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Review
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Review

Advance in yellowing mechanism and the regulation technology of post-harvested broccoli

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Abstract

Yellowing is one of the main problems of quality deterioration in the storage, transportation, and sales of post-harvested broccoli, which seriously affects the commodity value of broccoli. Therefore, it is of significance to understand the mechanism of the process and develop effective regulation technology. In this review, we expounded the changes in the appearance of the flower ball, bud morphology, and calyx cell structure, as well as endogenous pigment metabolism, accompanying the yellowing process of broccoli. In addition, recent research on the molecular mechanism of yellowing was summarized from the aspects of transcriptome analysis and transcription regulation. Finally, the progress on the control technology of broccoli yellowing was reviewed.

Key words: broccoli; yellowing; pigment metabolism; transcriptome; transcription factor.

Introduction

Broccoli (*Brassica oleracea* L. var. *italica*), originated in Italy along the coast of Europe, is widely grown in countries all over the world (Sudhir *et al.*, 2018). The statistics of the International Food and Agriculture Organization database in 2019 showed that the country with the largest acreage of broccoli in the world is China, with an annual output of nearly 10 million tons, accounting for more than 45% of the global total (Branham *et al.*, 2019). The annual increase in the global production of broccoli is attributed to the demand of the market, which is the result of consumers' pursuit of the nutritional components in broccoli (Zhu *et al.*, 2018). Broccoli is a low-calorie nutritious vegetable with great potential to prevent many diseases, such as cancer and cardiovascular diseases. In the ranking of anti-cancer vegetables released by the Japan Cancer Research Center (Yagishita *et al.*, 2019), broccoli is among the best. Long-term consumption of broccoli can effectively improve the health of the general population and enhance the body's ability to fight cancer. The nutritional value of broccoli comes from its various bioactive ingredients, which can boost the immune system and antioxidant capacity of the body. Among the bioactive substances in broccoli, flavonoids, ascorbic acid, glucosinolates, sulforaphane, polyphenols, and selenium are representative. The secondary metabolites of glucosinolates and antioxidants such as selenium and flavonoids have a synergistic effect on cancer prevention. Besides, broccoli is a good source of many minerals such as calcium, magnesium, phosphorus, potassium, and sodium (He *et al.*, 2018).

The flower head of broccoli, composed of small clusters of green buds, is a reproductive organ and a part of the food. The freshly harvested flower head is dark green, with round shape and compact buds. Broccoli is a kind of delicate vegetable that is resistant to low temperatures. After harvesting, the metabolism of broccoli is still vigorous. Broccoli's head will wilt, and the buds will turn green to yellow, which is accompanied by a massive loss of nutrients. In severe cases, broccoli head can be spoiled by moulds and produces an unpleasant odour, which seriously affects the quality and commercial value of it (Moreirarodríguez *et al*., 2017). The researchers found that as the yellowing process intensified, the antioxidant activity in broccoli decreased, sulforaphane and vitamin C significantly decreased, and the loss of various high-quality amino acids and minerals was detected (Luo *et al*., 2018). In this review, we present the recent findings on the changes in the appearance of the flower ball, bud morphology and calyx cell structure, the endogenous pigment metabolism, and the molecular mechanism accompanying the yellowing process, as well as the strategies to control yellowing of broccoli.

Changes in the Appearance of the Flower Ball, Bud Morphology, and Calyx Cell Structure

Freshly harvested broccoli flower balls are dark green and round shape and formed by tightly clustered buds. With the extension of storage under inappropriate conditions, the flower balls show visible colour changes. In severe cases, the flower balls become loose and waxy layer subsides, accompanied by mildew and off-flavour. Generally, colour analysis is used to evaluate the overall appearance of fruits and vegetables, International Commission on illumination Lab Value (CIELAB) colour space, and parameters. The values of a^* and b^* detected by colorimeter combined with $h°$ calculated are used to evaluate the change of colour. a^* value auto-displayed represents the deviation between red and green, and a negative value is biased toward green. b^* value, directly read from the instrument, implies deviation between yellow and blue, the larger the value, the more yellow the sample tends to be. $h°$ value calculated from $a^*$and b^* value represents the hue angle. In the process of broccoli yellowing, the levels of a^* and b^* value increase progressively and rise sharply when evident yellowing is observed. Meanwhile, $h°$ value declines gradually. The changes in colour parameter are irreversible in the above execution. Under anatomic microscope, it can be seen that the buds turn yellow from the bottom to the top, and the buds do not swell and bloom, which indicates that the initiation of yellowing is not due to bloom. By scanning electron microscope, the cell of calyx tissue in fresh broccoli is smooth, plump with an ordered arrangement. Besides, the elliptical stomata are evenly distributed, and the guard cell structure is complete (Shimoda *et al.*, 2016). However, the cells in yellowed calyx tissue are irregularly arranged and there is a large area of cell morphological damage. The stomata are significantly open and the contraction rate is gradually increased, and abnormal stomata appear. More subtle changes of cell ultrastructure in the sepal tissue can be observed under a transmission electron microscope. In fresh green samples, the thylakoid matrix layer is regularly arranged and clearly visible. The fusiform chloroplast has a complete double membrane and contains a small amount of osmium globules and starch granules. The difference is that in the yellowed broccoli calyx tissue, the thin layer of the thylakoid matrix is sparse, the chloroplast turns into a circle, and the number of osmium globules and starch granules increases considerably. In addition, the chloroplasts in the yellowed calyx develop abnormally and shift to chromoplast, which means that the plastid gradually shifts from a place where chlorophyll is stored to the chromoplast storing carotenoids (Luo *et al*., 2019a). During the aging process of broccoli, the conversion of chlorophyll to carotenoids reveals the changes in cell ultrastructure on the one hand, and on the other hand, it realizes the effective storage of nutrients.

Changes in Endogenous Pigment Metabolism in the Process of Yellowing

The yellowing of fruits and vegetables can be induced by many factors, including senescence, pathogen infection (yellow leaf curl virus and chlorosis virus), free radicals, energy, and metal ions. However, the specific endogenous pigments in fruits and vegetables are the main factors that affect the appearance of plants. Generally, yellowing is the result of coordinated multi-pathway pigment metabolisms, such as carotenoid, anthocyanin, and capsaicin biosynthesis and chlorophyll degradation (Rubio *et al*., 2008). Chlorophyll degradation and carotenoid synthesis are the main metabolic pathways that induce broccoli yellowing. Studies have shown that when broccoli is significantly yellowed, the content of chlorophyll a decreases, while the contents of chlorophyll b, zeaxanthin, β-carotene, lutein, and β-cryptoxanthin accumulated to varying degrees. The $h°$ value was positively correlated with the content of chlorophyll b ($P < 0.05$) and β-carotene ($P < 0.01$), and was negatively correlated with the content of zeaxanthin ($P < 0.05$) and β-cryptoxanthin ($P < 0.05$), which indicates the increasing contributions of carotenoid components to yellowing (Luo *et al*., 2019a). In the severe yellowing stage, the contents of chlorophyll b and chlorophyll a decreased significantly, while the contents of various carotenoid components increased. The correlation analysis results showed that the $h°$ value was significantly positively correlated with chlorophyll b and β-carotene content ($P < 0.01$) and was significantly negatively correlated with β-cryptoxanthin content ($P < 0.01$). From the above analysis, it can be seen that chlorophyll b, β-carotene, zeaxanthin, and β-cryptoxanthin may be the main pigment components leading to the yellowing of broccoli (Luo *et al*., 2019a).

The phenotypic changes in fruits and vegetables are regulated by endogenous complex physiological metabolism. The obvious sign of plant senescence is caused by the rapid degradation of chlorophyll, namely degreening/yellowing. Chlorophyll and intermediate products of chlorophyll degradation and metabolism can generate free radicals under high concentration of oxygen or strong light, which causes membrane lipid oxidative damage, nucleic acid base hydroxylation, cells programmed death, and potentially phototoxic to plant cells. Therefore, the efficient and orderly degradation of chlorophyll is a detoxification method for senescent cells, which helps to maintain the cellular activity in the senescence process, to ensure the full mobilization and reuse of nutrients (Yang *et al.*, 2019). The chlorophyll degradation pathway can be divided into photodegradation and enzymatic degradation. The former usually occurs under light conditions, the latter is a degradation process that takes place under the action of a series of enzymes. Chlorophyll degradation is achieved by an ordered series of chlorophyll catabolism enzymes (CCEs). As the starting point of chlorophyll degradation, chlorophyll b and chlorophyll a participate in the chlorophyll cycle under the catalysis of Non-Yellow Coloring 1 (NYC1) and chlorophyll a oxygenase (CAO). Chlorophyll a further removes magnesium atoms by cleavage of phytes. Under the catalysis of pheophorbide a oxygenase (PaO) and red chlorophyll catabolism reductase (RCCR), pheophytin a (Pheide a) is converted into primary fluorescent chlorophyll (Figure 1).

Cai *et al.* (2019a) found that the activities of chlorophyll degradation-related enzymes (MDCase and PaO) and carotenoids biosynthetic enzyme (LCY-e) activity increased significantly accompanied by the intensification of the yellowing process. Currently, two kinds of chlorophyllase (Chlase) isozymes have been isolated from broccoli. Chlase has no chloroplast transit peptide and

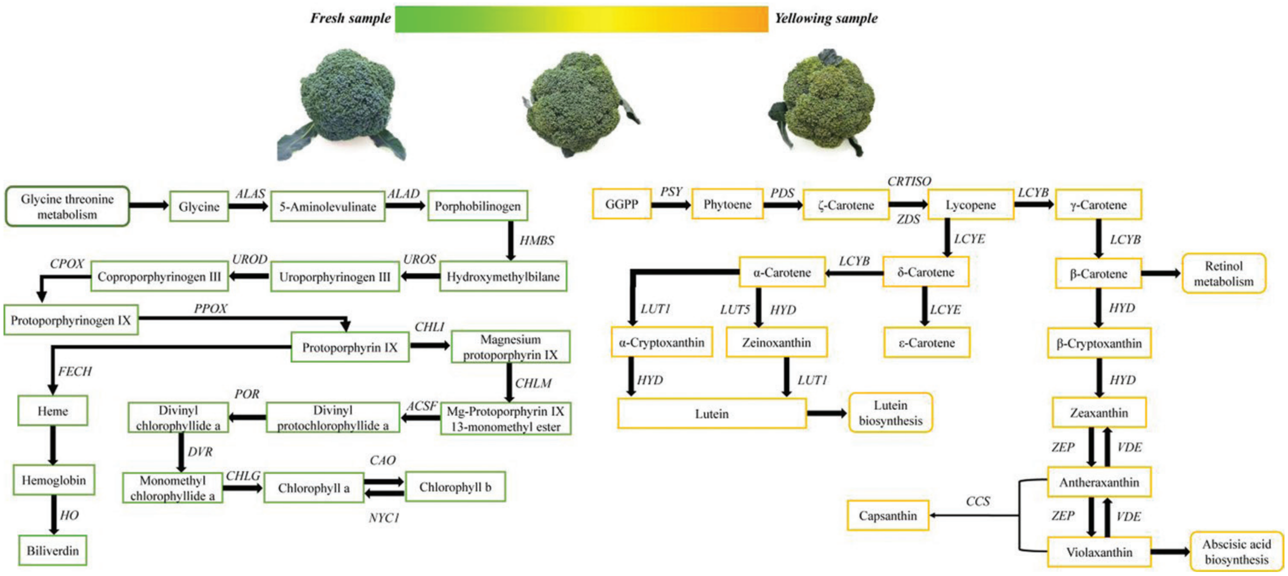

Figure 1. Changes in endogenous pigment metabolism in the process of yellowing.

could not respond to hormone regulation, but Chlase is involved in hormone regulation, thereby degrading chlorophyll. In studies involving ethanol-treated broccoli, the activities of MDCase and peroxidase (POX) were increased with changes in chlorophyll content, indicating that they were the key enzymes in chlorophyll degradation. Gómez-Lobato (2012) cloned the gene (*BoPAO*) segment encoding pheophorbide a oxygenase (PaO) from broccoli and found that it was vital for chlorophyll degradation, and ethylene could accelerate the increase of *BoPAO* expression. The expression of *BoPPH* gene increased during the yellowing of broccoli, and the expression of it was induced by ethylene, but inhibited by the cytocytoins treatment. In addition, the expression level of *BoRCCR*, a gene involved in chlorophyll degradation, in broccoli increased with yellowing, which was positively correlated with the degree of chlorophyll catabolism. Similarly, Yu *et al.* (2019) confirmed that post-harvest tea wilting and yellowing were directly related to the content of various chlorophyll derivatives and the expression level of the *PAO* gene. Li *et al.* (2017) studied the leaf colouration of Japanese maple leaves (green wild-type and yellow mutant). The phenotype of Japanese maple leaves was regulated by the genes involved in chlorophyll metabolism pathway. The flower organs of Qingtian pineapple gradually changed colour during the development process, which was accompanied by yellowing. In addition, Liu *et al.* (2016) found that the pheophytin hydrolase (*PPH*) gene was the key gene isolated during the bracts yellowing. Cadrain (2006) inferred that the yellowing of *Arabidopsis* leaves was caused by a combination of chlorophyll degradation and anthocyanin accumulation. Thomas (2010) confirmed that chlorophyll degradation was the key factor for the *sage* mutants' yellowing. Calvo and Santamaria (2008) revealed that the colouration of ripe tomato was the result of lycopene biosynthesis and chlorophyll degradation.

Carotenoids, one of the main plant pigment systems, are responsible for providing orange, yellow, or red pigments to the flowers, fruits, and other organs of many plants, which mainly consist of violaxanthin, neoxanthin, α-carotene, β-carotene, γ-carotene, antheraxanthin, lutein, β-cryptoxanthin, capsanthin, capsorubin, bixin, astaxanthin, canthaxanthin, etc. Among them, β-carotene, β-cryptoxanthin, and lutein are closely related to the yellowing of broccoli. Luo *et al.* (2019b) carried out the transcriptome of broccoli yellowing, which indicated that the carotenoid biosynthesis was another key pathway associated with the broccoli yellowing post-harvest. The biosynthesis of carotenoids accompanies the whole process of fruits and vegetables ripening and senescence. The colouration of fruits and vegetables occurs in plastids (chromoplast and chloroplast), which are controlled by a series of proteases. Geranylgeranyl diphosphate (GGPP) is a precursor of carotenoids. Under the action of rate-limiting enzyme phytoene synthase (PSY), two molecules of GGPP are condensed into phytoene. The phytoene is further transformed into lycopene under the catalytic action of phytoene desaturase (PDS) and zeta-carotene desaturase. Lycopene cyclization is a demarcation point, which is further divided into two pathways, α-carotene and β-carotene. Lycopene is converted into β-carotene through a two-step reaction of lycopene β-cyclase (LCY-b), while α-carotene is composed of lycopene ε-cyclase (LCY-e), and LCY-b obtained by continuous action. Under the action of β-carotene hydroxylase (HYD) α-carotene is catalyzed to zeaxanthin, while β-carotene is hydroxylated to β-cryptoxanthin by HYD. After that, the α-carotene and β-carotene branch merge, leading to enter the lutein biosynthetic pathway (Figure 1).

Tao *et al.* (2006) studied the colour formation of red meat navel orange and Washington navel orange fruits, and *PSY*, *LCY-b*, and *LCY-e* genes were the key enzyme genes in the carotenoid biosynthesis pathway, which were the molecular basis for the sweet orange fruits yellowing. Lv *et al.* (2015) revealed that *GGPS*, *PSY*, and *LCY-b* genes were the genes in the carotenoid biosynthesis pathway during the colouration process of watermelon pulp, which directly participate in regulating the colour transformation. In the study of *spr2* and *def1* mutants during tomato fruit ripening, it was found that lycopene synthesis is directly related to the expression of 1-deoxy-d-xylose-5-phosphate synthase (*DXS*) gene, *GGPS*, *PSY1*, and *PDS* were selected as the main genes of tomato yellowing, which induced by methyl jasmonate (MeJA). Guzman *et al.* (2010) inferred that carotenoids accumulation was the main pigment basis for colour evolution in summer orange fruit. *PSY* gene was the key pigment regulation gene during the period of summer orange turning yellow, and the up-regulated expression of the *PSY* gene triggered the yellowing of fruits during the later greening period. In addition, there have been some reports on the research of PSY, LCY-b, and LCY-e, such as tomato (Bu *et al.*, 2014), dried apricots (Fratianni *et al.*, 2017), and apple (Greco *et al.*, 2012).

Molecular Basis of Endogenous Pigment Metabolism Associated With Yellowing

Yellowing is the main problem of quality deterioration in post-harvested broccoli. Understanding the molecular mechanism of this process is helpful to further explore the corresponding regulatory techniques. However, the yellowing of fruits and vegetables involves a complex endogenous pigment metabolism network. Finding the main metabolic pathways, critical structural genes, and their regulatory factors are the problem that researchers are committed to breaking through. In recent years, with the development of omics and other molecular biology techniques, great progress has been made in the research of the broccoli yellowing mechanism.

Research on Transcriptome Analysis

Recently, transcriptomics techniques have been successfully applied in the field of post-harvest biology. Kong *et al.* (2019) revealed that the gene regulation network and cold injury (CI) mechanisms were in response to cold stress on bell pepper by using high-throughput RNA-Seq. The similar study of adversity and stress is also reported in banana, plantain, tomato, and peach. With the help of transcriptome techniques, researchers have also achieved gratifying results in elucidating the mechanisms of yellowing in fruits and vegetables. Ge *et al.* (2019) revealed that carotenoid biosynthesis was directly related to the colouration of pigment in avocado peel through transcriptome analysis. Luo *et al.* (2019b) used transcriptome sequencing technology to analyze the differentially expressed genes (DEGs) and explore key enzyme genes involved in the main metabolic pathways that are related to the yellowing process of post-harvested broccoli. The results showed that 1717 DEGs were annotated on 92 KEGG pathways. In addition, 6 (*BoCPOX*, *BoCHLI*, *BoPOR*, *BoCAO*, *BoHO1*, and *BoNYC1*), 5 (*BoLCYE*, *BoHYD*, *BoVDE*, *BoCCS*, and *BoZEP*), and 4 (*Bo4CL*, *BoCHS*, *BoFLS*, and *BoF3H*) DEGs were screened from chlorophyll metabolism, carotenoid biosynthesis, and flavonoid biosynthetic metabolism pathways, respectively. There were 579 differentially expressed transcription factors. Gene ontology analysis showed that various members of the MYBs, bHLHs, and bZips transcription factor families were enriched in the chlorophyll metabolism pathway. The transcription factor families enriched in the carotenoid biosynthesis pathway were NACs and ethylene response factors (ERFs). The flavonoid biosynthesis pathway was regulated by the MYBs, NACs, WRKYs, MADSs, and bZips transcription factor families. BobHLH66, BoPIF4, BoLOB13, BoNAC92, and BoAPL transcription factors were the candidate transcription factors involved in regulating chlorophyll and carotenoid metabolic pathways. Through proteomics and transcriptome techniques, Liu *et al.* (2013) revealed the mechanism of delaying broccoli yellowing by cytokinin (CK) and *N*(6)-benzylaminopurine (BA). The results showed that BA treatment reduced the number of proteins involved in energy and carbohydrate metabolism and amino acid metabolism and provided cell protection during post-harvest storage. Both treatments were involved in regulating multiple metabolic pathways, including signalling pathways, sugar transport, energy, and carbohydrate metabolism pathways, and antagonized the function of ethylene, thereby maintaining the bright green of broccoli.

Research on Transcriptional Regulation Mechanism

In recent years (Rajagopal *et al.*, 2018), researchers have performed a lot of research on transcription factors in drought, cold damage, hormones, high salt, and the formation of quality of harvested vegetables, which in turn regulated the plant quality. Transcription factors play a crucial role in regulating post-harvest quality changes in fruits and vegetables. A large number of studies have shown that transcription factors are actively involved in regulating various pigment metabolism pathways. In papaya fruits, NAC1/2 and EIN3a transcription factors were involved in carotenoid biosynthesis by activating the regulation of *PDS2/3/4*, *LCYE*, and *CHY-b* genes, which in turn induced the papaya to turn yellow. Wang *et al.* (2018) confirmed that the transcription factors associated with post-harvest tea colouration were MYB2 and MYB26. Jiang *et al.* (2018) revealed that BoERF1 transcription factor was involved in regulating the post-harvest yellowing process of broccoli by inhibiting the activities of catalase, peroxidase, and superoxide dismutase, reducing the relative conductivity and malondialdehyde content of transgenic plants, increasing the content of free proline, protecting the morphological structure of plant leaf cells, in order to delay the chlorophyll degradation. Luo *et al.* (2019b) explored the molecular mechanism of post-harvest broccoli yellowing by transcription analysis and screened out a variety of BoMYB, BobHLH, and BobZip transcription factor family members to participate in chlorophyll metabolism pathways. More than 16% of BoNAC and BoERF transcription factor family members were enriched in the carotenoid biosynthetic pathway. In addition, qPCR results indicate that BobHLH66, BoPIF4, BoLOB13, BoNAC92, and BoAPL were potential positive regulators of broccoli yellowing key genes (*BoCAO* and *BoHYD*) and actively participated in the regulation of broccoli yellowing metabolism network.

Technologies of Relieving the Yellowing of Post-Harvested Broccoli

The yellowing of post-harvested broccoli is accompanied by changes in taste and nutritional composition, which has been the researcher's commitment to solve the problem of broccoli quality deterioration. In recent years, researchers have made some progress in controlling the yellowing of post-harvested broccoli and extending its fresh-keeping period. The reported regulation techniques are mainly divided into physical and chemical measures (Kumar *et al.*, 2016).

Physical measures

Physical measures are mainly used to change the external environmental conditions and to interfere with the physiological and biochemical metabolism of products. Most physical measures are the non-toxic and pollution-free preservation methods, which affect physical, biochemical, and mutagenic reactions by inhibiting the respiration of fruits and vegetables, killing pathogens, inhibiting pathogen activity, changing enzyme activity, and inducing fruits and vegetables stress resistance, and then achieve the purpose of preservation. In addition to the above advantages, physical measures also have the disadvantages of high cost and strict requirements on equipment. In recent years, a variety of physical measures have been applied to broccoli preservation, including modified atmosphere, film bagging [polyethylene (PE) film and polylactic acid (PLA) film], high electrostatic field, electrostatic atomized water, light irradiation [ultraviolet-B (UV-B), UV-C, low-intensity light-emitting diodes (LEDs), and fluorescent], and temperature control (drying, controlled freezing point, hot air, low temperature, and heat shock).

The modified atmosphere with low O_2 and high CO_2 concentration could effectively resist the induction of alternative oxidase, thereby maintaining the freshness of post-harvest broccoli and delaying the yellowing for 7 days (Makino *et al.*, 2020). Compared with the control group, PE film and PLA film packaging significantly maintained the reduction of chlorophyll, prevented broccoli yellowing, and effectively prolonged the shelf life to 4 days (Li *et al.*, 2016). Studies have shown that controlled freezing point technology combined with modified atmosphere packaging maintained the texture of broccoli to varying degrees, inhibited the loss of biologically active ingredients, PPO activity, and the release of ethylene, which also reduced the accumulation of MDA, thereby effectively postponing the yellowing of broccoli and the shelf life extended to 12 days (Jing *et al.*, 2016). Ozone was released under high electrostatic field voltage and treatment time at 278.8 kV and 20 min, respectively, which could effectively reduce the total bacterial count in broccoli, suppress respiratory intensity, maintain the sensory quality of broccoli, and the shelf life was 3 days longer than the control group (Yao *et al.*, 2012). The treatment of electrostatic atomized water particles could inhibit the activity of respiratory enzymes and had a significant preservation effect on broccoli. The broccoli yellowing was delayed by 50.5 h compared to the untreated group (Wang *et al.*, 2017). Aiamla-Or *et al.* (2012) studied the effect of UV-B treatment on the quality of post-harvest broccoli and found that UV-B treatment reduced the contents of chlorophyll a and chlorophyll b, inhibited the activities of Chlase and MDCase enzymes, effectively delayed chlorophyll degradation, and the shelf life was postponed by 5 days. Loi *et al.* (2019) studied the influence of five kinds of LEDs on the quality of broccoli and assessed the content of chlorophyll, carotenoids, and other pigments. The results showed that the green LED effectively increased the chlorophyll content in broccoli. Moreover, LED treatment prolonged the shelf life of broccoli during cold storage to 15 days. Jin *et al.* (2015) found that fluorescent treatment could effectively inhibit the decrease of chlorophyll, thus extending the shelf life of broccoli post-harvest. Hot air drying at 40 °C, 50 °C, and 60 °C combined with microwave-assisted hot air drying technology could delay the yellowing of broccoli under low-temperature storage for 7 days (Salim *et al.*, 2017). Furthermore, post-harvest yellowing of broccoli under low-temperature storage was delayed for 10 days (Mølmann *et al.*, 2015). In addition, optimized temperature and light intensity effectively delayed the post-harvest broccoli yellowing and maintained the sensory quality of it (Johansen *et al.*, 2017). Hot air combined with UV-C treatment effectively delayed the yellowing of broccoli and maintained shelf-life quality by improving the anti-oxidation system capacity of post-harvest broccoli, and the shelf life was 5 days (Zhang *et al.*, 2013).

Chemical measures

According to the reports, certain chemical treatments can delay the yellowing process of broccoli post-harvest, although the mechanism is quite different. In general, chemical measures have the characteristics of good preservation effect, low cost, simple process, easy degradation, high practicability, etc., which maintain the stability of the internal water structure of fruits and vegetables, block direct contact between fruits and vegetables and adversity, maintain cell membrane permeability, and inhibit the accumulation of dialdehydes, and then delay the senescence process of fruits and vegetables. However, the residue and pollution of chemical preservatives have not been well resolved, especially the impact of plant hormones on human metabolism has always been a matter of great concern to consumers. Chemical measures used can be divided into two categories, one is exogenous hormones, including 6-benzylaminopurine (BAP), 24-epibrassinolide (EBR), melatonin, MeJA, and jasmonate (JA), and the other is preservatives, including diethyldithiocarbamic acid (DIECA), calcium propionate, natamycin, putrescine, calcium sulfate, hydrogen sulfide, dl-propargylglycine (PAG), ethanol, 1-methylcyclopropene (1-MCP), ClO_2, and sodium benzoate (SBN). Moreover, there are some reports on the effects of combination treatment of chemicals.

Researchers showed that the expression level of the *BoPPH* gene was mediated by BAP, and the shelf life of broccoli has been delayed for 5 days. However, ethylene treatment could enhance the transcription level of *BoPPH* and then participate in the regulation of broccoli yellowing post-harvest (María et al., 2005). Cai *et al.* (2019b) set a variety of EBR treatment concentrations, and the results show that the same chemical treatment with low- and high-concentration treatment effects was exactly the opposite. Among them, 2 μM EBR-treated broccoli, the expression levels of ACC, ACC3, pheophytinase, and polyamine oxidase were more effectively inhibited than that of other concentrations. In addition, the activity of chlorophyll degradation-related enzymes was reduced, the chloroplast structure was intact, and the degradation of chlorophyll was slow, which deferred the broccoli yellowing for more than 3 days. Fang *et al.* (2020) explored the effect of different concentrations of MeJA treatment on the post-harvest quality of broccoli. The results showed that 1 mM JA could obviously inhibit the yellowing of broccoli post-harvest and block the synthesis of endogenous JA and inferred the potential promotion of JA on broccoli yellowing. In addition, DIECA treatment effectively reduced the accumulation of endogenous JA, AOC, and 12-oxo-phyto-dienoic acid reductase and inferred the potential promotion of JA on broccoli yellowing for 2 days. Treating broccoli with putrescine inhibited the gene expression of *BoPPH* and *BoPaO*, which prevented chlorophyll degradation, and the shelf life has been postponed to more than 5 days (Zheng *et al.*, 2019). Calcium sulfate treatment enhanced the content of broccoli glucosinolates and improved antioxidant capacity, resulting in the delay of yellowing for 6 days (Guo *et al.*, 2018). Limwachiranon *et al.* (2017) inferred that 0.8 mM hydrogen sulfide and 0.5 mM PAG treatment significantly maintained post-harvested broccoli green and prolonged the shelf life to 5 days. Balschun *et al.* (2010) found that ethanol steam treatment inhibited the activity of CCEs and genes expression, thereby delaying the yellowing process of broccoli for more than 4 days. About 1 μl/L of 1-MCP treatment significantly maintained the content of chlorophyll components, which delayed the yellowing of broccoli stored at 20 °C for 3 days (Reyes Jara *et al.*, 2019). About 2.5 μl/L of 1-MCP combined with 50 mg/L of ClO_2-treated post-harvest broccoli, this combined treatment was better to effectively reduce chlorophyll degradation, inhibited MDA accumulation and PPO enzyme activity, thus effectively prolonging broccoli yellowing to 4 days (Bi *et al.*, 2018). About 40 mg/L of BAP and 0.2% of SBN compound preservatives actively blocked chlorophyll degradation and reduced the degree of membrane lipid peroxidation, thereby inhibiting the yellowing and senescence of broccoli during storage for 2–4 days (Fernández-León *et al.*, 2013). Considering the complexity of the influencing factors, it is recommended to conduct a small-scale verification test before application.

Conclusions

According to the present study, it is reasonable to consider endogenous pigment changes other than bloom as the cause of commercial broccoli yellowing during the post-harvest period. In the early stage of yellowing, the changes in chlorophyll content and its metabolism were the main inducement. However, carotenoids become the main factor related to serious yellowing and even losing the commodity value of broccoli. Through extensive screening of transcriptome, it can be found that chlorophyll degradation and carotenoid synthesis were the main metabolic pathways enriched in DEGs and related to pigments in the process of yellowing , and some candidate genes and potential transcription factors in the pathways are screened out. Furthermore, great progress has been made in the transcriptional regulation mechanism of some transcription factors, such as BoMYB, BobHLH, and BobZip, on critical genes involved in chlorophyll degradation and carotenoid synthesis. On the basis of the above research, a series of physical and chemical treatments have been explored to reduce the post-harvest yellowing of broccoli.

Yellowing is a complex metabolic process in plants, which may be coordinately regulated by multiple genes and transcription factors. Therefore, further research is needed to reveal the yellowing mechanism of broccoli post-harvest. Extensive screening of genes, metabolites, and related metabolic pathways involved in the yellowing process through multi-omics analysis techniques, and analysis of transcriptional regulatory mechanisms of key structural genes in metabolic pathways. Moreover, by means of co-immunoprecipitation (Co-IP), pull-down, protein modification, and other technical means, the study at the protein level will be conducive to a deeper understanding of the mechanism of post-harvest yellowing of broccoli. Based on mechanism research, further perfection and innovation of control technology, which could effectively solve the production problem, is required so as to extend the shelf life of broccoli, broaden its sales area, and provide consumers with high-quality broccoli.

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Conflict of Interest

The authors declare no conflicts of interest.

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