

Article

Molecular Hydrogen Improves Storage Quality of Bok Choy by Reducing Water Loss and Maintaining Cell Wall Integrity

Guanjie Zhu ¹ , Ronghui Yu ¹ , Yuhao Wang ¹ , Pengfei Cheng ¹, Ke Jiang ¹ , Xin Zhou ² , Feng Cao ² , Zhe Wang ²  and Wenbiao Shen ^{1,*} 

- ¹ Laboratory Center of Life Sciences, College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, China; 2023816117@stu.njau.edu.cn (G.Z.); 2025816133@stu.njau.edu.cn (R.Y.); 2025816132@stu.njau.edu.cn (Y.W.); t2024051@njau.edu.cn (P.C.); 2022216005@stu.njau.edu.cn (K.J.)
- ² Preservation Technology, Advanced Research Center, Hefei Hualing Co., Ltd., Midea Group, Hefei 230601, China; zhouxin911@midea.com (X.Z.); caofeng21@midea.com (F.C.); wangzhe9@midea.com (Z.W.)
- * Correspondence: wbsenh@njau.edu.cn; Tel.: +86-25-84-399-032; Fax: +86-25-84-396-542

Abstract

Bok choy (*Brassica rapa* var. *chinensis*) experiences rapid postharvest quality decline, primarily due to water loss. This study aims to evaluate the effect of H₂-modified atmosphere packaging (MAP; containing 0.01%, 0.1%, and 1% H₂) on extending the shelf life of bok choy. First, we observed that the storage of bok choy for 9 d (4 °C and 85% relative humidity) was accompanied by a decreasing tendency of molecular hydrogen (H₂) production. Subsequently, the effects of hydrogen (H₂) administered via modified atmosphere packaging on the preservation of bok choy were investigated. The results showed that among the treatments, 0.1% H₂ MAP could effectively improve the storage quality of bok choy under identical storage conditions. Compared to the control groups at 9 d, the contents of chlorophyll a/b, carotenoids, total phenols and flavonoids, and total soluble solids were increased by 43.75%, 40%, 27.78%, 28.89%, 102.38%, and 7.29%, and weight loss rate and electrolyte leakage were reduced by 31.77% and 50.19% ($p < 0.05$). Meanwhile, the combined water fraction was increased and respiration rate was decreased. Biochemical evidence further showed that *xyloglucan endotransglycosylase 2/3* (XET2/3) transcripts and xyloglucan endotransglycosylase (XET) activity, responsible for cell wall degradation, were decreased. By contrast, *peroxidase 31/37* (POD31/37) genes and peroxidase (POD) activity, key to the synthesis of lignin, were increased. Importantly, these changes were positively matched with the maintenance of cell wall integrity by H₂ MAP. Together, the results clearly suggested that H₂ MAP could maintain the postharvest quality of bok choy via alleviating water loss and maintaining cell wall integrity. These findings thus provide a useful technology for improving the storage quality of vegetables prone to water loss.

Keywords: *Brassica rapa* var. *chinensis*; hydrogen gas; preservation; shelf life; moisture status



Received: 17 December 2025

Revised: 17 February 2026

Accepted: 17 February 2026

Published: 19 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Bok choy (*Brassica rapa* var. *chinensis*), one of the Brassica plants in the Cruciferae family, is a vegetable native to China that is commonly grown and consumed on a daily basis. Rich in carotenoids, total phenols, flavonoids, and soluble solids, bok choy is an indispensable element on people's tables [1]. Due to harvested bok choy being prone to rapid senescence, characterized by leaf yellowing, water loss, and rotting, short shelf life and significant economic losses become two important concerns. Among these, leaf

moisture status is one of the important parameters that has a strong influence during storage, since transpiration and respiration are still occurring after harvest [2]. Previous studies have shown that the growing conditions of vegetables before harvest significantly affect a series of physiological processes, which in turn influence the postharvest quality of vegetables, including water retention, firmness, and texture. This indicates that preharvest management is an important approach to ensure the postharvest quality of vegetables [3]. However, for postharvest circulation and storage, the physiological characteristics formed in the preharvest environment are already fixed and cannot be altered. Therefore, it is crucial to seek a cost-effective method that has potential use in industrial packing for preventing water loss during the storage period of bok choy and other leafy vegetables.

Ample evidence reveals that cyanobacteria, soil bacteria, plants, and animals (microbes in the gut, etc.) can produce hydrogen (H_2), although the pathways for its production by plant and animal cells are poorly understood compared to those elucidated for cyanobacteria and soil bacteria [4,5]. A related investigation speculated that H_2 might be a possible candidate gasotransmitter, functioning in both animals and plants [6]. This possibility was partially supported by subsequent findings showing that this gas has myriad functions in development and orchestrates responses in response to stress in higher plants, during which cellular metabolic reprogramming occurs [7–11].

Ample evidence has also shown that H_2 has applications in the food industry, mainly including H_2 fumigation, H_2 -modified atmosphere packaging (H_2 MAP), and hydrogen-rich water (HRW). For example, H_2 fumigation could reduce ethylene production and extend the shelf life of postharvest kiwifruit [12]. The deterioration of dried shrimp and the oxidation level of strawberries during storage were positively improved by H_2 MAP [13,14]. The application with HRW not only delayed the ripening of bananas [15] and the softening of okra [16], but also influenced nitrite accumulation in ripened tomato [17]. The reestablishment of redox homeostasis achieved by H_2 supplementation was discovered [7,9,11]. However, the above experiments were mostly conducted at room temperature or higher temperatures. Few studies have been conducted on the effects of H_2 addition on perishable postharvest horticultural products under low-temperature conditions and the interplay between H_2 supplementation and improved postharvest storage quality in terms of changes in the kinetics of endogenous H_2 [18,19]. Since HRW could modulate stomatal closure in *Arabidopsis thaliana* [20], as well as increase the fresh weight of cut flowers by adjusting stomatal size [21], the possibility that H_2 might alleviate the water loss of leafy vegetables during low-temperature storage cannot be easily ruled out. H_2 has shown promising application potential in the postharvest preservation of horticultural products. However, the short residence time of H_2 in HRW and the safety issues associated with H_2 fumigation limit its application in agricultural practice. The controllability of H_2 when applied in MAP gives it greater commercialization potential [22]. Therefore, this study used MAP to investigate the effects of H_2 on bok choy.

In this study, we clearly observed impaired H_2 homeostasis when bok choy was stored for 9 d (4 °C and 85% relative humidity). The role of H_2 in extending the shelf life of bok choy was elucidated by adopting H_2 MAP (containing 0.01%, 0.1%, and 1% H_2). Further investigation aimed to discover how H_2 MAP improves the storage quality of bok choy, especially exploring the detailed mechanisms at physiological and molecular levels. The involvement of improved water loss and cell wall integrity was accordingly suggested. Together, these findings have theoretical and practical significance and also provide a new and efficient strategy for improving the storage quality of leafy vegetables prone to water loss.

2. Results

2.1. H_2 Homeostasis and Shelf Life of Bok Choy Are Linked

As can be observed in Figure 1A, severe yellowing in the control leaves appeared on the 9th day of storage. Meanwhile, a decrease in endogenous H_2 contents (as determined by GC) occurred in a time-dependent fashion (Figure 1B). These results clearly indicated the possible interaction between bok choy leaf yellowing and impaired H_2 homeostasis during storage. Furthermore, since the addition of 0.1% N_2 (air + 0.1% N_2) did not affect the endogenous H_2 content in bok choy (Figure S1), we speculated that the increase in H_2 content in bok choy may primarily originate from H_2 MAP rather than its endogenous synthesis.

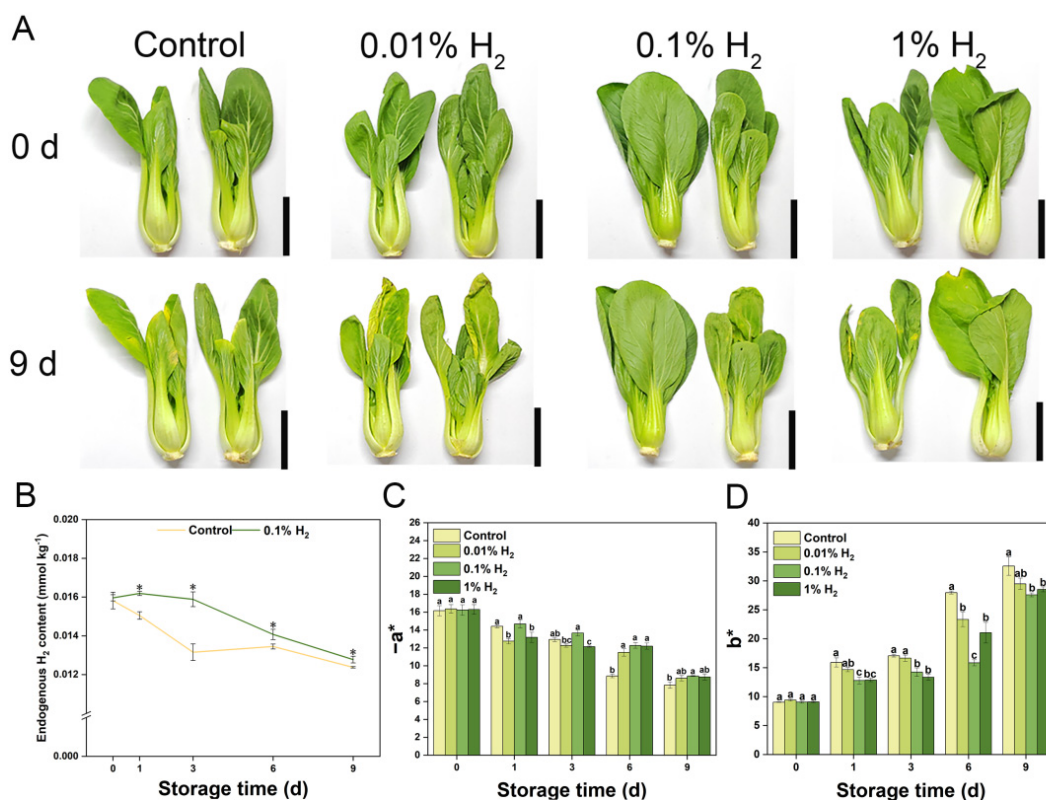


Figure 1. Altered appearance (A), endogenous H_2 content (B), chroma $-a^*$ (C), and chroma b^* (D) in bok choy during storage for 0, 1, 3, 6, and 9 d after H_2 MAP. Scale bar = 5 cm. The vertical bar represents the standard error ($n = 3$ replicates). According to Duncan's multiple range test, different letters for each storage time indicate statistically significant difference ($p < 0.05$). The asterisks indicate significant differences for each storage time (t -test; $* p < 0.05$).

Further results uncovered that compared to the control, the H_2 MAP-treated bok choy exhibited differentially delayed yellowing, with the most significant effect being in the presence of 0.1% H_2 MAP, remaining almost green until the end of the storage experiment (day 9). Meanwhile, a decrease in H_2 contents was delayed by the addition of 0.1% H_2 MAP, partially confirming the reestablishment of H_2 homeostasis.

Then, the color of bok choy was further determined. As illustrated in Figure 1C, the decrease in $-a^*$ value was significantly retarded by 0.1% H_2 MAP at 9 d ($p < 0.05$) (Figure 1C). H_2 MAP groups were also effective in impairing the degrees of increase in the b^* value (Figure 1D); in particular, 0.1% H_2 MAP was only 27.59 ± 0.34 at 9 d, compared to 32.56 ± 1.60 in the control group. The above results clearly indicated that H_2 MAP was effective at delaying the yellowing of bok choy and maintaining its green color.

2.2. H₂ MAP Maintains Chlorophyll, Carotenoid, Total Soluble Solids (TSS), Phenol, and Flavonoid Contents During Low-Temperature Storage

As illustrated in Table 1, the decreasing trend of chlorophyll a/b, carotenoid contents, and TSS during storage was differentially abolished or slowed down by H₂ MAP groups. At 9 d, the contents of chlorophyll a/b and carotenoids in the 0.1% H₂ MAP group were 43.75%, 40%, and 27.78% higher than those of the control groups, respectively. Likewise, the TSS content of the 0.01% H₂, 0.1% H₂, and 1% H₂ MAP groups was 1.19%, 7.29%, and 2.88%, respectively, higher than that of the control group at 9 d.

Table 1. Changes in chlorophyll a, chlorophyll b, carotenoids, total soluble solids, phenolic, and flavonoids contents in bok choy during storage for 0, 1, 3, 6, and 9 d after H₂ MAP.

Indices	Treatment	Storage Time (Days)				
		0	1	3	6	9
Chlorophyll a content (g kg ⁻¹ FW)	Control	1.01 ± 0.03 ^a	0.77 ± 0.01 ^c	0.75 ± 0.01 ^d	0.61 ± 0.01 ^c	0.32 ± 0.01 ^c
	0.01% H ₂	1.04 ± 0.01 ^a	0.93 ± 0.00 ^{ab}	0.85 ± 0.00 ^c	0.77 ± 0.01 ^b	0.39 ± 0.01 ^b
	0.1% H ₂	1.02 ± 0.00 ^a	0.94 ± 0.01 ^a	0.92 ± 0.01 ^a	0.83 ± 0.01 ^a	0.46 ± 0.01 ^a
	1% H ₂	1.03 ± 0.01 ^a	0.91 ± 0.00 ^b	0.89 ± 0.01 ^b	0.78 ± 0.01 ^b	0.46 ± 0.00 ^a
Chlorophyll b content (g kg ⁻¹ FW)	Control	0.40 ± 0.01 ^a	0.33 ± 0.01 ^b	0.30 ± 0.01 ^c	0.22 ± 0.00 ^b	0.15 ± 0.00 ^b
	0.01% H ₂	0.41 ± 0.00 ^a	0.35 ± 0.01 ^{ab}	0.32 ± 0.00 ^b	0.23 ± 0.00 ^b	0.19 ± 0.01 ^a
	0.1% H ₂	0.41 ± 0.01 ^a	0.36 ± 0.01 ^a	0.35 ± 0.01 ^a	0.25 ± 0.01 ^a	0.21 ± 0.01 ^a
	1% H ₂	0.40 ± 0.01 ^a	0.34 ± 0.01 ^{ab}	0.33 ± 0.01 ^b	0.25 ± 0.01 ^a	0.19 ± 0.01 ^a
Carotenoid content (g kg ⁻¹ FW)	Control	0.42 ± 0.00 ^a	0.29 ± 0.01 ^{ab}	0.25 ± 0.01 ^b	0.20 ± 0.00 ^c	0.18 ± 0.00 ^c
	0.01% H ₂	0.41 ± 0.00 ^a	0.29 ± 0.00 ^{ab}	0.26 ± 0.01 ^{ab}	0.22 ± 0.00 ^b	0.20 ± 0.01 ^b
	0.1% H ₂	0.41 ± 0.00 ^a	0.30 ± 0.00 ^a	0.27 ± 0.00 ^a	0.24 ± 0.00 ^a	0.23 ± 0.00 ^a
	1% H ₂	0.42 ± 0.01 ^a	0.29 ± 0.00 ^b	0.24 ± 0.00 ^b	0.21 ± 0.00 ^b	0.21 ± 0.00 ^b
Total soluble solids (%)	Control	9.57 ± 0.09 ^a	7.03 ± 0.13 ^c	6.50 ± 0.21 ^b	6.10 ± 0.12 ^b	5.90 ± 0.06 ^b
	0.01% H ₂	9.37 ± 0.09 ^a	7.70 ± 0.12 ^{ab}	7.23 ± 0.13 ^a	6.47 ± 0.12 ^{ab}	5.97 ± 0.09 ^{ab}
	0.1% H ₂	9.53 ± 0.15 ^a	7.93 ± 0.03 ^a	7.37 ± 0.09 ^a	6.73 ± 0.09 ^a	6.33 ± 0.17 ^a
	1% H ₂	9.60 ± 0.15 ^a	7.43 ± 0.03 ^b	7.13 ± 0.07 ^a	6.60 ± 0.15 ^a	6.07 ± 0.09 ^{ab}
Phenolic content (g GAE kg ⁻¹ FW)	Control	1.65 ± 0.02 ^a	1.27 ± 0.01 ^c	1.11 ± 0.04 ^c	1.07 ± 0.05 ^b	0.90 ± 0.04 ^c
	0.01% H ₂	1.63 ± 0.04 ^a	1.53 ± 0.01 ^b	1.30 ± 0.04 ^b	1.07 ± 0.04 ^b	1.01 ± 0.04 ^{bc}
	0.1% H ₂	1.65 ± 0.03 ^a	1.61 ± 0.03 ^a	1.45 ± 0.03 ^a	1.30 ± 0.03 ^a	1.16 ± 0.06 ^a
	1% H ₂	1.65 ± 0.04 ^a	1.59 ± 0.03 ^a	1.28 ± 0.03 ^b	1.19 ± 0.03 ^{ab}	1.09 ± 0.03 ^{ab}
Flavonoid content (g CE kg ⁻¹ FW)	Control	1.10 ± 0.05 ^a	0.83 ± 0.05 ^c	0.76 ± 0.02 ^b	0.68 ± 0.06 ^b	0.42 ± 0.04 ^c
	0.01% H ₂	1.13 ± 0.05 ^a	0.89 ± 0.04 ^{bc}	0.87 ± 0.06 ^b	0.73 ± 0.06 ^{ab}	0.58 ± 0.06 ^{bc}
	0.1% H ₂	1.08 ± 0.07 ^a	1.10 ± 0.05 ^a	1.08 ± 0.08 ^a	0.89 ± 0.05 ^a	0.85 ± 0.06 ^a
	1% H ₂	1.12 ± 0.04 ^a	0.99 ± 0.04 ^{ab}	0.81 ± 0.04 ^b	0.75 ± 0.06 ^{ab}	0.72 ± 0.04 ^{ab}

Data for quantification analyses are presented as mean ± SE (*n* = 3 replicates). According to Duncan's multiple range test, different letters for each storage time indicate statistically significant difference (*p* < 0.05).

Similarly, H₂ MAP obviously mitigated the decreases in total phenol and flavonoid contents, and, in particular, the contents of the 0.1% H₂ MAP group were 28.89% and 102.38% higher than those of the control group at 9 d. Flavonoids are important components of phenolic compounds [23]. Therefore, we mainly analyzed the changes in total flavonoid content. Further analysis obtained different data on the total flavonoid contents when using rutin (Figure S2) or catechin (Table 1) as a standard for measurements. We also observed that the total flavonoid content even exceeded the total phenol content when rutin was used as a standard (Figure S2), and a similar phenomenon was previously reported [24]. This discrepancy may be due to the inherent bias of determining a complex class of compounds using a single standard.

2.3. Changes in O₂ and CO₂ Content

The peak area of O₂ and the percentage of CO₂ were used to represent the relative content of O₂ and CO₂ for trend analysis during storage (Figure S3). The results showed that the residual O₂ levels in the H₂ MAP groups were higher than in the control group during storage. In contrast, the residual CO₂ level was relatively lower than in the control group, especially in the 0.1% H₂ MAP group.

2.4. The Increases in Weight Loss Rate, Electrolyte Leakage, Respiration Rate, and Firmness of Bok Choy Were Delayed by H₂ MAP

The weight loss rate of bok choy in the control group changed rapidly and reached the highest level of about 9.6% at the end of storage (9 d; Table 2). Comparatively, the weight loss rate in the 0.1% H₂ MAP group was only about 6.55% ($p < 0.05$). Water loss can greatly affect the textural properties of horticultural products post-harvest. The decreasing tendency of firmness was also reduced by 0.1% H₂ MAP (Figure S4).

Table 2. Changes in weight loss rate, electrolyte leakage, respiration rate, hemicellulose, cellulose, and lignin contents in bok choy during storage for 0, 1, 3, 6, and 9 d after H₂ MAP.

Indices	Treatment	Storage Time (Days)				
		0	1	3	6	9
Weight loss rate (%)	Control	0	1.42 ± 0.05 ^a	3.31 ± 0.23 ^a	8.54 ± 0.69 ^a	9.60 ± 0.79 ^a
	0.01% H ₂	0	1.16 ± 0.23 ^a	2.70 ± 0.46 ^{ab}	7.45 ± 0.68 ^{ab}	9.26 ± 0.19 ^a
	0.1% H ₂	0	1.33 ± 0.21 ^a	2.25 ± 0.15 ^b	6.03 ± 0.17 ^b	6.55 ± 0.55 ^b
	1% H ₂	0	1.12 ± 0.38 ^a	2.66 ± 0.25 ^{ab}	7.31 ± 0.40 ^{ab}	8.57 ± 0.46 ^a
Electrolyte leakage (%)	Control	3.02 ± 0.60 ^a	5.54 ± 0.71 ^a	8.80 ± 0.62 ^a	12.25 ± 0.51 ^a	13.49 ± 0.26 ^a
	0.01% H ₂	3.26 ± 0.10 ^a	6.77 ± 0.06 ^a	8.51 ± 0.27 ^a	10.97 ± 0.15 ^a	11.91 ± 0.35 ^b
	0.1% H ₂	3.01 ± 0.29 ^a	3.85 ± 0.39 ^b	4.61 ± 0.65 ^b	5.83 ± 0.24 ^b	6.72 ± 0.31 ^c
	1% H ₂	3.33 ± 0.14 ^a	3.75 ± 0.23 ^b	4.89 ± 0.38 ^b	7.11 ± 0.65 ^b	11.03 ± 0.51 ^b
Respiration rate (mg CO ₂ kg ^{−1} h ^{−1})	Control	105.95 ± 2.78 ^a	74.96 ± 1.52 ^a	70.63 ± 1.62 ^a	65.82 ± 1.62 ^a	40.13 ± 1.62 ^a
	0.01% H ₂	104.34 ± 1.44 ^a	68.64 ± 1.33 ^b	64.52 ± 1.36 ^b	59.15 ± 2.00 ^b	32.64 ± 2.04 ^b
	0.1% H ₂	105.52 ± 1.43 ^a	40.47 ± 1.44 ^c	24.57 ± 1.45 ^c	18.79 ± 1.46 ^d	8.68 ± 2.51 ^d
	1% H ₂	101.99 ± 1.97 ^a	65.28 ± 2.08 ^b	63.24 ± 2.09 ^b	52.17 ± 1.34 ^c	19.22 ± 1.36 ^c
Hemicellulose content (g kg ^{−1} DW)	Control	7.03 ± 0.25 ^a	4.27 ± 0.02 ^c	3.51 ± 0.06 ^d	3.05 ± 0.06 ^b	0.94 ± 0.07 ^d
	0.01% H ₂	6.92 ± 0.06 ^a	5.57 ± 0.06 ^a	4.13 ± 0.03 ^c	3.01 ± 0.11 ^b	1.95 ± 0.03 ^c
	0.1% H ₂	6.99 ± 0.14 ^a	5.62 ± 0.05 ^a	4.91 ± 0.02 ^a	3.82 ± 0.12 ^a	3.51 ± 0.03 ^a
	1% H ₂	7.00 ± 0.13 ^a	4.90 ± 0.05 ^b	4.66 ± 0.02 ^b	3.83 ± 0.13 ^a	2.51 ± 0.05 ^b
Cellulose (g kg ^{−1} DW)	Control	13.07 ± 0.47 ^a	11.86 ± 0.11 ^b	10.36 ± 0.22 ^a	9.81 ± 0.18 ^b	9.23 ± 0.10 ^c
	0.01% H ₂	13.57 ± 0.76 ^a	12.47 ± 0.13 ^a	11.09 ± 0.08 ^a	9.84 ± 0.20 ^b	9.41 ± 0.14 ^{bc}
	0.1% H ₂	13.07 ± 0.42 ^a	12.26 ± 0.12 ^{ab}	11.45 ± 0.13 ^a	10.88 ± 0.16 ^a	10.40 ± 0.08 ^a
	1% H ₂	13.07 ± 0.35 ^a	12.28 ± 0.25 ^{ab}	11.02 ± 0.20 ^a	10.49 ± 0.35 ^{ab}	9.71 ± 0.13 ^b
Lignin (g kg ^{−1} DW)	Control	3.18 ± 0.04 ^a	2.58 ± 0.10 ^b	2.16 ± 0.02 ^d	1.90 ± 0.03 ^c	1.78 ± 0.04 ^c
	0.01% H ₂	3.19 ± 0.02 ^a	3.11 ± 0.07 ^a	2.84 ± 0.06 ^b	2.24 ± 0.10 ^b	2.07 ± 0.05 ^b
	0.1% H ₂	3.24 ± 0.03 ^a	3.25 ± 0.07 ^a	3.09 ± 0.05 ^a	2.82 ± 0.02 ^a	2.57 ± 0.06 ^a
	1% H ₂	3.26 ± 0.02 ^a	2.80 ± 0.06 ^b	2.52 ± 0.10 ^c	2.47 ± 0.11 ^b	2.18 ± 0.09 ^b

Data for quantification analyses are presented as mean ± SE ($n = 3$ replicates). According to Duncan's multiple range test, different letters for each storage time indicate statistically significant difference ($p < 0.05$).

The changes in electrolyte leakage characterize the degree of damage in the cell membrane of leafy vegetables. The results showed that the electrolyte leakage of the control bok choy was mostly kept at the highest levels, from $3.02 \pm 0.6\%$ (0 d) to $13.49 \pm 0.26\%$ (9 d; Table 2). Although the electrolyte leakage in the 0.1% H₂ MAP group increased substantially from the initial value of $3.01 \pm 0.29\%$ (0 d), this amount was still 50.19%, 43.58%, and 39.08% lower than the control, 0.01% H₂, and 1% H₂ MAP groups, respectively (9 d). Consistently, time course changes in damaged cells stained with Evans blue exhibited similar tendencies, and, in particular, the 0.1% H₂ MAP group displayed the weakest staining (Figure S5).

During the first three days of storage, the respiration rate of bok choy in all groups showed a decreasing trend (Table 2), which might be due to the inhibition of respiration by low temperature [25]. At 9 d, the respiratory rate in the control group reached 40.13 ± 1.62 mg CO₂ kg^{−1} h^{−1}. Comparatively, the respiration rate of H₂ MAP groups was lower than that of the control group at 9 d ($p < 0.05$). In particular, the 0.1% H₂ MAP group only reached 8.68 ± 2.51 mg CO₂ kg^{−1} h^{−1}. This result was further supported by a subsequent evaluation of the residual O₂ and CO₂ in the boxes during storage, showing relatively lower levels of residual CO₂ in particular, especially in the 0.1% H₂ MAP group, compared to the control (Figure S3). Since the respiration of vegetables postharvest leads to weight loss, softening, and shriveling [26], these differences may indicate that H₂ MAP

could reduce the loss of water by inhibiting the respiration rate of bok choy. It should be noted that in this experiment, the O_2 content was expressed as the relative peak area in GC, reflecting its relative change rather than the absolute concentration. In the present study, H_2 MAP treatment reduced the respiration rate of bok choy and resulted in lower residual CO_2 and higher residual O_2 in the H_2 MAP groups compared with the control group (Figure S3). This pattern of gas changes was opposite to the typical characteristics of classical MAP. Therefore, we speculated that the postharvest quality preservation effect of H_2 treatment on bok choy may mainly originate from the specific biological effects of H_2 . Nonetheless, changes in gas composition during storage were influenced by both respiratory kinetics and the microenvironment inside the box. Therefore, the potential contribution of O_2/CO_2 -driven respiration change cannot be completely ruled out. Further studies could be carried out to precisely control the background gas concentrations, thus clarifying the independent effect of H_2 itself.

2.5. Changes in Hemicellulose, Cellulose, and Lignin Contents in Response to H_2 MAP

The composition and structure of the cell wall determine cell morphology and function, and also play important roles in environmental response [27]. Here, we observed that the hemicellulose contents of the control group decreased from $7.03 \pm 0.25 \text{ g kg}^{-1}$ (0 d) to $0.94 \pm 0.07 \text{ g kg}^{-1}$ (9 d; Table 2). Comparatively, the hemicellulose contents of the 0.01% H_2 ($1.95 \pm 0.03 \text{ g kg}^{-1}$), 0.1% H_2 ($3.51 \pm 0.03 \text{ g kg}^{-1}$), and 1% H_2 MAP ($2.51 \pm 0.05 \text{ g kg}^{-1}$) groups were significantly higher than that of the control group at 9 d.

The cellulose content of the control group decreased from $13.07 \pm 0.47 \text{ g kg}^{-1}$ (0 d) to $9.23 \pm 0.1 \text{ g kg}^{-1}$ (9 d). Similarly, the cellulose contents of the 0.01% H_2 ($9.41 \pm 0.14 \text{ g kg}^{-1}$), 0.1% H_2 ($10.40 \pm 0.08 \text{ g kg}^{-1}$), and 1% H_2 MAP groups ($9.71 \pm 0.13 \text{ g kg}^{-1}$) were still higher than that of the control at 9 d.

The lignin content of the control group decreased from $3.18 \pm 0.04 \text{ g kg}^{-1}$ (0 d) to $1.78 \pm 0.04 \text{ g kg}^{-1}$ (9 d). Among the H_2 MAP groups, the lignin content of the 0.1% H_2 MAP group ($2.57 \pm 0.06 \text{ g kg}^{-1}$) was the highest compared to that of the control. These results indicated that 0.1% H_2 MAP significantly impaired the decreases in hemicellulose, cellulose, and lignin content, thus maintaining cell wall integrity.

2.6. Moisture Status Changes in the Presence of H_2 MAP

As leaves senesce in storage, transverse relaxation time is correlated with senescence, reflecting the fluidity of water molecules. Figure 2 shows the distribution of transverse relaxation time of bok choy after different treatments at 1 d, 3 d, 6 d, and 9 d of storage. T_{21} , T_{22} , and T_{23} , three obvious proton relaxation peaks in the relaxation time spectrum of bok choy, represent the presence of combined water, semi-bound water, and free water in the tissues, and the corresponding peak area is used to indicate the relative content of water in the three states. As storage time was extended, the T_{21} (combined water) peak area of all groups showed a decreasing trend and the onset value showed increasing tendencies (Figure 2, Table S1), indicating that the internal water-binding capacity of bok choy was decreased. For example, on 0 d, the T_{21} signal appeared at 0.577 ms, with a peak area of 230.734. After 9 d, the T_{21} signal of the control group appeared at 5.941 ms, with a peak area of 32.838. The 0.01% H_2 MAP, 0.1% H_2 MAP, and 1% H_2 MAP groups showed signals at 2.967, 2.768, and 3.181 ms, with peak areas of 55.879, 62.335, and 55.764. Regarding peak areas, the H_2 MAP groups (especially 0.1% H_2 MAP) had much higher combined water activity than the control group on the same day.

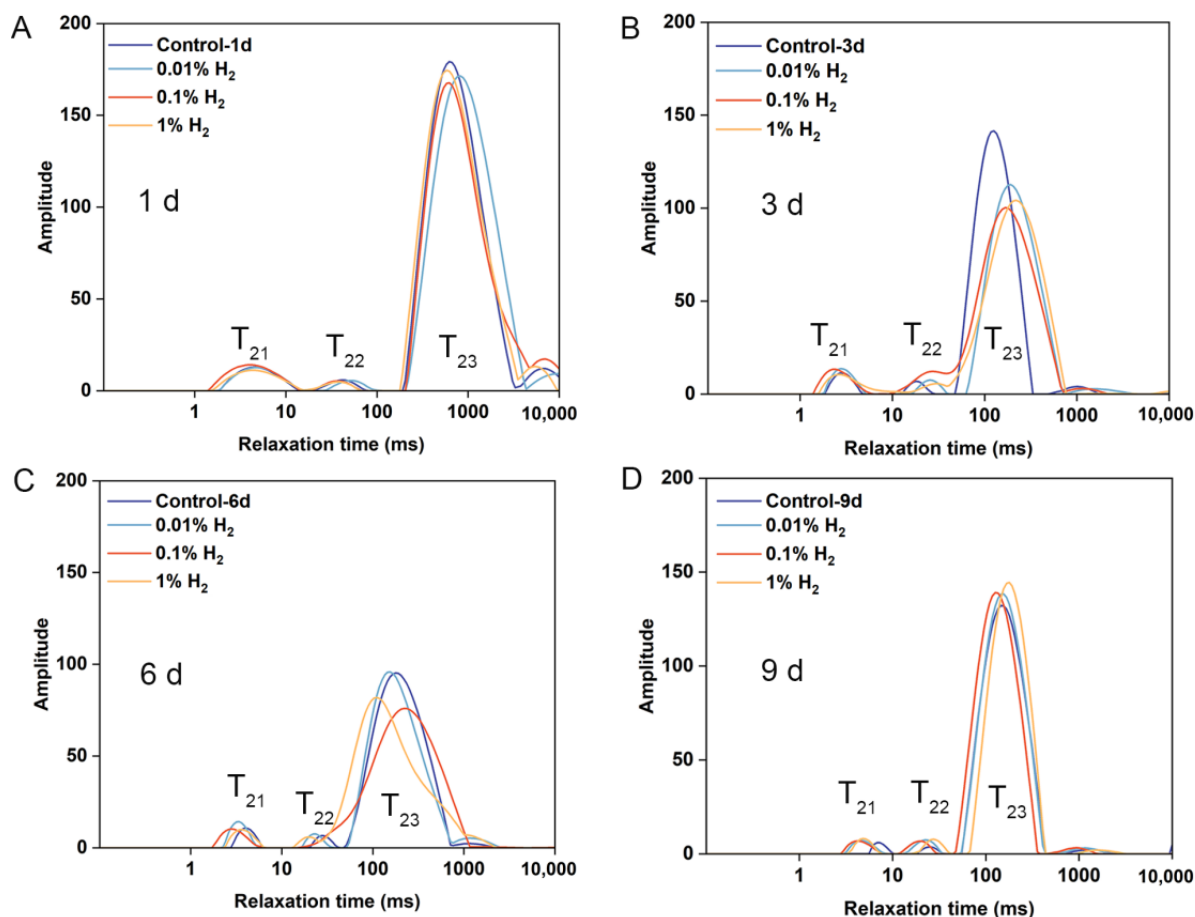


Figure 2. T_2 relaxation time spectrum (1, 3, 6, and 9 d for (A–D)) in the stored bok choy after H_2 MAP. T_{21} , T_{23} , and T_{22} denote combined water, free water, and semi-bound water, respectively.

Meanwhile, we observed that the free water activity (T_{23}) of the control group was greater than that of the H_2 MAP groups (except 1% H_2 MAP) on the sixth day. A high level of free water activity (T_{23}) in plants often means that the binding of water molecules is reduced, making it easier for water to be lost [28]. These results suggested that H_2 MAP significantly inhibited the decrease in the binding capacity of combined water and differentially delayed the conversion of combined water to free water.

2.7. Light Microscopy Observation

Figure S6 shows an optical micrograph of the stem of bok choy at 0 d and 9 d of storage. Compared with the control group, H_2 MAP groups (especially 0.1% H_2 MAP) had a clearer and less damaged cell wall profile.

Open stomata tend to accelerate water loss, leading to a decrease in quality. We observed that during storage, the stomata of bok choy were open until day 6 in the control, whereas they were inhibited to varying degrees in the H_2 MAP groups. Among these, the 0.1% H_2 MAP treatment obviously reduced bok choy stomatal opening (Figure S7).

2.8. Reprogramming of Gene Expression Related to Cell Wall Degradation and Lignin Synthesis

$XET2/3$ is associated with cell wall polysaccharide degradation, and $POD31/37$ is responsible for lignin synthesis [29]. In order to further elucidate the molecular mechanisms driven by H_2 MAP, an analysis of the transcriptional abundance of $XET2/3$ and $POD31/37$ genes was conducted by RT-qPCR.

As illustrated in Figure 3A,B, transcriptional profiles of *XET2/3* were differentially reduced by H₂ MAP treatments (especially 0.1% H₂ MAP), reflecting that H₂ may down-regulate the expression of genes related to cell wall degradation, thus maintaining storage quality. Changes in XET activity displayed a similar tendency (Figure 3C).

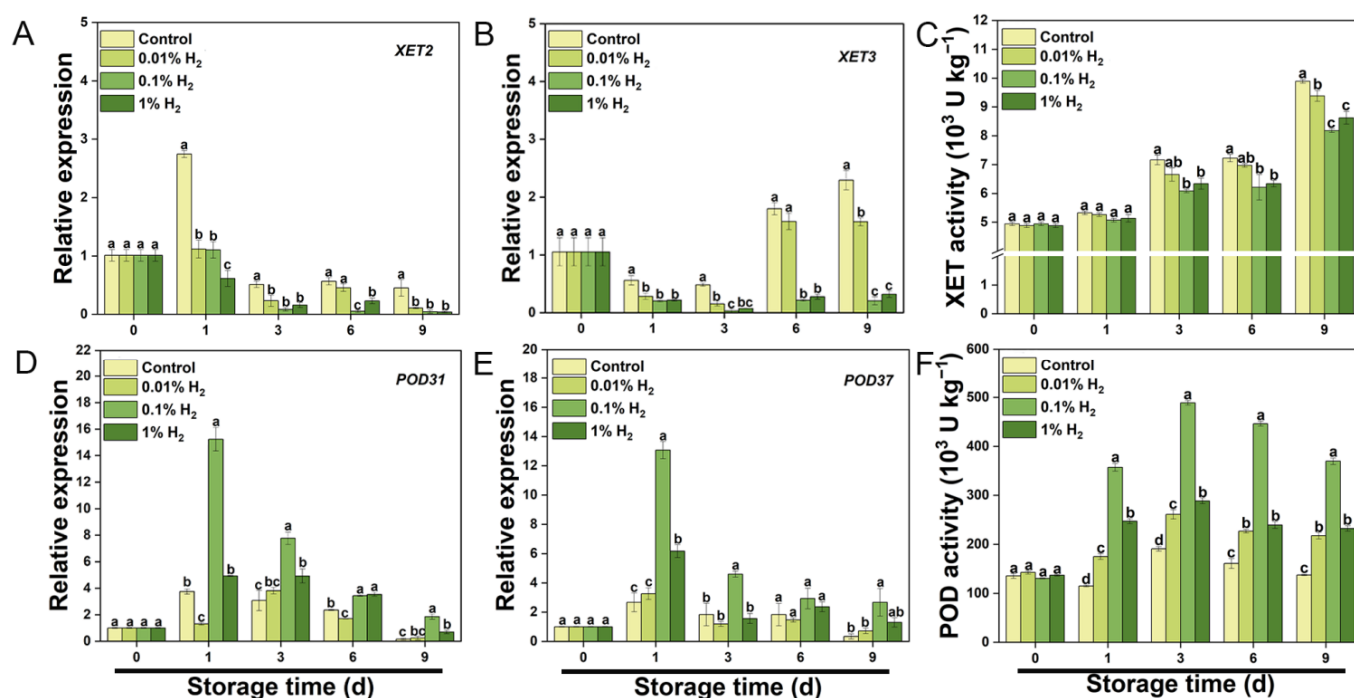


Figure 3. Changes in gene expression of *XET2* (A) and *XET3* (B) and XET activity (C) and gene expression of *POD31* (D) and *POD37* (E) and POD activity (F) in bok choy during storage for 0, 1, 3, 6, and 9 d after H₂ MAP. The vertical bar represents the standard error ($n = 3$ replicates). According to Duncan's multiple range test, different letters for each storage time indicate statistically significant difference ($p < 0.05$).

During the storage period of bok choy, the expression pattern of *POD31/37* and POD activity showed an increase followed by a decreasing tendency (Figure 3D–F). For H₂ MAP treatments (especially 0.1% H₂ MAP), the above-mentioned changes were further differentially stimulated.

2.9. Correlation Analysis Between Cell Wall Components and Physicochemical Indexes

The cell wall strengthens the plant body and plays a key role in water movement and defense [30]. As shown in Figure 4, in the presence of 0.1% H₂ MAP, the total contents of cell wall components displayed a positive correlation with firmness, $-a^*$, chlorophyll a/b, total phenols, total flavonoids, total soluble solids, carotenoids, and endogenous H₂ content ($p < 0.05$), and a negative correlation with weight loss rate, electrolyte leakage, and b^* . Also, we noticed that firmness was negatively correlated with weight loss. Previous studies have shown that high moisture retention can reduce weight loss and maintain firmness, which is consistent with what can be observed in Figure 2 [31]. This further confirms that the storage under air-tight conditions or in a controlled atmosphere may significantly support water retention, thereby reducing weight loss and decline in firmness.

Importantly, we observed that after treatment with 0.1% H₂ MAP for 9 d, lignin content and POD (a key enzyme for lignin synthesis) activity were positively correlated, while hemicellulose and cellulose content were negatively correlated with XET activity, which could be functionally associated with cell wall polysaccharide degradation [32,33].

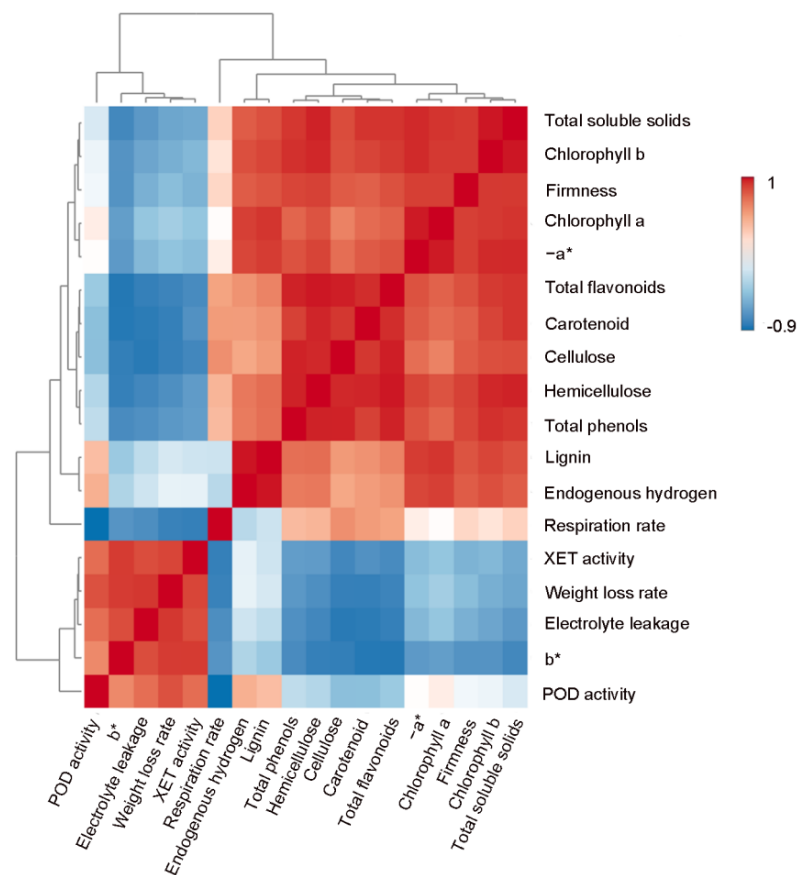


Figure 4. Pearson's correlation heatmap of cell wall components, enzyme activity changes, nutritional indexes, and quality-related parameters in bok choy after 0.1% H₂ MAP for 9 d. $p < 0.05$ was considered statistically significant and highly significant. Colored bars indicate correlation coefficients (r) from low to high. XET: xyloglucan endotransglycosylase. POD: peroxidase. The asterisk * is part of the standard nomenclature of the CIELAB color system.

3. Discussion

Improving the nutritional and postharvest quality of agricultural products is an important topic of hydrogen agriculture research, although a number of key questions still remain to be elucidated [34]. In particular, given the current absence of clarity regarding the possible enzymatic sources of H₂ in plants, it would be helpful to understand whether or how H₂ supply might influence H₂ homeostasis and alter physiological responses via gene or metabolic reprogramming. In this report, H₂ MAP treatment was implemented, and we discovered that this approach could not only reestablish H₂ homeostasis occurring during bok choy storage, but also improve storage quality through reducing water loss and influencing cell wall composition, although other mechanisms may also be involved. Consistently, the transcriptional profiles of representative genes related to lignin synthesis and cell wall degradation were regulated.

As illustrated in Figure 1, we clearly observed that H₂ MAP treatment, especially 0.1% H₂ MAP, significantly delayed or slowed down the yellowing of bok choy during storage (Figure 1A,C,D). This conclusion was evaluated by assessing the rescuing influence achieved by 0.1% H₂ MAP in the reduction in chlorophyll, carotenoids, total soluble solids, total phenols, and flavonoids contents during storage (Table 1). Meanwhile, 0.1% H₂ MAP obviously maintained the firmness of bok choy during storage (Figure S4). Most importantly, impaired H₂ homeostasis during storage was observed in the 0.1% H₂ MAP group (Figure 1B). By contrast, 0.1% H₂ MAP significantly restored the decline of endogenous H₂ content during the storage of bok choy, suggesting that the exogenous application of H₂

may be able to maintain the quality of bok choy during storage by reestablishing H₂ homeostasis in bok choy. It should be noted that endogenous H₂ was only measured in the 0.1% H₂ MAP and control groups, which limits the dose–response analysis between exogenous H₂ and endogenous H₂ response. However, this mechanism is consistent with the findings of Jin et al. [18], who demonstrated that H₂ MAP improved quality by reestablishing H₂ homeostasis in fresh-cut apple slices. These findings reflected the possibility of using it as a potential gasotransmitter [6] and that the spatial and temporal regulation of H₂ production is essential for maintaining storage quality.

Leafy vegetables still have vigorous metabolic activity after harvesting. The weight loss rate, respiration rate, and the electrolyte leakage of bok choy continued to increase, thus seriously reducing its quality during the storage period [25]. In the H₂ MAP groups, we herein found that the improved quality of stored bok choy was greatly and positively linked to the inhibition of water loss rate, electrolyte leakage, and respiration rate (Table 2). The evaluation of moisture status changes further showed that 0.1% H₂ MAP significantly inhibited the reduction in the binding capacity of combined water and differentially slowed down the conversion of combined water to free water during storage (Figure 2; Table S1). Similarly, it was found that a high-voltage electrostatic field combined with light-emitting diode irradiation reduced the free movement of water in pak choi and delayed postharvest aging [25]. Additionally, similar results were previously obtained when H₂ MAP was applied in strawberries [13], fresh apple slices [18], and fresh potato slices [19]. These results propose a common beneficial function of H₂ when applied to horticultural products, especially the aforementioned leafy vegetables. Concurrently, Fanourakis et al. [3] found that preharvest conditions and species variation affect vegetable water relations and physiological status, thereby influencing postharvest water retention. The efficacy of H₂ MAP shows the potential to modulate this multi-factor quality pathway (cultivation × genotype × packaging), offering a strategy to optimize nutritional quality and storage tolerance of leafy vegetables.

It is well known that plant transpiration cannot be separated from stomatal movement, and the opening and closing movement of stomata must rely on the elasticity of the guard cell wall for the requirements of stomatal action [35]. Cellulose, hemicellulose, and lignin in the cell wall are required for proper stomata function [36]. We subsequently found that hemicellulose, cellulose, and lignin levels continued to decrease during storage (Table 2). By contrast, the 0.1% H₂ MAP treatment effectively maintained the above-mentioned cell wall components. We speculated that these alterations may alleviate the loss of the normal physiological function of stomata, which in turn decreases the water loss of bok choy. This conjecture was further confirmed in the microstructure observation of bok choy, where 0.1% H₂ MAP displayed the maximal closing of stomata during the storage period (Figure S7). An in-depth investigation into the molecular and biochemical mechanisms underlying this regulatory pathway will be the focus of our future work.

Previous studies have shown that high expression of *XET2/3* weakens the cell wall and leads to loosening of the cell wall structure, leading to the softening and deterioration of fruit and vegetables during storage [28,37]. Lignin provides mechanical support for plant organs and improves sap conduction through lignified vascular components, and peroxidase is considered to be a key enzyme involved in lignin synthesis [31]. Previous studies have reported that cellulose and hemicellulose contents in amaranth decreased with the decline of fresh weight loss [38], and the increase in lignin content in celery slowed down softening [39]. These findings are consistent with our observations in the present study (Table 2; Figure S4). In this study, the expression of *XET2/3* and *XET* activity were relatively higher in the control than in 0.1% H₂ MAP group, especially after 1 d of storage (Figure 3A–C). By contrast, *POD* activity and expression of *POD31/37* transcripts [28] were

differentially stimulated by 0.1% H₂ MAP during storage (Figure 3D–F). Observation of bok choy stem tissues under a microscope showed that 0.1% H₂ MAP treatment reduced cell wall damage (Figure S6). These results further suggest that H₂ MAP could maintain cell wall integrity by regulating enzyme activity and the transcriptional abundance of related genes. The above conclusions were only based on evidence at the transcriptional and enzymatic levels, and the molecular mechanisms involved remain to be further elucidated.

Similarly, a previous report revealed that acidic electrolyzed water inhibited the expression of cell wall-degrading enzymes and encoding genes, thus maintaining the cell wall integrity of longans and therefore extending their storage quality after harvest [40]. These results reflect the important role of maintaining cell wall integrity during storage when using an effective post-harvest commercial handling method.

4. Materials and Methods

4.1. Preparation of H₂

Pure H₂ (H₂, 99.999% (v/v)) was prepared by electrolysis in a H₂ generator (SHC-500; Saikesaisi Hydrogen Energy Co., Ltd., Shandong, China) according to its operation manual, and H₂ was immediately injected into the gas collection bag at a flow rate of 150 mL/min for backup.

4.2. Plant Materials and Treatments

The fresh bok choy (*Brassica rapa* var. *chinensis*; Shanghaiqing), picked before 6:00 am and transported to the Suguo Supermarket (Nanjing, China), was purchased at 9:00 am. Yellowed and wilted bok choy were discarded, and fresh bok choy with no bolting, free from defects and physical damage, and similar in the number of leaves, color, and appearance were immediately chosen for the subsequent experiments.

The selected bok choy was randomly divided into four groups (treatments). Subsequently, every two bok choy, as a single evaluation unit, were stored in an airtight polypropylene box 205 mm long, 134 mm wide, and 84 mm high (1.4 L, Lock & Lock, Suzhou, China). All boxes were placed into a refrigerator (BCD-531WKPZM, Midea, China) with 4 ± 1 °C and 85% relative humidity for 9 d storage experiments. The bok choy was taken out before storage and at four different storage times (1, 3, 6, and 9 d) for the assessment of quality indicators (firmness, weight loss rate, color, chlorophyll, total phenol, and total flavonoid content); biochemical indicators (endogenous H₂ and cell wall component content); and water retention-related indicators. Each experiment was performed in triplicate, and three biological replicates (each biological replicate consisting of 3 evaluation units, and 2 bok choy per evaluation unit $\times$ 3 boxes) included 18 bok choy (6 bok choy $\times$ 3). Meanwhile, to avoid interference from immature top leaves or aging bottom leaves, leaves were collected between the second and fourth leaves from the bottom of bok choy from the four treatment groups, and quickly frozen in liquid nitrogen; they were then ground and stored at -80 °C. With the exception of color, firmness, weight loss rate, total soluble solids, electrolyte leakage, respiratory rate, moisture status measurement, light microscopy analysis, and cell damage, which were assessed in six individual bok choy per biological replicate, all other biochemical measurements were measured using the pooled sample from each biological replicate. According to the previous research on the effects of hydrogen gas on the preservation of other foods [13,14], 0.01%, 0.1%, and 1% H₂ MAP were adopted as the treatment concentrations, and the control was filled with air.

After the calculation of the volume of bok choy, a certain volume of air was drawn from the injection port of the boxes using a syringe, and then the prepared H₂ was immediately injected into the boxes, which included filling the boxes with 0.01% H₂, 0.1% H₂, and 1% H₂ (v/v), to create H₂-modified atmosphere packing (H₂ MAP; Figure S8). Boxes without

H₂ injection were used as the control (with air). The boxes were synchronously opened and the gas renewed on days 1, 3, 6, and 9 of storage under the same environmental conditions. Specifically, the lids of all groups were opened simultaneously and equilibrated with ambient air in a fume hood (air velocity: 0.5 m/s) for 5 min, allowing the headspace gas composition to return to a near-atmospheric baseline. The lids were then closed tightly at the same time. The opening time, equilibration duration, and environmental conditions were identical for the control and all H₂ MAP groups. Afterwards, the same filling method was used for the renewal of the H₂ in the boxes during storage. Additionally, gas chromatography (GC) was used to check and confirm whether H₂ could penetrate into bok choy during the storage period.

For three H₂ MAP treatments, the H₂ in the boxes retained at least 60–70% of its initial concentration after being stored in a sealed plastic box for 72 h (Figure S9) or during the whole 9-day storage period (Figure S10).

4.3. Measurement of H₂ Content and Gas Composition Analysis

Endogenous H₂ content was determined by gas chromatography (GC7900, Tianmei, Shanghai, China) [7]. Samples of 0.5 g from the control and 0.1% H₂ MAP group were ground to a homogenate with 2 mL of distilled water and 200 µL of sulfuric acid (2 M) was added; this mixture was then transferred to a 10 mL gas chromatograph bottle. Subsequently, the sample was rapidly heated for 1 h with the lid closed, cooled, and 1 mL of headspace gas was extracted and analyzed by GC. The system used N₂ as the carrier gas, with an air pressure of 0.2 MPa, and H₂ was delivered to the thermal conductivity detector. The injection and detector temperatures were set to 150 °C and 70 °C, respectively.

The O₂ concentration in the boxes was measured daily by GC during storage, and the result was expressed as the O₂ peak area. Prior to detection, the GC system was equilibrated until a stable baseline was achieved. The injection volume and chromatographic conditions were kept identical for all samples to ensure stable and reproducible peak area responses.

The CO₂ concentration (%) in the box during storage was assessed by an air detector (B36, Zhenghe Qingyuan, Shanghai, China).

4.4. Measurement of Color and Firmness

*a** and *b** colorimetric values were determined using a portable colorimeter (CR-400, Konica Minolta Inc, Tokyo, Japan). The colorimeter was calibrated with a white board, and the CIELAB color system was used to represent the color of bok choy. *a** is the degree of redness or greenness, with positive values indicating red and negative values indicating green; *b** is the degree of yellow–blue coloration, with positive values indicating yellow color [24]. Measurements were taken at any three points on the surface of the outermost leaf of each bok choy.

The firmness of bok choy was determined according to the previous method [41]. The bok choy was removed from the 4 °C refrigerator and left at room temperature for 1 h. The firmness of the stems of bok choy was measured using a texture analyzer (TMS-Pro, FTC, USA), fitted with a cylinder probe (1 cm diameter). Measurements were carried out using a texture profile analysis (TPA) procedure with a test speed of 60 mm/min, a trigger force of 0.15 N, and a compression degree of 60%. Results were expressed as the N.

4.5. Determination of Contents of Chlorophyll, Carotenoid, and Total Soluble Solids (TSS)

Chlorophyll and carotenoids were determined using the spectrophotometric method [42]. The absorbance values were measured at 645 nm, 663 nm, and 470 nm, respectively, using a spectrophotometer (UV-4802 UV/VIS, UNICO, Shanghai, China). Results were expressed as g kg^{−1} fresh weight.

About 0.5 g of bok choy tissue was ground, and the juice, filtered through gauze, was used for the measurement of total soluble solids [43]. The total soluble solids were measured using a digital refractometer (BM-03, Tianjin Lookout Optoelectronics Technology Co., Ltd., Tianjin, China). Results were expressed as a percentage (%).

4.6. Determination of Total Phenol and Flavonoid Contents

The total phenol content of the sample was determined using the Folin–Ciocalteu method [44]. The total phenolic content was calculated based on the gallic acid standard curve. The results were expressed as gallic acid equivalent (GAE) in g kg^{−1} fresh bok choy.

The assessment of total flavonoid content referred to a previous method [13], and its content was calculated from the standard curve of catechin. The results were expressed as catechin equivalent (CE) or rutin equivalent (RE) in g kg^{−1} fresh bok choy.

4.7. Measurement of Weight Loss Rate, Electrolyte Leakage, and Respiration Rate

The weight loss rate of bok choy was determined with reference to the previous method [45]. Six bok choy per replicate were randomly selected from each treatment group. The weight of each individual bok choy was measured on the initial day of storage and recorded as W_0 . Subsequently, the weight of each bok choy was determined on days 0, 1, 3, 6, and 9, and recorded as W_1 . The weight loss rate was calculated according to the equation $\text{weight loss rate (\%)} = (W_0 - W_1) \times 100 / W_0$.

For the determination of electrolyte leakage (%), a previous method [24] was referred to. Electrolyte leakage was measured using an electrical electrolyte rate (EC) meter (DDS-11A, Shanghai INESA Scientific Instrument Co., Ltd., Shanghai, China). The initial conductivity of the solution (E_1) was recorded. After the bok choy was treated in a boiling water bath for 20 min, the conductivity (E_2) of the killed tissue was measured again. The electrolyte leakage was calculated according to the equation $E (\%) = E_1 / E_2 \times 100 (\%)$.

The respiratory rate of the bok choy was determined by the static-measuring method [46] and expressed as mg CO₂ kg^{−1} h^{−1}. Six bok choy per replicate were stored in a 2.3 L sealed box (Lock & Lock, Suzhou, China) for 30 min with 10 mL 0.4 mol/L NaOH in a Petri dish. Residual NaOH was titrated with 0.2 mol/L oxalic acid. The bok choy on its own was regarded as a blank control. Respiratory rate was calculated using the following formula:

$$\text{Respiratory rate (mg CO}_2\text{ kg}^{-1}\text{ h}^{-1}) = ((V_1 - V_2) \times C \times 22) / M \times t$$

where V_1 and V_2 are the volumes (mL) of oxalic acid solution used in the blank and test titrations, respectively, C is the oxalic acid solution concentration (mol/L), M is the sample mass (kg), and t is the measurement duration (h).

4.8. Measurement of Hemicellulose, Cellulose, and Lignin Contents

The hemicellulose content was measured using a Hemicellulose Determination Kit (Cat. No. H930817, Macklin, Shanghai, China). The bok choy was dried to constant weight at 80 °C to extract hemicellulose. Hemicellulose was decomposed into reducing sugar by acid hydrolysis, and reducing sugar was reacted with 3, 5-dinitrosalicylic acid (DNS) reagent under alkaline conditions to produce a brown–red compound. The absorbance was measured at 540 nm, and the reducing sugar content was calculated by comparing it with the glucose standard curve, and the results were expressed as g kg^{−1} dry weight.

Cellulose content was determined using a Cellulose Determination Kit (BC4280, Solarbio, Beijing, China). Cellulose was extracted by adding a strong acid, at which point the lignin was thermally decomposed to 5-hydroxymethylfurfural. Subsequently, the cellulose lysate was reacted with anthrone reagent, and the absorbance was measured at 620 nm. The

cellulose content was calculated by the glucose standard curve, and results were expressed as g kg^{-1} dry weight.

Lignin content was determined using a Lignin Assay Kit (AC10656, Acme, Shanghai, China). Dried bok choy samples were used, and lignin was extracted by adding petroleum ether. After the acetylation of lignin, there was a characteristic absorption peak at 280 nm. The lignin content was calculated by the glucose standard curve, and results were expressed as g kg^{-1} dry weight.

4.9. Light Microscopic Analysis

According to the previous method [47], the stems of bok choy were cut into small pieces of about 5 mm^3 , and the thin slices were cut longitudinally into $15 \mu\text{m}$ with a Leica cryomicrotome (CM1950, Leica, Nussloch, Germany), stained with toluidine blue, and observed at 100 times magnification using a microscope (CX21, Olympus, Tokyo, Japan).

The stomatal state of the leaf surface was observed according to a previous method [24]. The lower epidermis near the leaf veins was torn with forceps, quickly placed on a slide, and magnified 400 times to observe the changes in stomatal status.

4.10. Detection of Cell Damage

Cell damage was determined according to previous methods [48,49], with some modifications. After treatments, the leaves were stained in 0.25% Evans blue solution for 30 min, and then decolorized in 95% ethanol. Afterwards, the leaves were punched with a square mold (side length 20 mm) for photographing.

4.11. Measurement of the Moisture State Change

The moisture state changes in bok choy were determined using a low-field nuclear magnetic resonance (LF-NMR) instrument (MesoMR23, Shanghai Newmark Electronic Technology Co., Ltd., Shanghai, China) [50].

On days 1, 3, 6, and 9 of storage, 0.5 g of bok choy of uniform specification were cut, wrapped in plastic wrap and placed at the bottom of a glass NMR tube, inserted into the inner lumen of the instrument, and then the change in moisture status and relaxation time (T_2) were determined using the Carr–Purcell–Meiboom–Gill (CPMG) sequence parameters (Table S1 in Supplementary Materials). The parameters of SW (spectral width), TW (waiting time), and TE (echo time) were 200 kHz, 4000 ms, and 1.000 ms, respectively. The NS (number of repeated scans) and NECH (number of echoes) were 4 and 1000.

4.12. Measurement of Peroxidase (POD) and Xyloglucan Endotransglycosylase (XET) Activities

To determine peroxidase (POD) activity and xyloglucan endotransglycosylase (XET) activity, the crude enzyme solution was extracted according to a previous method [51]. The unit (U) of POD activity was defined as the amount of enzyme required to increase the absorbance by 0.001 at 420 nm per minute. The results were expressed as U kg^{-1} fresh weight. The enzyme activity unit (U) of the XET enzyme was defined as the amount per μmol of xylose released per hour per g of fresh weight sample, and the result was expressed as U kg^{-1} fresh weight.

4.13. Transcription Analysis of Cell Wall Degradation and Lignin Synthesis Related Genes

RNA was extracted and reverse transcribed to cDNA using the RNA Reversal Kit (+gDNA wiper; Vazyme, Nanjing, China). Subsequently, qPCR was performed on a Mastercycler ep[®]realplex real-time PCR system (Eppendorf, Germany) using SYBR qPCR Master Mix (Vazyme, Nanjing, China). The relative expression of genes was expressed as values relative to the corresponding control (day 0 control) using the $2^{-\Delta\Delta\text{CT}}$ method [52], normalized to the expression levels of the genes encoding glyceraldehyde 3-phosphate

dehydrogenase (*GAPDH*) and actin (*Actin*) [53]. All primers used for the RT-qPCR are listed in Table S2 in the Supplementary Materials. Each biological replicate was performed in three technical replicates, which were averaged before statistical analysis. The final results are expressed as the mean $\pm$ standard error (SE) of the three biological replicates.

4.14. Statistical Analysis

Experiments employed a completely randomized design. All results in this study are expressed as the mean $\pm$ standard error (SE) of three independent biological replicates. Data were analyzed using SPSS 16.0 software (IBM SPSS Inc., Chicago, IL, USA), and image mapping was performed using Origin 2024 software. Duncan's multiple range test and one-way analysis of variance (ANOVA; treatment as a single factor) were performed to test the significance of difference ($p < 0.05$), or a *t*-test was used ($* p < 0.05$, $** p < 0.01$).

Correlation analyses of data on nutritional metrics, cell wall composition, weight loss rate, and other metrics were also performed using MetaboAnalyst 6.0 (<https://www.metaboanalyst.ca>, accessed 23 February 2025). Raw data were normalized (sample medians were normalized, and data were converted to cube roots; data scaling was done using range scaling).

5. Conclusions

Given the above results, H₂ MAP might not only reestablish H₂ homeostasis (Figure 1B) but also effectively maintain storage quality (including alleviating nutrient loss) by reducing weight loss rate and maintaining cell wall integrity. A schematic working model for H₂ MAP improving the quality of bok choy during storage is further illustrated in Figure 5. Therefore, our findings provided a more efficient method for the storage of leafy vegetables prone to water loss. Certainly, a sensitive and effective hydrogen supply system is also required.

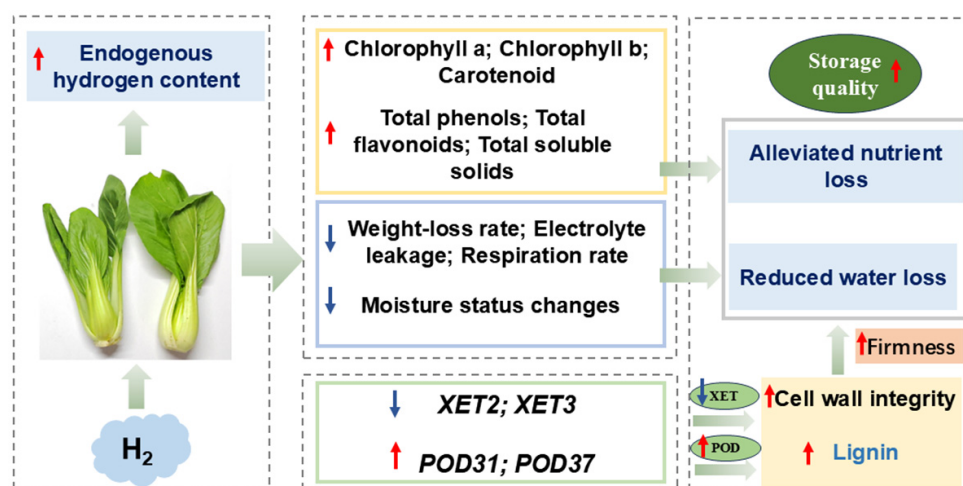


Figure 5. Schematic model of H₂ MAP maintaining the quality of postharvest bok choy. *XET2/3*: Xyloglucan endotransglycosylase2/3. *POD31/37*: Peroxidase31/37. Red arrows indicate increase and blue arrows indicate decrease, compared to control group.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/plants15040649/s1>: Table S1: T₂ changes and peak areas in bok choy stored for 0, 1, 3, 6, and 9 d after H₂ MAP; Table S2: Primer sequences used in RT-qPCR for bok choy; Figure S1: H₂ content of bok choy under control, 0.1% H₂ MAP, and 0.1% N₂ (air + 0.1% N₂) conditions during storage; Figure S2: The effect of H₂ MAP on the total flavonoid content of bok choy after 0, 1, 3, 6, and 9 d of storage; Figure S3: O₂ peak area (A) and CO₂ concentration (B) in sealed

plastic boxes; Figure S4: The effect of H₂ MAP on the firmness of bok choy after 0, 1, 3, 6, and 9 d of storage; Figure S5: The leaves of the bok choy were stained with Evans blue after 0, 3, 6, and 9 d of storage; Figure S6: The effect of H₂ MAP on the optical micrographs of bok choy stems at 0 d and 9 d of storage; Figure S7: The effect of H₂ MAP on the leaf stomatal status of bok choy under 4 °C stored for 0, 3, 6, and 9 d; Figure S8: A schematic diagram illustrating the experimental process of H₂ supply and the injection of H₂ into the storage boxes; Figure S9: Hydrogen gas concentration in the sealed plastic boxes under control (with air) vs. 0.01% H₂ MAP (A), control (with air) vs. 0.1% H₂ MAP (B), and control (with air) vs. 1% H₂ MAP (C); Figure S10: Hydrogen concentration in sealed plastic boxes within nine days under control (with air) vs. 0.01% H₂ MAP (A), control (with air) vs. 0.1% H₂ MAP (B), control (with air) vs. 1% H₂ MAP (C), and control (with air) vs. 0.01% H₂ MAP, or vs. 0.1% H₂ MAP, or vs. 1% H₂ MAP (D).

Author Contributions: G.Z. and R.Y. conceived and designed the experiments. G.Z., R.Y. and W.S. performed the research. G.Z., Y.W., P.C., K.J., X.Z., F.C., Z.W. and W.S. analyzed the data. G.Z., R.Y. and W.S. wrote the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by Preservation Technology, Advanced Research Center, Hefei Hualing Co., Ltd., Midea Group, China.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article or Supplementary Materials.

Conflicts of Interest: The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Xin Zhou, Feng Cao, and Zhe Wang are employees and hold ownership interests in Preservation Technology, Advanced Research Center, Hefei Hualing Co., Ltd, Midea Group, China.

Abbreviations

The following abbreviations are used in this manuscript:

CO ₂	carbon dioxide
DW	dry weight
FW	fresh weight
H ₂	molecular hydrogen
ROS	reactive oxygen species
XET	xyloglucan endotransglycosylase
POD	peroxidase
HRW	hydrogen-rich water
O ₂	oxygen
SE	standard error
MAP	modified atmosphere packaging
GC	gas chromatography
LF-NMR	low-field nuclear magnetic resonance
CPMG	Carr–Purcell–Meiboom–Gill
SW	spectral width
TW	waiting time
TE	echo time
NS	number of repeated scans
NECH	number of echoes
U	unit
GAPDH	glyceraldehyde 3-phosphate dehydrogenase

References

- Liu, W.; Liu, Y.; Kleiber, T. A review of progress in current research on Chinese flowering cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen et Lee). *J. Elem.* **2021**, *26*, 149–162. [\[CrossRef\]](#)
- Lipton, W.J. Senescence of leafy vegetables. *Hortscience* **1987**, *25*, 854–858. [\[CrossRef\]](#)
- Fanourakis, D.; Makraki, T.; Spyrou, G.P.; Karavidas, I.; Tsaniklidis, G.; Ntatsi, G. Environmental drivers of fruit quality and shelf life in greenhouse vegetables: Species-specific insights. *Agronomy* **2026**, *16*, 48. [\[CrossRef\]](#)
- Aguilera-Campos, K.I.; Stairs, C.W. Hydrogen metabolism: A eukaryote taps into the electron sink. *Curr. Biol.* **2022**, *32*, R49–R51. [\[CrossRef\]](#)
- Renwick, G.M.; Giumarro, C.; Siegel, S.M. Hydrogen metabolism in higher plants. *Plant Physiol.* **1964**, *39*, 303–306. [\[CrossRef\]](#)
- Wang, R. Gasotransmitters: Growing pains and joys. *Trends Biochem. Sci.* **2014**, *39*, 227–232. [\[CrossRef\]](#)
- Zhang, X.; Su, N.; Jia, L.; Tian, J.; Li, H.; Huang, L.; Shen, Z.; Cui, J. Transcriptome analysis of radish sprouts hypocotyls reveals the regulatory role of hydrogen-rich water in anthocyanin biosynthesis under UV-A. *BMC Plant Biol.* **2018**, *18*, 227. [\[CrossRef\]](#)
- Chen, Y.; Wang, M.; Hu, L.; Liao, W.; Dawuda, M.M.; Li, C. Carbon monoxide is involved in hydrogen gas-induced adventitious root development in cucumber under simulated drought stress. *Front. Plant Sci.* **2017**, *8*, 128. [\[CrossRef\]](#)
- Ren, A.; Liu, R.; Miao, Z.G.; Zhang, X.; Gao, P.F.; Chen, T.X.; Li, C.Y.; Shi, L.; Jiang, A.L.; Zhao, M.W. Hydrogen-rich water regulates effects of ROS balance on morphology, growth and secondary metabolism via glutathione peroxidase in *Ganoderma lucidum*. *Environ. Microbiol.* **2017**, *19*, 566–583. [\[CrossRef\]](#)
- Su, N.; Wu, Q.; Chen, H.; Huang, Y.; Zhu, Z.; Chen, Y.; Cui, J. Hydrogen gas alleviates toxic effects of cadmium in *Brassica campestris* seedlings through up-regulation of the antioxidant capacities: Possible involvement of nitric oxide. *Environ. Pollut.* **2019**, *251*, 45–55. [\[CrossRef\]](#)
- Wu, Q.; Huang, L.; Su, N.; Shabala, L.; Wang, H.; Huang, X.; Wen, R.; Yu, M.; Cui, J.; Shabala, S. Calcium-dependent hydrogen peroxide mediates hydrogen-rich water-reduced cadmium uptake in plant roots. *Plant Physiol.* **2020**, *183*, 1331–1344. [\[CrossRef\]](#) [\[PubMed\]](#)
- Hu, H.; Zhao, S.; Li, P.; Shen, W. Hydrogen gas prolongs the shelf life of kiwifruit by decreasing ethylene biosynthesis. *Postharvest Biol. Technol.* **2018**, *135*, 123–130. [\[CrossRef\]](#)
- Alwazeer, D.; Özkan, N. Incorporation of hydrogen into the packaging atmosphere protects the nutritional, textural and sensorial freshness notes of strawberries and extends shelf life. *J. Food Sci. Technol.-Mysore* **2022**, *59*, 3951–3964. [\[CrossRef\]](#) [\[PubMed\]](#)
- Jiang, K.; Zhang, Y.; Cai, C.; Lin, W.; Li, L.; Shen, W. Hydrogen-based modified atmosphere packaging delays the deterioration of dried shrimp (*Fenneropenaeus chinensis*) during accelerated storage. *Food Control* **2023**, *152*, 109897. [\[CrossRef\]](#)
- Yun, Z.; Gao, H.; Chen, X.; Duan, X.; Jiang, Y. The role of hydrogen water in delaying ripening of banana fruit during postharvest storage. *Food Chem.* **2022**, *373*, 131590. [\[CrossRef\]](#)
- Dong, W.; Shi, L.; Li, S.; Xu, F.; Yang, Z.; Cao, S. Hydrogen-rich water delays fruit softening and prolongs shelf life of postharvest okras. *Food Chem.* **2023**, *399*, 133997. [\[CrossRef\]](#)
- Zhang, Y.; Zhao, G.; Cheng, P.; Yan, X.; Li, Y.; Cheng, D.; Shen, W. Nitrite accumulation during storage of tomato fruit as prevented by hydrogen gas. *Int. J. Food Prop.* **2019**, *22*, 1425–1438. [\[CrossRef\]](#)
- Jin, S.; Zhu, G.; Wang, H.; Zhou, X.; Cao, F.; Wang, Z.; Shen, W. Fresh apple slice preservation driven by molecular hydrogen. *Food Chem.* **2025**, *488*, 144886. [\[CrossRef\]](#)
- Jin, S.; Zhu, G.; Shan, H.; Zhou, X.; Cao, F.; Wang, Z.; Shen, W. H₂ MAP improves fresh potato slice preservation by keeping starch content and regulating antioxidant capacity. *Food Packag. Shelf Life* **2025**, *50*, 101547. [\[CrossRef\]](#)
- Xie, Y.; Mao, Y.; Zhang, W.; Lai, D.; Wang, Q.; Shen, W. Reactive oxygen species-dependent nitric oxide production contributes to hydrogen-promoted stomatal closure in *Arabidopsis*. *Plant Physiol.* **2014**, *165*, 759–773. [\[CrossRef\]](#)
- Ren, P.; Jin, X.; Liao, W.; Wang, M.; Niu, L.; Li, X.; Xu, X.; Zhu, Y. Effect of hydrogen-rich water on vase life and quality in cut lily and rose flowers. *Hortic. Environ. Biotechnol.* **2017**, *58*, 576–584. [\[CrossRef\]](#)
- Li, L.; Zeng, Y.; Cheng, X.; Shen, W. The applications of molecular hydrogen in horticulture. *Hortic. Sci.* **2021**, *7*, 513. [\[CrossRef\]](#)
- Williamson, G. Bioavailability of food polyphenols: Current state of knowledge. *Annu. Rev. Food Sci. Technol.* **2025**, *16*, 315–332. [\[CrossRef\]](#) [\[PubMed\]](#)
- Mou, Z.; Wang, L.; Zeng, Z.; Su, X.; Ji, S.; Shan, W.; Kuang, J.; Lu, W.; Chen, Y.; Zhao, Y.; et al. Metabolomics integrated with transcriptomics unveil the regulatory pathways of modified atmosphere packaging-maintained leaf quality of Chinese flowering cabbage. *Food Chem.* **2023**, *405*, 134910. [\[CrossRef\]](#)
- Zhang, X.; Zhang, M.; Bhandari, B.; Guo, Z. High-voltage electrostatic field combined with light-emitting diode irradiation: A new method for optimizing the quality of modified atmosphere packaged pakchoi. *Food Packag. Shelf Life* **2023**, *40*, 101203. [\[CrossRef\]](#)
- Ferdousi, J.; Hossain, M.I.; Saha, S.R.; Rob, M.; Afroz, T.; Pramanik, S.; Nath, D.D. Postharvest physiology of fruits and vegetables and their management technology: A review. *JAPS J. Anim. Plant Sci.* **2024**, *34*, 291–303. [\[CrossRef\]](#)

27. Barnes, W.J.; Anderson, C.T. Release, recycle, rebuild: Cell-wall remodeling, autodegradation, and sugar salvage for new wall biosynthesis during plant development. *Mol. Plant.* **2018**, *11*, 31–46. [\[CrossRef\]](#)
28. Chen, Y.; Zhang, Y.; Jiang, L.; Chen, G.; Yu, J.; Li, S.; Chen, Y. Moisture molecule migration and quality changes of fresh wet noodles dehydrated by cold plasma treatment. *Food Chem.* **2025**, *328*, 127053. [\[CrossRef\]](#)
29. Wang, L.; Chen, Y.; Wu, M.; Dai, F.; Ye, M.; Chen, F.; Qi, Y.; Luo, Z.; Huang, H. Involvement of lignin deposition and cell wall degradation in stem senescence of Chinese flowering cabbage during storage. *Postharvest Biol. Technol.* **2023**, *198*, 112256. [\[CrossRef\]](#)
30. Cosgrove, D.J. Growth of the plant cell wall. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 850–861. [\[CrossRef\]](#)
31. Maghenzani, M.; Chiabrando, V.; Giacalone, G. The effect of different MAP on quality retention of fresh-cut nectarines. *Int. Food Res. J.* **2016**, *23*, 1872–1878.
32. Boudet, A.M. Lignins and lignification: Selected issues. *Plant Physiol. Biochem.* **2000**, *38*, 81–96. [\[CrossRef\]](#)
33. Chen, Y.; Yu, J.; Lin, H.; Zheng, Y.; Fan, Z.; Wang, H.; Chen, Y.; Lin, Y. *Phomopsis longanae* chi-induced longan pulp breakdown and softening in relation to cell wall polysaccharides disassembly. *Postharvest Biol. Technol.* **2022**, *186*, 111837. [\[CrossRef\]](#)
34. Wang, Y.; Liu, Y.; Wang, S.; Du, H.; Shen, W. Hydrogen agronomy: Research progress and prospects. *J. Zhejiang Univ. SCI. B* **2020**, *21*, 841–855. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Jones, L.; Milne, J.L.; Ashford, D.; McQueen-Mason, S.J. Cell wall arabinan is essential for guard cell function. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 11783–11788. [\[CrossRef\]](#)
36. Wang, Y.; Li, P.; Sun, W.; Zhang, T. Plant cell walls: Emerging targets of stomata engineering to improve photosynthesis and water use efficiency. *New Crops* **2024**, *1*, 100021. [\[CrossRef\]](#)
37. Brummell, D.A. Cell wall disassembly in ripening fruit. *Funct. Plant Biol.* **2006**, *33*, 103–119. [\[CrossRef\]](#)
38. Gogo, E.O.; Opiyo, A.M.; Hassenberg, K.; Ulrichs, C.; Huyskens-Keil, S. Postharvest UV-C treatment for extending shelf life and improving nutritional quality of African indigenous leafy vegetables. *Postharvest Biol. Technol.* **2017**, *129*, 107–117. [\[CrossRef\]](#)
39. Zhu, Z.; Yu, J.; Qiao, X.; Yu, Z.; Xiong, A.; Sun, M. Hydrogen sulfide delays yellowing and softening, inhibits nutrient loss in postharvest celery. *Sci. Hortic.* **2023**, *315*, 111991. [\[CrossRef\]](#)
40. Sun, J.; Chen, H.; Xie, H.; Li, M.; Chen, Y.; Hung, Y.; Lin, H. Acidic electrolyzed water treatment retards softening and retains cell wall polysaccharides in pulp of postharvest fresh longans and its possible mechanism. *Food Chem. X* **2022**, *13*, 100265. [\[CrossRef\]](#)
41. Wang, R.; Xing, Y.; Li, X.; Guo, X.; Xu, Q.; Li, W.; Che, Z. Microstructure and quality of cabbage slices (*Brassica oleracea* L. var. *capitata* L.) as affected by cryogenic quick-freezing treatment. *Int. J. Food Prop.* **2019**, *22*, 1815–1833. [\[CrossRef\]](#)
42. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol.* **1987**, *148*, 350–382. [\[CrossRef\]](#)
43. Fan, X.; Shu, C.; Zhao, K.; Wang, X.; Cao, J.; Jiang, W. Regulation of apricot ripening and softening process during shelf life by post-storage treatments of exogenous ethylene and 1-methylcyclopropene. *Sci. Hortic.* **2018**, *232*, 63–70. [\[CrossRef\]](#)
44. Xue, Z.; Feng, W.; Cao, J.; Cao, D.; Jiang, W. Antioxidant activity and total phenolic contents in peel and pulp of Chinese jujube (*Ziziphus jujuba* Mill) fruits. *J. Food Biochem.* **2009**, *33*, 613–629. [\[CrossRef\]](#)
45. Vázquez-Celestino, D.; Ramos-Sotelo, H.; Rivera-Pastrana, D.M.; Vázquez-Barrios, M.E.; Mercado-Silva, E.M. Effects of waxing, microperforated polyethylene bag, 1-methylcyclopropene and nitric oxide on firmness and shrivel and weight loss of ‘Manila’ mango fruit during ripening. *Postharvest Biol. Technol.* **2016**, *111*, 398–405. [\[CrossRef\]](#)
46. Li, W.; Zhang, M.; Yu, H. Study on hypobaric storage of green asparagus. *J. Food Eng.* **2006**, *73*, 225–230. [\[CrossRef\]](#)
47. Sorin, C.; Mariette, F.; Musse, M. NMR study of fresh cut salads: Influence of temperature and storage time on leaf structure and water distribution in escarole. *Magn. Reson. Chem.* **2019**, *57*, 626–637. [\[CrossRef\]](#)
48. Li, R.; Chen, S.; Liu, G.; Han, R.; Jiang, J. Characterization and Identification of a woody lesion mimic mutant lmd, showing defence response and resistance to *Alternaria alternata* in birch. *Sci Rep* **2017**, *7*, 11308. [\[CrossRef\]](#)
49. Lin, Z.; Liu, N.; Lin, G.; Peng, C. In situ localisation of superoxide generated in leaves of *Alocasia macrorrhiza* (L.) shott under various stresses. *J. Plant Biol.* **2009**, *52*, 340–347. [\[CrossRef\]](#)
50. Fan, K.; Zhang, M.; Bhandari, B.R.; Jiang, F. A combination treatment of ultrasound and ϵ -polylysine to improve microorganisms and storage quality of fresh-cut lettuce. *LWT Food Sci. Technol.* **2019**, *113*, 108315. [\[CrossRef\]](#)
51. Chen, Y.; Sun, J.; Lin, H.; Hung, Y.C.; Zhang, S.; Lin, Y.; Lin, T. Paper-based 1-MCP treatment suppresses cell wall metabolism and delays softening of Huanghua pears during storage. *J. Sci. Food Agric.* **2017**, *97*, 2547–2552. [\[CrossRef\]](#)

52. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **2001**, *25*, 402–408. [[CrossRef](#)]
53. Qi, J.; Yu, S.; Zhang, F.; Shen, X.; Zhao, X.; Yu, Y.; Zhang, D. Reference gene selection for real-time quantitative polymerase chain reaction of mRNA transcript levels in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *Plant Mol. Biol. Rep.* **2010**, *28*, 597–604. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.