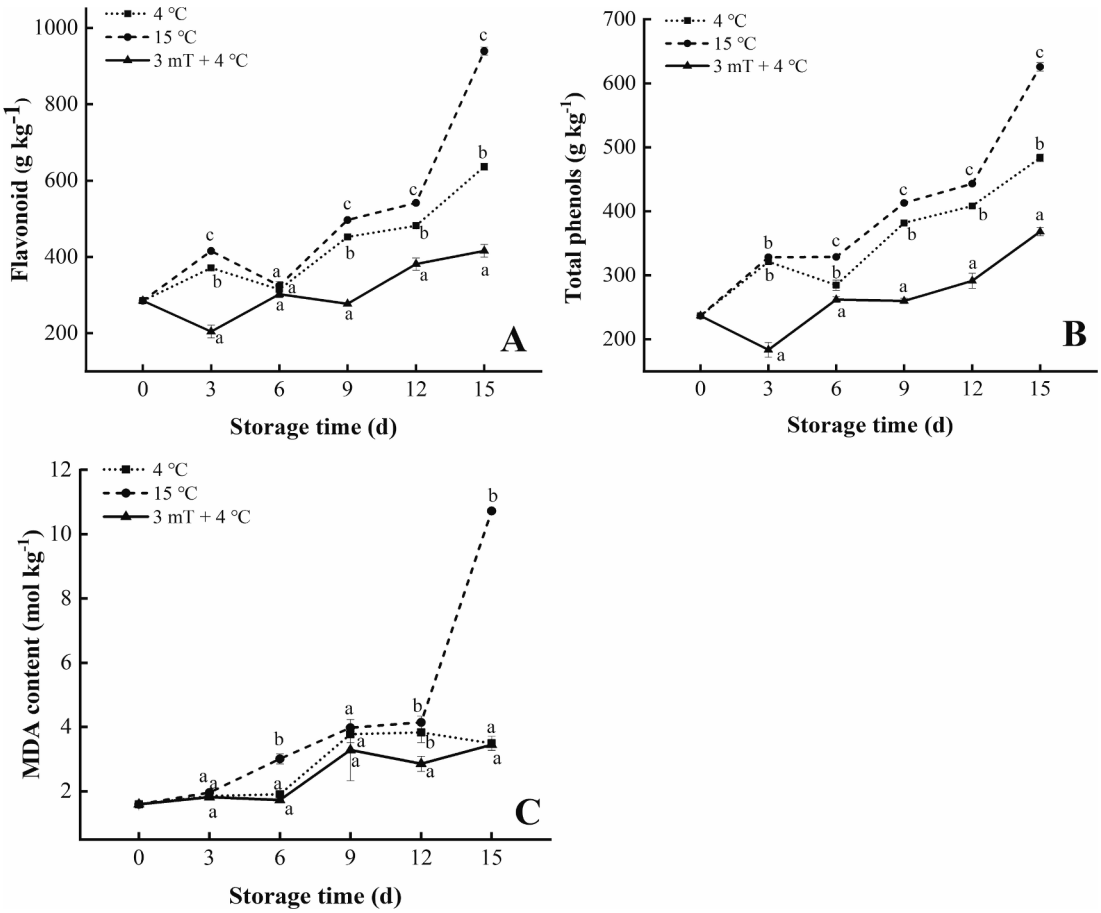

Fig. 3. Effects of magnetic field on flavonoid (A), total phenols (B) and MDA content (C) of wampee during storage.

and it even became a single broad peak with less retention time at 15 d of storage in 15 °C group (Fig. 4D-F). It was worth noting that more intense peak at around 8.5 min was observed in 3 mT + 4 °C group compared with 4 °C group, indicating the existence of larger proportion of carbohydrate with higher molecular weight. Among all chromatograms of SSP in three groups, there was only one main peak on the whole with different intensities (Fig. 4G-I). Obviously, dramatic decrease appeared in abundance of the main peak from 3 d to 15 d of storage in 15 °C group. On the contrary, it even rose up in the late storage in 4 °C and 3 mT + 4 °C groups.

3.5. Monosaccharide compositions of cell wall pectic-polysaccharides

Monosaccharide compositions of cell wall pectic-polysaccharides revealed that GalA and Glu were the most abundant acid and neutral monosaccharide in WSP, CSP and SSP in three groups, respectively (Fig. 5). It could be seen that there was no difference in varieties of monosaccharides but concrete contents in WSP of three groups during storage. And the contents of Xyl and Ara were only second to that of Glu in WSP (Fig. 5A). Compared with WSP, the biggest distinction in CSP was sharp decrease in contents of Glu, especially in 15 °C group followed by 4 °C group and 3 mT + 4 °C group. Besides, contents of GalA seemed to exhibit an overall increase in 3 mT + 4 °C group while those in other two groups not. The appearance of Glu in CSP in 15 °C and 3 mT + 4 °C group was also a visible difference (Fig. 5B). As with SSP, contents of Glu in 3 mT + 4 °C group were the lowest on the whole over the storage period compared with 15 °C and 4 °C groups (Fig. 5C).

3.6. Cell wall pectin-degrading enzymes activity

As shown in Fig. 6A, PG activities in three groups went up steadily on the whole during storage except 6 d in 15 °C group. Different from PG, inconsistent variation trends were exhibited in PME activities and they peaked at various storage time in three groups (Fig. 6B). It was interesting that PME activities always stayed at the lowest level after wampee was treated by magnetic field combined with low temperature storage. Similar phenomenon was also observed in β-Gal (Fig. 6C).

4. Discussion

Fruit softening was the result of texture changes in almost all postharvest fruit during storage, especially in tropical fruit, and seriously affected its commodity value. Therefore, appropriate approach to delay fruit softening would help extend shelf life and enhance fruit quality. In this study, magnetic field (3 mT, 30 min) combined with low temperature storage was applied in the preservation of wampee fruit and its effect was investigated. Wampee fruit treated by magnetic field combined with low temperature storage still have commodity value and was edible after storage of fifteen days, while those in 15 °C group had seriously shrank and browned (Fig. 1A). During storage, the color of wampee fruit, reflected by L, a and b values, tended to be more dark, red and blue (Fig. 1B-D). However, this trend was inhibited by combined treatment, which was consistent with the observation result of its appearance. Fruit browning was a complex progress and involved factors were also various, such as microorganism contaminate, inappropriate storage temperature, water loss, ATP and ADP, enzymatic browning and so on. In general, enzymatic browning was considered to play a key role in fruit discoloration and its substrates were phenolic

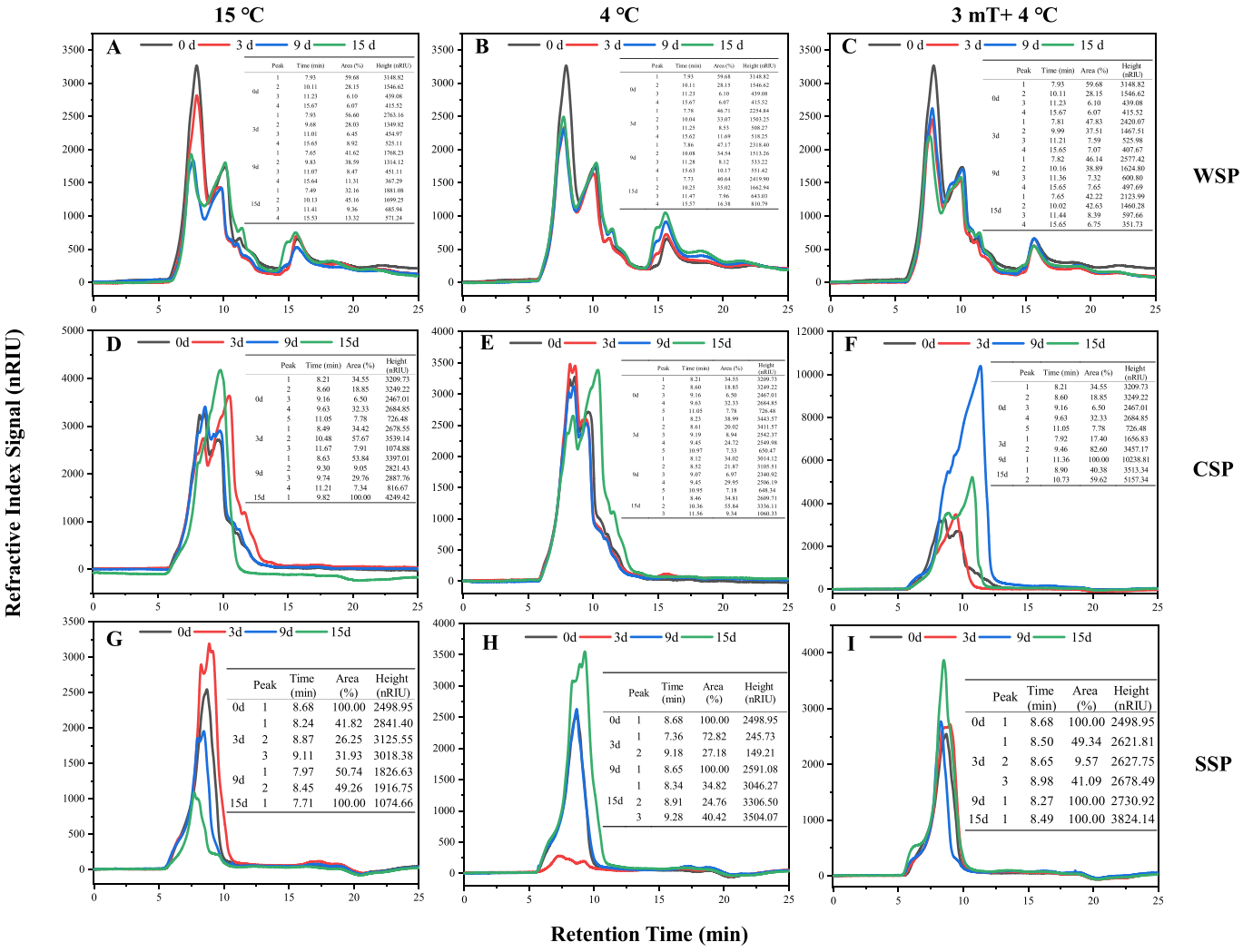

Fig. 4. Molecular weight distribution of WSP (A-C), CSP (D-F) and SSP (G-I) in wampee during storage.

compounds produced by carbohydrate metabolism in fruit (Zhang et al., 2015). Our study showed that the accumulation of flavonoid and total phenols in wampee was weakened by magnetic field treatment combined with low temperature storage (Fig. 3A-B), which might contribute to the preservation of fruit color in this group. Otherwise, previous report indicated that weight loss in fruit would also accelerate browning (Zhang et al., 2023), which was consistent with our result in Fig. 2A that weight loss rate in 15 °C group was higher compared with those of other two groups.

Fruit weight loss was an important factor to influence fruit appearance, texture, flavor and saleable weight (Lufu et al., 2020). The weight loss of wampee fruit was strongly affected by storage temperature instead of magnetic field treatment (Fig. 2A). It was understandable for this phenomenon that higher storage temperature would make moisture diffusion across fruit peel more rapid. Besides, high temperature could also enhance permeability of fruit peel and accelerate the consumption of respiration substrate (Nguyen et al., 2006; Xanthopoulos et al., 2017). In terms of fruit firmness, during storage within 3 d, the firmness of wampee in 3 mT + 4 °C group did not have advantage even was lower than those of other two groups (Fig. 2B). Interestingly, its decline trend was greatly limited by magnetic field treatment combined with low temperature storage and even had slight increasement after three days' storage, which might owe to individual difference of wampee fruit. Jia et al. (2015) also reported that the firmness of cantaloupe melons increased after magnetic field treatment. During fruit softening of peach and banana, it had been observed obvious depolymerization of cell wall components which led to the disruption of cell wall structure and eventually the decline of fruit firmness (Zhao et al., 2021; Shinga and Fawole, 2023). As with TSS, it was always lower in 3 mT + 4 °C group than those of other two groups while TA content was maintained by combined treatment during the whole storage (Fig. 2C, D). In general, TSS and TA content both reflected the taste of fruit, representing carbohydrate content and acidity of fruit, respectively. Previous report also found that magnetic field treatment (2 mT, 15 min) on cantaloupe melons resulted in reduced flows of soluble solids and titratable acid (Jia et al., 2015). With regard to the significant increasement of TA content at 15 d in 15 °C group, this phenomenon might be related with fruit quality of wampee at the moment, which was severe dehydration as well as severe browning, even a small amount of white mold appeared, completely different from that of fruit at the same day in other two groups. MDA content was one index of lipid peroxidation and could reflect the degree of deterioration, including disease, chilling and so on. Fig. 3A-C showed that lower storage temperature (4 °C) could result in relatively lower contents of flavonoid, total phenols and MDA in wampee fruit compared with those at 15 °C. And magnetic field combined with low temperature storage had a positive or synergistic effect in this decline tendency, which was in agreement with change of browning index. It was reported that magnetism could alter the formation of radicals and reduce the probability of triplet radical formation, thus leading to the decreased MDA content after magnetic field treatment

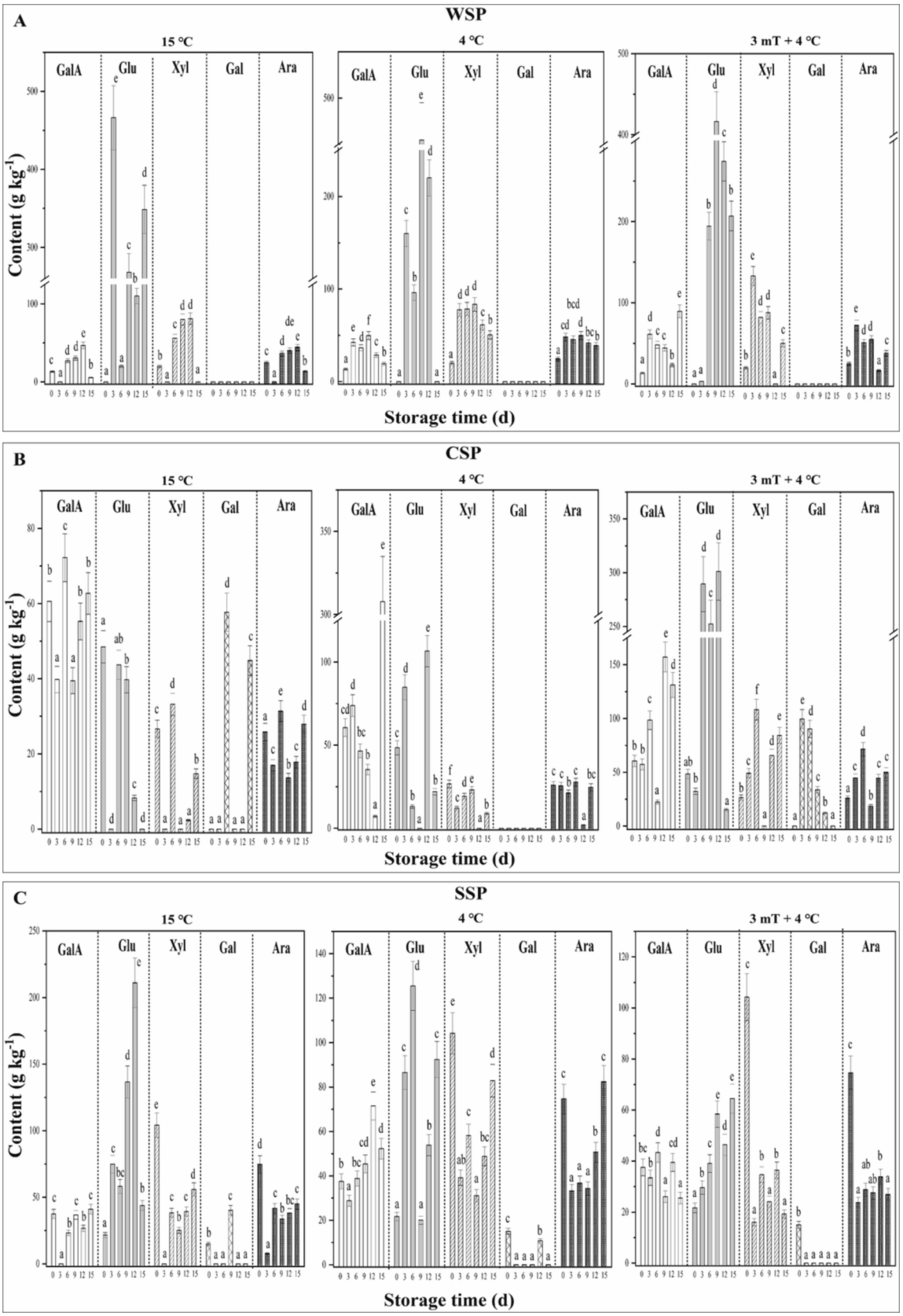

Fig. 5. Monosaccharide compositions of WSP (A), CSP (B) and SSP (C) in wampee during storage. Glucuronic acid (GluA), galacturonic acid (GalA), glucose (Glc), xylose (Xyl), galactose (Gal), arabinose (Ara).

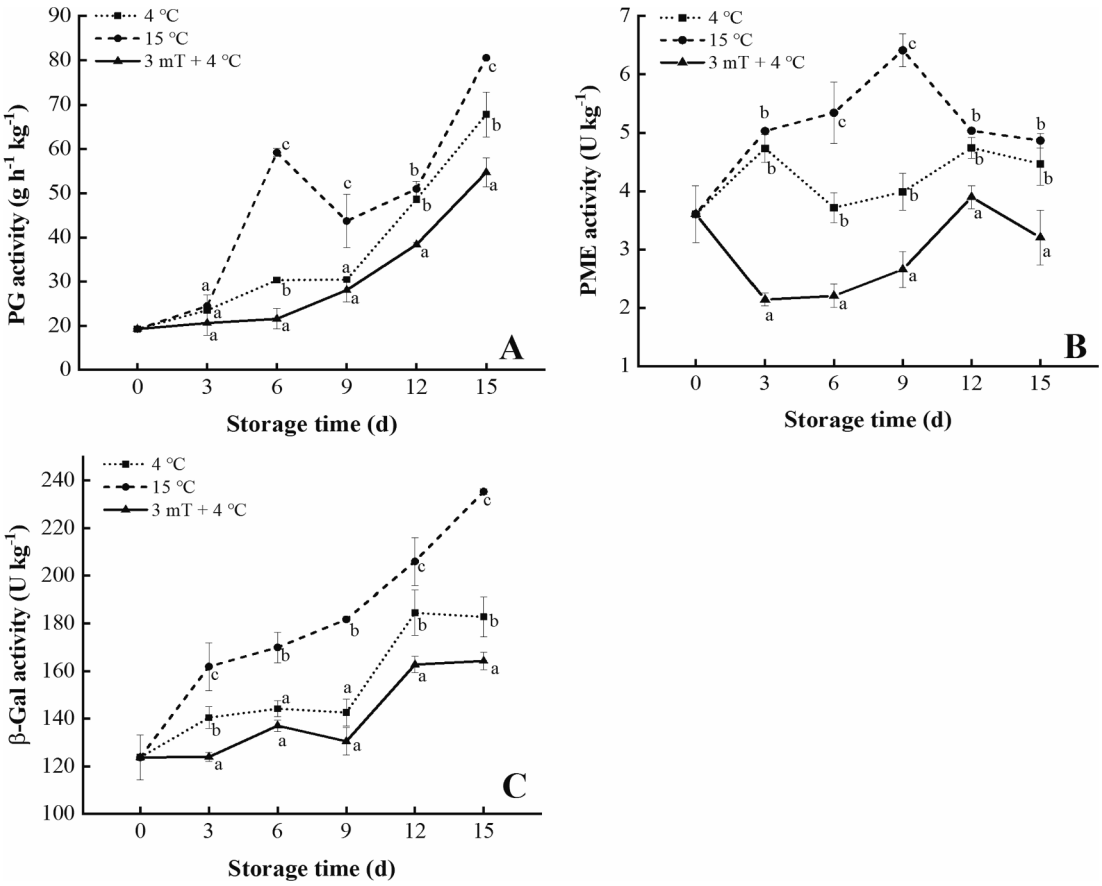

Fig. 6. Effects of magnetic field on PG (A), PME (B) and β-Gal (C) activities of wampee during storage.

(Yang et al., 2020).

Pectin, mainly comprised of covalently linked polysaccharide and rich in galacturonic acid (GalA), is the second most important component of cell wall after cellulose in fruit. More and more studies have demonstrated that pectin plays a crucial role in fruit softening, especially the degradation of pectin (Chen et al., 2023; Li et al., 2022; Yun et al., 2021). Therefore, pectic-polysaccharide metabolism under the effect of magnetic field was paid high attention in our research and cell wall pectic-polysaccharide of wampee was prepared. Three pectin fractions extracted from wampee, including water soluble pectin (WSP), chelate soluble pectin (CSP) and sodium carbonate soluble pectin (SSP), represented loosely-, ionically- and tightly-bound polymers, respectively (Ren et al., 2020). Their structural changes, especially molecular weight distribution and monosaccharide compositions, which had direct influence on storage characteristics of postharvest fruit and closely related with carbohydrate metabolism, were explored. Clearly, our experimental results demonstrated that carbohydrate with relatively higher molecular weight in cell wall pectic-polysaccharides degraded in different extent and their contents varied during storage, while magnetic field combined with low temperature storage could postpone this phenomenon (Fig. 4). Specifically, the area of peak 1 representing high molecular weight in WSP at storage of 15 d in 3 mT + 4 °C group was the highest (42.22 %), followed by 4 °C group (40.64 %) and 15 °C group (32.16 %) in sequence (Fig. 4A-C), which declared that the combined treatment was effective in controlling the degradation of WSP. It was reported that pectin isolated from more ripen fruit displayed lower molecular weight and it might be the consequence of pectin hydrolysis by enzyme such as PG and PME (Yuliarti et al., 2015). Besides, common phenomenon existed in all three groups that carbohydrate with higher molecular weight appeared with the extension of storage time, which might be caused by the conversion of insoluble pectin to water-soluble pectin during fruit softening process (Deng et al., 2019). For CSP, in three groups, the area and height of the first main peak with high molecular weight decreased gradually, and the second main peak increased correspondingly on the whole.

The single broad peak at 15 d of storage in 15 °C group manifested the disappearance of carbohydrate with higher molecular weight, while it was more intense in 3 mT + 4 °C group compared with 4 °C group. As with SSP, lower storage temperature (4 °C) altered the dramatic decreasing tendency in abundance of the main peak in 15 °C group, which might be associated with the depolymerization of SSP long pectin chains during ripening (Wang et al., 2021). When magnetic field treatment was combined with low temperature storage, the intensity or area of the main peak at 0–9 d of storage almost remained unchanged. This phenomenon further elucidated the availability of combined treatment in controlling the degradation of pectin. Most notably, as illustrated in Fig. 5, there seemed to be no obvious pattern in terms of magnetic field effect on monosaccharide compositions of pectic-polysaccharides except fluctuation of contents in certain monosaccharides. In our previous study, it was proved that magnetic field (3 mT, 30 min) could inhibit activity of pectinase as well as alter its secondary structure and then delayed fruit softening of postharvest sapodilla, which was also prefect explanation of present results (Sun et al., 2023).

The solubilization and depolymerization of cell wall pectic-polysaccharides were the consequence of coordinated action of cell wall modifying enzymes related with pectin degradation, including polygalacturonase (PG), pectin methyl esterase (PME), β-galactosidase (β-Gal) and xylanase. It was generally believed that demethylesterification of pectin by PME generated suitable substrate for PG, which was the foremost responsible enzyme for fruit softening associated with pectin modifications and solubilization (Lin et al., 2018). Besides, β-Gal, which could cause obvious loss of galactose independently in cell wall,

was another key pectin debranched enzyme involved in cell wall disassembly during fruit softening (Chang et al., 2017). Commonly, storage condition in low temperature could restrict these enzymic activity in postharvest fruit (Fig. 6) and make fruit texture property better due to decreasing biochemical reaction in fruit relating enzymes. Meanwhile, PG, PME, and β-Gal in wampee treated by magnetic field combined with 4 °C showed lower activity than these of 4 °C condition, especially for PME. Extensive literatures have been reported on magnetic field to regulate activity of enzymes, such as laccase, lysozyme and peroxidase (Prando, et al., 2017; Wasak, et al., 2019; Emamdadi, et al., 2021). The intuitive and measurable changes were shifting enzymic structure with activity alteration for these enzymes mentioned above. And our previously published research also reported that magnetic field treatment could change the polarity of aromatic amino acid residues and cause the modification of functional groups as well as secondary structure in pectinase, thus altering pectinase activity (Sun et al., 2023). Therefore, altering enzymic activity is one of directly reasons to degrade cell wall pectic-polysaccharide and disassemble fruit's cell wall construction, which led to the delay of fruit softening. Beside enzymic structure changes, magnetic field might affect genes expression levels of relating enzymes in fruit. For example, Fang (2020) found the relative expression levels for β-glucosidase and polygalacturonidase in *Cynanchum thesioides (Freyn)* K. Schum could be reduced by alternating magnetic field with intensity of 1.28 mT for 15 min. Furthermore, delay of fruit softening may relate to enhancement of the antioxidant system by exposure in magnetic field, although our research didn't revolve in it. Yang et al., (2020) reported the delay of postharvest cherry tomato ripening process due to the higher activity of the catalase with treatment of magnetic field. And this phenomenon also be observed in seedlings. Celik, et al., (2009) found SOD and catalase activities in soybean roots increased while soybean seeds were exposed to magnetic field. These antioxidant substances could scavenge reactive oxygen species that were produced and accumulated during fruit respiration, thereby maintaining the integrity of the cell membrane and delaying fruits or plant senescence (Song, et al., 2009). Earlier research (de Swardt & Rousseau, 1973) reported the rising membrane permeability of tomatoes coinciding with its climacteric and senescence, and this shifting permeability would result in the loss or transformation of compartmentation and disruptions of the normal distribution of substrates and enzymes in intracellular structures. Therefore, enhancement of antioxidant substances by magnetic field treatment might be another reason for retarding pectic-polysaccharide dissolution and fruit softening. In addition, the growth of microorganisms such as *Escherichia coli* and yeast exposed to the magnetic field was inhibited, and their cell surface appeared obvious damages as well as even the expression of some genes was also affected (Filipic et al., 2012; Rakoczy et al., 2016). However, until now there is rare reports that link the anti-bacteria effect of magnetic field with postharvest fruit preservation. Hence, effects of magnetic field on antioxidant substances, genes expression of key enzymes, and microbial growth in postharvest wampee would be next research aim for us.

5. Conclusion

To sum up, fruit softening of postharvest wampee was accompanied by declined fruit quality and degradation of cell wall pectic-polysaccharides. However, magnetic field treatment combined with low temperature storage could postpone and regulate this phenomenon. To be specific, wampee treated with magnetic field combined with low temperature exhibited reduced browning rate, TSS, flavonoid, total phenols and MDA. In addition, results of molecular weight distribution and monosaccharide compositions indicated degradation of WSP, CSP as well as SSP were also affected, which resulted from the coordinated action of cell wall pectin degradation enzymes. Interestingly, magnetic field treatment combined with low temperature storage reduced activities of these enzymes during storage, including PG, PME and β-Gal. Therefore, magnetic field treatment combined with low temperature storage could be an effective non-thermal technology in preservation of postharvest wampee in future.

CRediT authorship contribution statement

Peng-peng Sun: Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Cheng Liu:** Validation, Software, Methodology, Data curation. **Chong-yang Yu:** Resources, Investigation. **Jue-jun Zhou:** Software, Investigation. **Yuan-yuan Ren:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101253.

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