

RESEARCH ARTICLE

Modified atmosphere packaging maintains the postharvest quality of baby mustard (*Brassica juncea* var. *gemmifera*)

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ABSTRACT

Baby mustard has increased in popularity among consumers. However, baby mustard is highly perishable. In this study, the influence of various modified atmosphere packaging (MAP) on the visual quality and content of soluble sugars and glucosinolates of baby mustard during postharvest storage was studied. Baby mustard was packed in no holes (M0), microholes (M1), or macroholes (M2) polyethylene bags respectively, and then stored at 20 °C. M1 and M2 inhibit the deterioration of visual quality of baby mustard, maintained the glucosinolates content, as well as prolonged the shelf life compared with non-wrapped plants, and M2 was the most effective in delaying the decline in visual parameters. By contrast, M0 accelerated the deterioration of postharvest quality. These findings indicate that M2 provides a promising approach for maintaining the visual and nutritional quality of postharvest baby mustard at ambient temperature.

Keywords: Baby mustard; Glucosinolate; Modified atmosphere packaging; Visual quality; Soluble sugar

INTRODUCTION

Baby mustard (*Brassica juncea* var. *gemmifera*) is a popular vegetable primarily grown in southwest China (Sun et al., 2018). It is rich in several health-promoting phytochemicals, such as glucosinolates, is some of the reasons for its increase in popularity among consumers (Sun et al., 2018; Sun et al., 2021; Zhang et al., 2021). Glucosinolates are substrates of the enzyme myrosinase, which is stored separately from glucosinolates in specialized cellular compartments. If tissue damage occurs, the enzyme encounters its substrates and catalyzes the loss of sugar, producing unstable aglycons. Aglycons decompose rapidly and release volatile isothiocyanates and nitriles. The major degradation products of glucosinolate, isothiocyanates, which have a protective effect against various types of cancer (e.g., colon, bladder, and lung cancer) (Casajús et al., 2021; Mandrich and Caputo, 2020; Miao et al., 2020). Long-term consumption of crucifers with high glucosinolates content decreases the risk of these cancers (Casajús et al., 2021; Managa et al., 2019; Zhang et al., 2021). However, baby mustard is highly perishable, as it

rapidly shrivels, the peel rapidly browns, and nutritional quality is rapidly lost after harvest (Sun et al., 2018). In addition, the heads of baby mustard are usually harvested, stored, transported, and sold at ambient temperature, which can hasten deterioration (Sun et al., 2018; Sun et al., 2021). There is thus a need to develop effective and sustainable postharvest methods to extend the shelf life and maintain the postharvest quality of baby mustard under ambient temperature storage.

Previous studies have shown that modified atmosphere packaging (MAP) was a promising technology for preserving visual and nutritional quality and extending the shelf life of products postharvest, such as broccoli florets (Jia et al., 2009; Paulsen et al., 2018), lettuce (Guo et al., 2019), *Toona sinensis* (Lin et al., 2019), and watercress (Pinela et al., 2016). MAP technology involved packaging horticultural products in permeable films (Paulsen et al., 2018). Inside the package, the gas composition around the product changed, which can slow the respiration rate, thereby retarding product senescence and deterioration (Guo et al., 2019; Jia et al., 2009; Paulsen et al., 2018). However, MAP can also damage

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the cell membrane and induce physiological injuries, such as enzymatic browning and loss of firmness (Burton et al., 1987). Thus, holes of various sizes were often made in the film to regulate the composition of the atmosphere within the package (Elwan et al., 2015; Jia et al., 2009; Kumpoun and Uthaibutra, 2010; Sanz et al., 1999).

Several studies of the postharvest storage of various horticultural products using MAP treatment have been conducted (Burton et al., 1987; Guo et al., 2019; Jia et al., 2008; Kumpoun and Uthaibutra, 2010; Lin et al., 2019; Paulsen et al., 2018; Pinela et al., 2016; Sanz et al., 1999). However, the respiration characteristics of various horticultural products vary, which can affect the efficacy of MAP (Paulsen et al., 2018). In addition, there is still a general lack of knowledge of how MAP treatment affected the postharvest quality of baby mustard. The aim of this study was to evaluate the effect of MAP treatment with different hole sizes in the film on the visual quality, soluble sugars, and glucosinolates content of baby mustard during postharvest storage.

MATERIALS AND METHODS

Plant materials

The heads of baby mustard (*Brassica juncea* var. *gemmifera* cv. Linjiang-Ercai) used in this study were collected from a local farm in Chengdu City, China, and transported to the laboratory immediately, where the baby mustard for uniformity of size, well-developed, and free of external damages. Healthy lateral buds, the main edible parts of baby mustard, were removed using a sharp stainless-steel knife and washed in a NaClO solution (50 mg kg⁻¹) for 3 min, rinsed with tap water for 1 min, and then air-dried.

MAP and storage

Lateral buds were randomly assigned to four treatment groups and stored in incubators at 20 °C with a relative humidity of 75% under continuous darkness. The baby mustard lateral buds (approximately 300 g per pack) from each group were placed in three types of transparent polyethylene bags (80 μm, 18 cm × 25 cm): (1) without holes (M0), (2) with eight microholes (6 mm in diameter, four holes on each side of the bag) (M1), and (3) with eight macroholes (12 mm in diameter, four holes on each side of the bag) (M2). As a control, lateral buds were stored without wrapping in transparent polypropylene containers without lids. Samples were taken after 0, 3, and 6 d. A bag of baby mustard lateral buds was collected as a repeat, and four repeats were used per sampling period. Several fresh samples were used for analyses of shelf life, visual quality, and weight loss, and other samples were lyophilized in a freeze dryer and stored at -20 °C for subsequent analyses of soluble sugars and glucosinolates.

Quality assessment

Shelf life and visual quality evaluation

Shelf life and visual quality of baby mustard were assessed daily and on sampling day, respectively. They were evaluated by a six-member panel, who were engaged in fresh produce research for at least two years. The samples were coded with random numbers to mask the treatment identity to minimize subjectivity and to ensure test accuracy. The lateral buds were considered to have reached the end of their shelf life when they became soft, shrank, and exhibited browning (Sun et al., 2021). Visual quality parameters were quantified on a 5-point scale. Color was graded using 5 = fresh green, 3 = lighter green, and 1 = yellowish lateral buds. Browning was graded using 5 = without browning, 3 = a few browning spots, and 1 = serious browning. Odor was graded using 5 = no unpleasant odor, 3 = slight unpleasant odor, and 1 = strong unpleasant odor. Texture was graded using 5 = tight and firm lateral buds, 3 = slightly softened but acceptable, and 1 = very softened. Decay was graded using 5 = no decay, 3 = slight but obvious severe, and 1 = severe decay. Acceptance was graded using 5 = excellent or having a freshly harvested appearance, 3 = average, and 1 = unmarketable.

Weight loss

Weight loss (%) was calculated by the formula $(W_x - W_0) / W_0 \times 100$, where W_0 was the weight at 0 day, and W_x was the weight at a certain day after storage (Sun et al., 2020).

Soluble sugars content

Freeze-dried samples (100 mg) were added in 5 mL distilled water and homogenized for 1 min. The mixture was then extracted in a water bath at 80 °C for 30 min. The supernatant was collected after centrifugation, filtered, and analyzed by high-performance liquid chromatography (HPLC) (Agilent Technologies, Inc., Palo Alto, USA). Content of glucose, fructose, and sucrose were determined using the standard curves for each sugar, respectively (Sun et al., 2020).

Glucosinolate composition and content

Freeze-dried samples (100 mg) were boiled in 5 mL water for 10 min. The supernatant was collected and applied to a DEAE-Sephadex A-25 column (Sigma Chemical Co., Saint Louis, USA). The glucosinolates were converted into their desulpho analogues by treated with aryl sulphatase. Then the desulphoglucosinolates were eluted and analyzed by HPLC (Sun et al., 2021).

Statistical analysis

Data were analyzed using one-way ANOVAs. A time-related trajectory analysis based on a two-dimensional principal component analysis map was used to visualize temporal changes in postharvest quality among different storage treatments (Sun et al., 2018).

RESULTS

External features

Baby mustard stored under control conditions gradually withered and browned, and their young leaves appeared yellow. M1 and M2 significantly delayed the deterioration of the external features of the lateral buds compared with the control. However, lateral buds rotted in M0 during storage; obvious rotting first appeared at 3 d of storage, and decay was severe at 6 d, which precluded subsequent measurements (Fig. 1).

Visual parameter scores

Visual parameter scores based on appearance are important for assessing the visual quality of baby mustard. Visual parameter scores of baby mustard gradually decreased in all treatments during storage (Fig. 2). M1 and M2 inhibited the decrease in color, browning, and texture score values. M2 also inhibited

the decline in acceptance score values, and the score of lateral buds was 1.9 times higher in M2 than in the control at 6 d. M0 and M1 accelerated the decline in odor and decay score values, especially in M0. There were no significant differences between M2 and the control during the entire storage period in the odor and decay score values. These results suggested that M2 was effective for delaying the decline in visual parameter scores during postharvest storage.

Weight loss

Weight loss is closely related to the visual quality of baby mustard, and it gradually increased in both MAP treatments and the control during storage (Fig. 3a). Weight loss under the control was severe and exceeded 39% at 6 d. However, MAP treatment significantly inhibited weight loss, which was less than 14% at 6 d. These results showed that MAP was an effective treatment for attenuating weight loss.

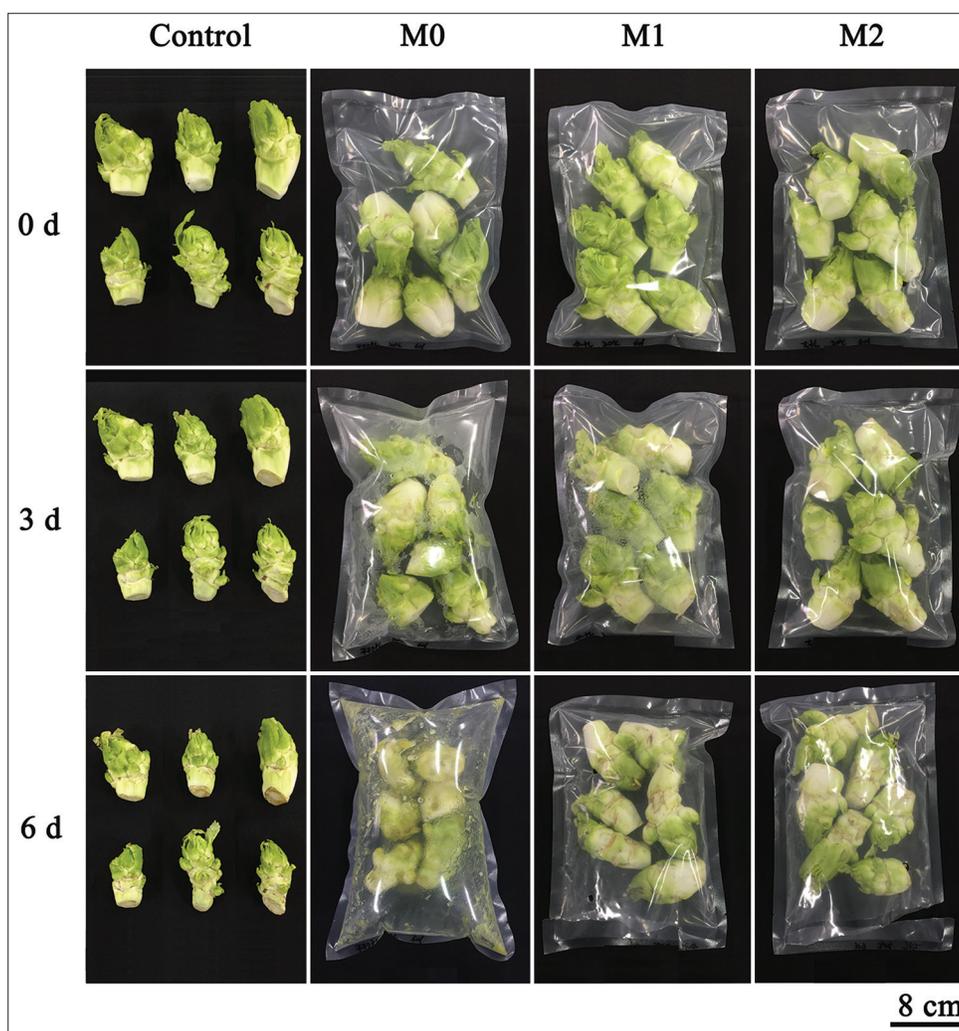


Fig 1. Visual external aspect of the lateral buds of baby mustard under different modified atmosphere packaging treatments during storage at ambient temperature. Scale bar = 8 cm.

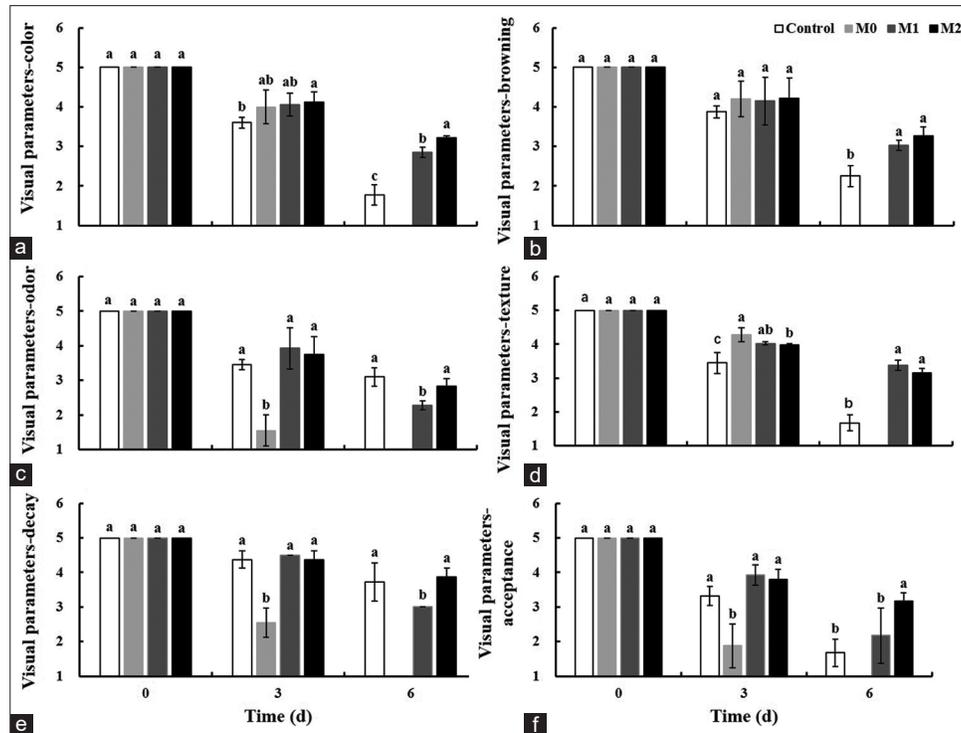


Fig 2. (a-f) Visual parameter scores of the lateral buds of baby mustard under different modified atmosphere packaging treatments during storage at ambient temperature. Values with different letters are statistically significant differences in various treatments within the same storage day ($P < 0.05$)

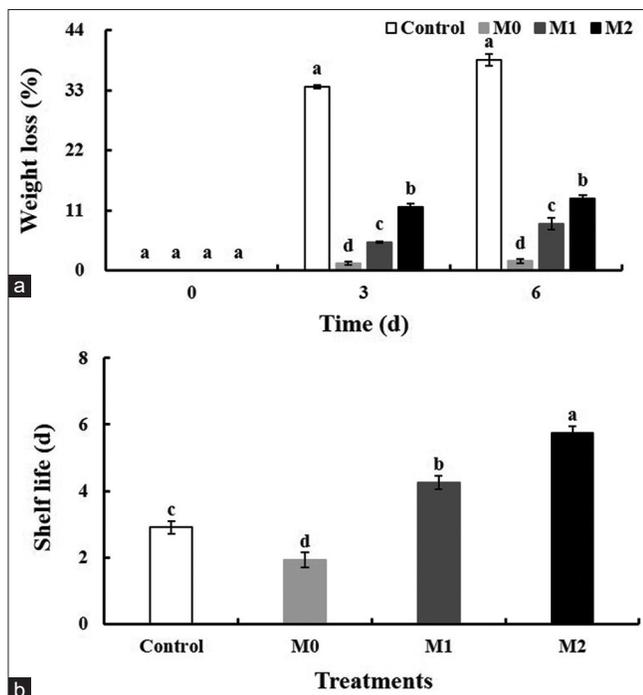


Fig 3. Weight loss (a) and shelf life (b) of the lateral buds of baby mustard under different modified atmosphere packaging treatments during storage at ambient temperature. Values with different letters in shelf life indicate statistically significant differences between treatments. Values with different letters in weight loss are statistically significant differences in various treatments within the same storage day ($P < 0.05$)

Shelf life

Baby mustard deteriorated rapidly and had a very short shelf life at ambient temperature. M1 and M2 extended the shelf life, whereas M0 shortened the shelf life (Fig. 3b). The shelf life of M2-treated baby mustard was increased nearly two-fold compared with the control.

Soluble sugars

Sucrose, fructose, and glucose were identified in baby mustard, and glucose was the most abundant (Fig. 4). Sucrose content increased in the control during storage, and its increase at the end of the storage period was 52% higher than the levels observed at 0 d; however, the sucrose content remained basically unchanged in M1 and M2 (Fig. 4a). The fructose content and glucose content decreased in both MAP treatments and the control during storage. No significant differences in fructose content between MAP treatments and the control were observed during storage. However, the glucose levels in M2 was higher than that of the control at 6 d (Fig. 4b and c).

Glucosinolates

Next, glucosinolate profiles in baby mustard samples were determined. Three aliphatic and four indolic glucosinolates were detected (Fig. 5). The most abundant glucosinolate was sinigrin, which accounted for 96% and 88% of the content of total aliphatic and total glucosinolates,

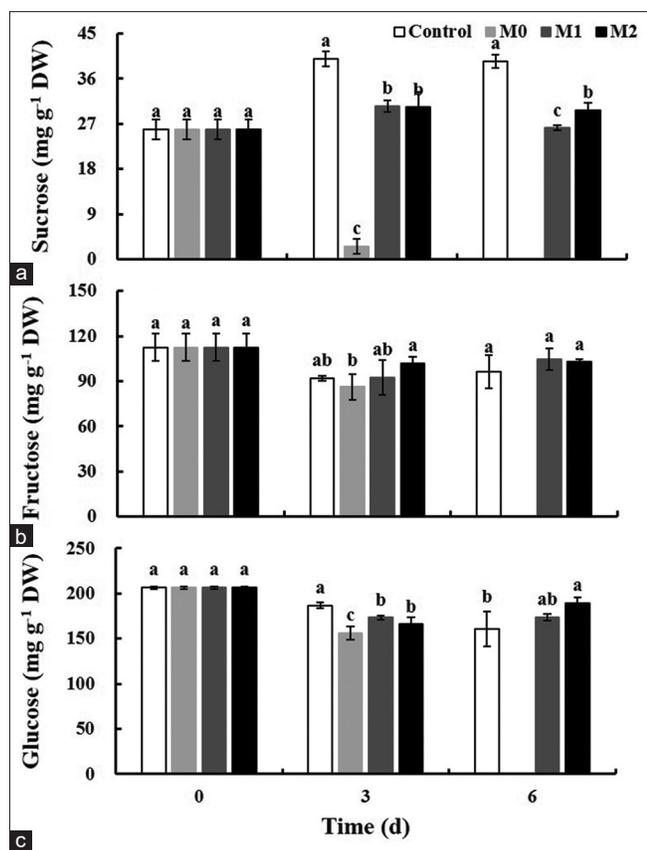


Fig 4. (a-c) Soluble sugars content of the lateral buds of baby mustard under different modified atmosphere packaging treatments during storage at ambient temperature. Values with different letters are statistically significant differences in various treatments within the same storage day ($P < 0.05$)

respectively. The predominant indole glucosinolates were 4-methoxyglucobrassicin and glucobrassicin.

The content of aliphatic glucosinolates (except for progoitrin) of the control decreased during storage, and M1 and M2 retarded the reduction of the content of these compounds. The most indole glucosinolates content declined significantly in the control during the last 3 d, and M1 and M2 significantly inhibited the decrease compared with the control. Because of the high proportion of total aliphatic glucosinolates, the changes in total glucosinolate content were similar to changes in total aliphatic glucosinolates during storage. The total glucosinolates content in M1 and M2 were 1.3- and 1.2-fold more than that of the control at 6 d. However, the content of most glucosinolates in M0 was less than that of the control. In short, M1 as well as M2 facilitated the accumulation of glucosinolates.

Time-related trajectory analysis

A time-related trajectory analysis was conducted to visualize the time-related responses of postharvest qualities under different treatments during storage (Fig. 6). Greater

distances from the origin (day 0) corresponded to higher degrees of postharvest deterioration of lateral buds. In the first 3 d of storage, the most variable distance was observed for M0, followed by the control, M1, and M2. At 6 d of storage, the total change in the distances of both M1 and M2 was less than half that of the control. These findings indicated that M0 promoted postharvest deterioration, whereas M1 and M2 significantly inhibited deterioration.

DISCUSSION

Previous studies examining the effect of MAP treatment on postharvest vegetables have shown that MAP treatment can create an atmosphere that slowed the deterioration of vegetables postharvest and increased their shelf life (D'Aquino et al., 2016; Ding et al., 2002; Frans et al., 2021; Lin et al., 2017; Xiao et al., 2014). Baby mustard is perishable and highly susceptible to withering, shriveling, and browning. M1 as well as M2 significantly inhibited the deterioration of visual quality and prolonged the shelf life compared with the control, and M2 was more effective than M1 (Fig. 1,2). This finding was consistent with the results of previous studies of lettuce (Guo et al., 2019), and sweet corn (Liu et al., 2021) suggesting that the changes in the gas composition that occur when baby mustard was packed could decrease respiration and maintain quality (Guo et al., 2019). In M0, plants rotted, odor and decay scores were lower, and shelf life was decreased (Fig. 1,2). The poor postharvest quality under M0 may stem from the induction of anaerobic metabolism due to the low O_2 concentration at ambient temperature, which contributed to rotting and the development of an unpleasant odor (Paulsen et al., 2018). Previous research of MAP technology had shown that the size of the holes in the film affected the phytochemical content and shelf life of products (Jia et al., 2009). The postharvest quality of baby mustard was retained to a greater degree under M2 compared with the other treatments. Studies of various vegetables such as broccoli florets (Jia et al., 2009), lettuce (Guo et al., 2019), and *Toona sinensis* (Lin et al., 2019) have reported that MAP treatment reduced weight loss during postharvest storage. Similarly, we found that MAP treatment suppressed the weight loss of baby mustard during storage, which may stem from the fact that MAP provided a better atmosphere for the storage of baby mustard and reduced weight loss by decreasing both respiration and transpiration (Kahramanoğlu, 2019).

Sugars not only affect the taste of vegetables but also the main substrates of primary metabolism (Li et al., 2018). In this study, sucrose content increased in the control during storage; however, M1 and M2

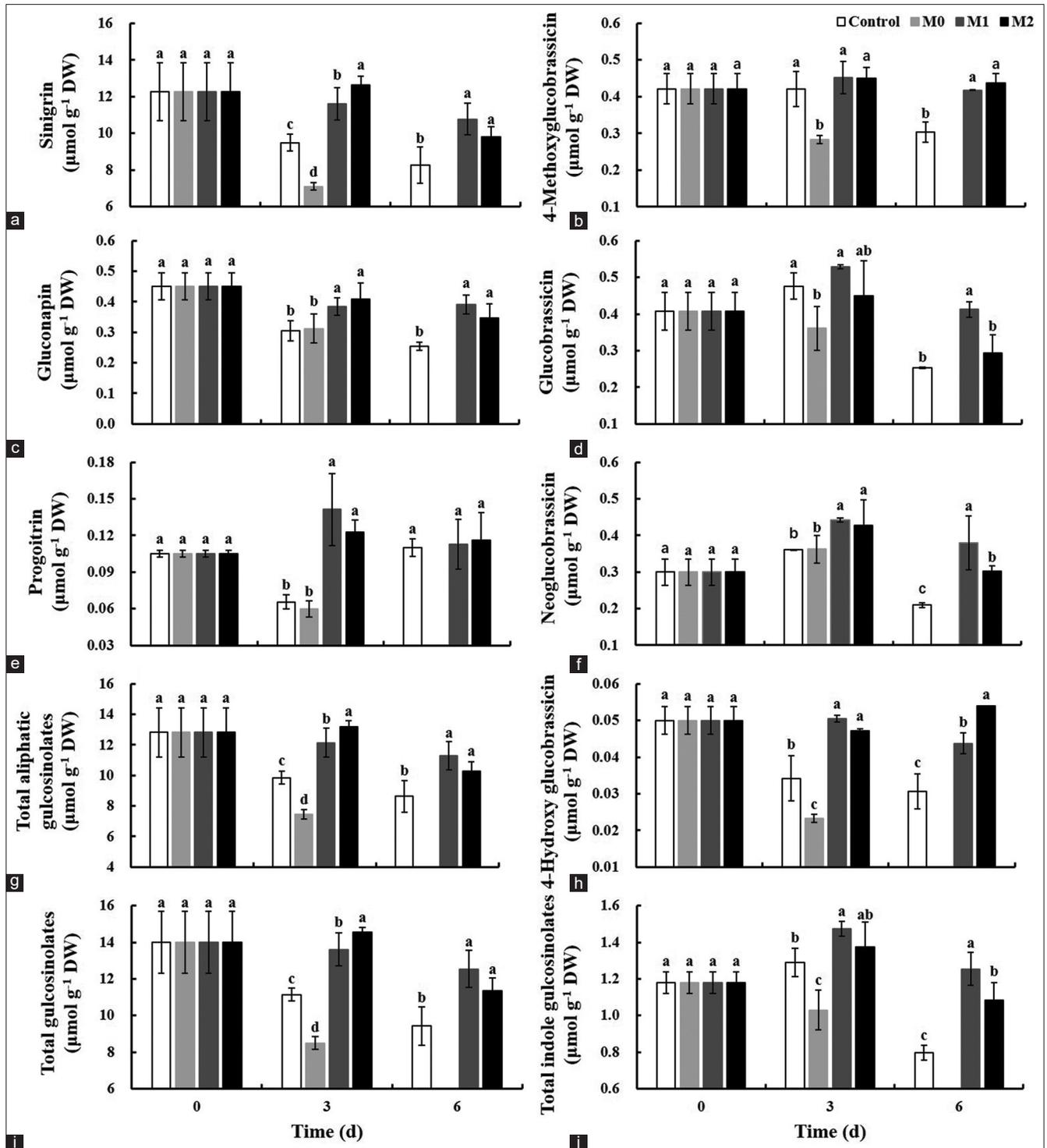


Fig 5. (a-j) Glucosinolates content of the lateral buds of baby mustard under different modified atmosphere packaging treatments during storage at ambient temperature. Values with different letters are statistically significant differences in various treatments within the same storage day ($P < 0.05$)

inhibited this increase. Previous research has shown that environmental stress during postharvest storage can increase the sucrose content (Itai and Tanahashi, 2007), and MAP can significantly reduce oxidative stress (Guo et al., 2019); consequently, in the control, sucrose content markedly increased but M1 and M2 restrained

the rise of sucrose levels. Moreover, fructose and glucose content decreased the most in M0; this might stem from the anaerobic conditions associated with the low oxygen concentration in the sealed environment, which accelerated the consumption of sugars (Kahramanoğlu, 2019).

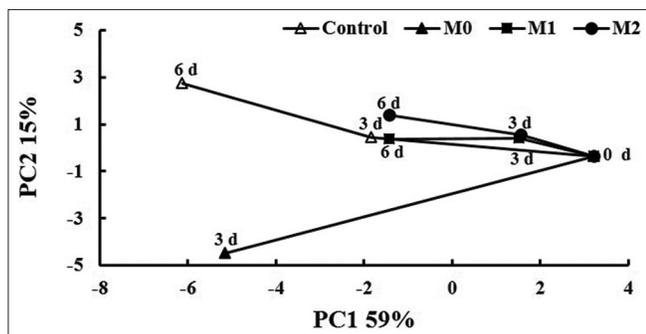


Fig 6. Time-related trajectory plot. This plot shows the dynamic time-related responses of postharvest quality in the lateral buds of baby mustard during storage at ambient temperature

Glucosinolates are a group of important health-promoting secondary metabolites in Brassica vegetables that contribute to not only anticarcinogenic activity but also taste and flavor (Jia et al., 2009; Miao et al., 2020; Wang et al., 2019; Wang et al., 2021). However, the glucosinolates content in baby mustard rapidly decreased postharvest under ambient temperature storage (Sun et al., 2020; Sun et al., 2021). In this study, compared with the control, M1 as well as M2 inhibited the decrease in most glucosinolate levels (Fig. 5). Baby mustard experienced several types of stress after harvest and during storage. The cutting of lateral buds from baby mustard heads brought myrosinase into contact with glucosinolates, which potentially led to a high degree of glucosinolate hydrolysis. The cell integrity of baby mustard gradually disappeared during the subsequent postharvest storage, which will also lead to glucosinolate hydrolysis. Previous research has shown that elevated CO₂ concentrations might facilitate reductions in glucosinolate degradation by inactivating myrosinase (Jia et al., 2009; Rangkadilok et al., 2002; Schreiner et al., 2006). This might explain the decreased glucosinolate degradation in M1 and M2. MAP treatment had also been shown to inhibit the decrease in glucosinolates content during the storage of broccoli florets (Jia et al., 2009; Paulsen et al., 2018). This led to the prediction that visual data should be positively correlated with cell integrity; consistent with this prediction, visual parameters were positively correlated with the content of glucosinolates. However, the content of most of the glucosinolates was lower in M0 than in the control. This may stem from the rapid destruction of baby mustard tissue during storage in M0, which was not conducive to the maintenance of cell integrity. As a result, myrosinase came into contact with glucosinolates, which resulted in a high degree of glucosinolate hydrolysis.

CONCLUSIONS

Appropriate MAP treatment (e.g., M1 and M2) delayed the deterioration in external features and weight loss, inhibited the decline in most visual parameters and the content

of glucosinolates, and prolonged the shelf life of baby mustard compared with the control, and M2 was more effective based on the visual analysis. However, unsuitable MAP treatment, such as M0, was not effective for the postharvest storage of baby mustard. In summary, M2 is an effective approach for maintaining the visual quality and content of glucosinolates in baby mustard at the same time during ambient temperature storage. Despite these findings, additional experiments are needed to assess whether MAP treatment could be combined with other technologies to better maintain the postharvest quality of baby mustard.

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Authors' contribution

Bo Sun, and Yang-Xia Zheng conceived and designed the experiments; Pei-Xing Lin, Hong-Mei Di, Gui-Yuan Wang, and Zhi-Qing Li performed the experiments; Ya-Ting Wang, Peng-Cheng Fang, Ce-Xian Cui, and Fen Zhang analyzed the data; Bo Sun, Pei-Xing Lin, and Hong-Mei Di wrote the paper.

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