Expression Analysis of *ERF*s during Storage under Modified Atmosphere Packaging (High-concentration of CO₂) of Fresh-cut Lotus Root

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Abstract. Ethylene response factor (*ERF*) genes have been characterized in numerous plants, where they are associated with responses to biotic and abiotic stress. Modified atmosphere packaging (MAP) is an effective treatment to prevent lotus root browning. However, the possible relationship between *ERF* transcription factors and lotus root browning under MAP remains unexplored. In this study, the effects of phenol, phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD) enzyme activities; and PPO, PAL, POD, and *ERF* gene expression on fresh-cut lotus root browning were studied with MAP. The expression pattern of *ERF2/5* correlated highly with the degree of browning. It is suggested that *NnERF2/5* can be used as an important candidate gene for the regulation of fresh-cut lotus root browning under MAP, and the correlation of each gene should be studied further.

Lotus root has high nutritional value and many bioactive substances. Lotus root is also widely grown in Asia and Southeast Asia. Its nutrients mainly include starch, sugar, protein, fat, and lecithin (Abohatem et al., 2011). Because it is not difficult to cut,

lotus root is more suitable for processing into fresh-cut produce (Christudass et al., 2012). Although fresh-cut lotus is a convenient and fast food, it is prone to browning during processing and storage. This has resulted in a short shelf life and has

hindered the sale and export of lotus root. Currently, methods for controlling browned lotus root in the world mainly include chemical treatments (Kwon and Baek, 2014) such as the use of salicylic acid, which inhibits enzymatic browning of fresh-cut chinese chestnut (*Castanea mollissima*) (Zhou et al., 2015); and physical treatments such as MAP (Waghmare and Annapure, 2018), heat treatment (Vigneault et al., 2017), and low-temperature storage (Razavi et al., 2018). Among these methods, MAP maintains the postharvest quality and safety of a wide range of whole, intact, minimally processed fruits and vegetables, such as yam and pear (Artés et al., 2006; Wilson et al., 2017). For most plants, high concentrations of CO₂ and low concentrations of O₂ may cause a hypoxic atmosphere and may stimulate the growth of anaerobic bacteria, thereby leading to the decay of fruits and vegetables (Conway et al., 2000). However, it is an effective means to delay browning of fresh-cut lotus root. MAP was shown to can delay the browning of fresh-cut lotus root significantly in our previous research (Min et al., 2017).

ERF genes have been characterized in numerous plants, where they are involved in responses to biotic and abiotic stress, including cold and heat stress (Francesco et al., 2013; Phukan et al., 2017), and especially hypoxia stress (Giordano, 2005; Yang et al., 2018). In fruits and vegetables, *ERF* genes are key targets for investigating the transcriptional regulatory roles in fruit and vegetable development, ripening, and senescence (Karlova et al., 2011; Francesco et al., 2013). *ERF* was studied previously under low-temperature storage and vacuum packaging in fresh-cut lotus root. MAP is similar to vacuum packaging in that it produces an hypoxic environment; however, in MAP, high CO₂ may affect the respiration of lotus roots. Compared with atmospheric packaging (AP), the respiration in corn and peaches was inhibited significantly under high CO₂ conditions. However, lettuce and spinach were barely inhibited, and progressive respiratory activity was enhanced (Kubo et al., 1990).

We found that the transcriptional levels of *NnERF3/4/5* were associated with fresh-cut lotus root browning, and that these levels could also be important regulators for the browning of fresh-cut lotus root during storage at low temperatures (Min et al., 2017). It has been proposed that the expression patterns of *NnERF4/5* are consistent with fresh-cut lotus root browning, which could be important regulators of fresh-cut lotus root browning under vacuum packaging (Min et al., 2019). MAP can delay the browning of fresh-cut lotus root significantly, as found in our previous research (Min et al., 2017). However, few studies have been carried out on the effect of MAP on fresh-cut lotus root on *ERF* genes. Thus, the main

Fig. 1. CO₂ and O₂ content change under two different types of packaging. (A) Modified atmosphere packaging (high concentration of CO₂ and low concentration of O₂). (B) "Atmospheric packaging."

Fig. 2. Effects of on the degree of browning of fresh-cut lotus root ('Wu zhi 2'). Fresh-cut lotus root was stored separately with modified atmosphere packaging (MAP; circles) and atmospheric packaging (AP; squares). Error bars represent SEs from three biological replicates. LSD = least significant difference.

purpose of our research was to evaluate the changes of related enzymes and gene expression in fresh-cut lotus root with MAP.

Materials and Methods

Sample preparation

Lotus root ('Wuzhi 2') was purchased in the commercial agricultural wholesale market (Four Seasons, Wuhan, China) in 2017 and then immediately shipped to the laboratory. The lotus roots were processed exactly the same as those in our previous study (Xie et al., 2018). Each sample treatment was carried out in triplicate. Some of the samples used MAP (100% CO₂) and some used AP. These samples were then stored at 4 °C.

Physicochemical parameters

The physicochemical parameters of fresh-cut lotus root slices, including their browning index and determination of total phenolic content, were measured according to the published method of Min et al. (2017).

Determination of browning degree. The browning index was determined with reference to the previous literature (Min et al., 2019). Samples were homogenized at 10:1 (sample:water) at 4 °C and centrifuged for 5 min (10,000 rpm). Then, the supernatant of the centrifuge tube was collected. After incubating for 5 min in a 25 °C water bath, the absorbance was measured at 410 nm using a spectrophotometer. The browning degree is expressed as $A_{410} \times 10$.

Determination of total phenolic content. Total phenolic content was measured according to the previous literature (Liu et al., 2018; Min et al., 2019). Most specific operations were similar to those conducted in the previous study. The result is the expression of gallic acid equivalent per kilogram fresh weight.

Determination of PAL, PPO, and POD enzyme activities. PAL, PPO, and POD activities were extracted and determined according to our previous study (Min et al., 2019).

RNA extraction and complementary DNA (cDNA) synthesis. Total RNA content and cDNA synthesis were prepared according to our previously used methods (Min et al., 2017). DNA traces of the contaminating genomes in the total RNA were cleared using TURBO Dnase (Ambion). Then, 1.0 mg of DNA-free RNA was used for cDNA synthesis using the iScript cDNA Synthesis Kit (Bio-Rad) protocol. At each sampling point, RNA extraction was repeated using three samples.

Gene expression analysis. Some primers were similar to our earlier articles (Min et al., 2014, 2019). According to previous literature (Min et al., 2017), real-time polymerase chain reaction (PCR) was carried out using the gene specific primers in an iCycler instrument (Bio Rad, Hercules, CA) with a Ssofast EvaGreen Supermix kit and IQ5 instrument for gene expression studies. The PCR reaction mixture (total volume, 20 µL) comprised 10 µL 2 × real-time PCR mix (Bio-Rad), 1 µL of each primer (10 µM), 2 µL diluted cDNA, and 6 µL diethyl pyrocarbonate H₂O. The relative abundance of each gene was calibrated with samples from the day 0 sample.

Data analyses

Images were drawn using Origin pro 9.0.0 (64-bit) b45 software. The statistical significance of the differences among and between treatments was calculated with the least significant difference test and Student's *t* test.

Results and Discussion

CO₂ and O₂ content in MAP. As shown in Fig. 1, the gas composition changes dynamically during controlled-atmosphere storage (MAP: high concentration of CO₂ and low concentration of O₂). The (a) "Modified atmosphere packaging" (high concentration of CO₂ and low concentration of O₂ on day 0: CO₂ 64.9%, O₂ 9.9%, N₂ 25.2%); the "Atmospheric packaging" on day 0 (CO₂ 1.2%, O₂ 20.8%, N₂ 78.0%). During storage (35 d) (Fig. 2), the CO₂ content in the (a) group decreased and the O₂ content did not change significantly. This may be due to MAP (a high

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Fig. 3. Effects of two different types of packaging on the total phenol content of fresh-cut lotus root ('Wu zhi 2'). Fresh-cut lotus root was stored separately by modified atmosphere packaging (MAP; squares) and atmospheric packaging (AP; circles). Error bars represent SES from three biological replicates. FW = fresh weight; LSD = least significant difference.

concentration of CO₂ and low concentration of O₂) inhibiting the use of O₂ by lotus root. The decrease in CO₂ content may be the result of the absorption of CO₂ by the lotus root's tissue structure. The CO₂ content in the air group increased by 5.7%, and the O₂ content decreased by 7.5%, possibly because the respiration of the lotus roots was enhanced, resulting in a decrease in O₂ and an increase in CO₂ content (Simpson et al., 2009).

Browning degree. Browning degree is the main indicator for evaluating the browning level of fruits and vegetables (Artés et al., 2006). As seen in Fig. 2, different packaging conditions (MAP and AP) were used to store fresh-cut lotus root pieces. The results show that the browning degree values of the two packaging methods were significantly different during storage (Fig. 2). Compared with AP, the browning degree of the fresh-cut lotus roots in MAP (a high concentration of CO₂ and low concentration of O₂) was delayed significantly, with a browning value between 0.26 and 0.28. The browning degree of the AP packaging varied greatly (0.26–0.62). Therefore, MAP can effectively delay the browning process of fresh-cut lotus root,

Fig. 4. Phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD) activity of different packaging of fresh-cut lotus roots. Effects of two different types of packaging on PAL, PPO, and POD activity ('Wu zhi 2'). Fresh-cut lotus root was stored separately with modified atmosphere packaging and atmospheric packaging (AP). Error bars represent SES from three biological replicates. LSD = least significant difference.

Fig. 5. Messenger RNA from phenylalanine ammonia lyase (PAL) genes in response to different temperature treatments. Fresh-cut lotus root was stored separately in modified atmosphere packaging (MAP; black) and atmospheric packaging (AP; white). Error bars represent SES from three biological replicates. LSD = least significant difference.

which is similar to the results of Gunes and Chang (2010).

Total phenolic content. The total phenolic content of fresh-cut lotus root had significant differences under different packaging methods (Fig. 3). The total phenolic content of the MAP samples increased slowly, but there was no significant difference (102.3 mg/kg on day 0 to 115.81 mg/kg on day 35). The total phenolic content of the AP samples increased from 96.891 mg/kg on day 0 to 159.252 mg/kg on day 35. The results also indicate that MAP delays the increase in total phenolic content, which is similar to the results of previous studies (Min et al., 2019).

PAL, PPO, and POD activities. PAL, PPO, and POD enzyme activities in two different packaging methods were analyzed. An increase was observed in the three activity levels in the AP samples, in comparison with the MAP samples in which the levels remained quite constant (Fig. 4). Moreover, PAL enzyme activity increased rapidly, ranging from 13.508 U/g on day 14 to 27.214 U/g on day 35 in the AP group, but the MAP group samples showed no significant change in PAL activity. The PPO activity in the MAP group was less than that of the AP group during the whole

storage period, with the initial value of PPO ranging from 0.526 to 0.594 U/g in the MAP group and 1.198 U/g in the AP group on day 35. These results indicate that PPO enzyme activity is very sensitive to CO₂ packaging, and that the PPO enzyme is the key enzyme for the browning of fruits and vegetables, which is consistent with our previous research results (Min et al., 2017). Similar to PAL activity, POD activity did not differ significantly during the early stage. After day 14, the POD activity in the MAP group increased slowly, from 0.034 U/g on day 0 to 0.056 U/g on day 35, compared with the AP group, with values from 0.034 U/g to 0.146 U/g on day 35. The PAL, PPO, and POD enzymatic activity in the MAP group was less than that of the AP group, which indicates that the MAP condition could inhibit the activity of the enzymes effectively, thereby inhibiting enzymatic browning (Qian et al., 2016).

In this experiment, the changes in PAL, PPO, and POD activities under different packaging conditions were studied, and the change law was found to be consistent with the browning of fresh-cut lotus root during storage. Moreover, this result is also consistent with the results of previous reports

(Dong et al., 2015; Min et al., 2015, 2017, 2019; Xie et al., 2018).

Gene expressions of NnPAL, NnPPO, and NnPOD. The two different packaging methods had different effects on the two *NnPAL* genes (Fig. 5). The expression of *NnPAL1* in the AP group increased gradually throughout the storage period and peaked around day 28; however, the expression of *NnPAL1* was continuously inhibited by MAP, and the relative abundance of the gene was reduced by more than 10 times around day 28. Messenger RNA (mRNA) expression of *NnPAL2* in the MAP group increased gradually and peaked at around day 21. In addition, mRNA expression of *NnPAL1* is similar to that of the PAL enzyme activity and browning degree. Therefore, *NnPAL1* is most likely involved in the synthesis of phenolic compounds in lotus root browning, which is consistent with the results of our previous study (Min et al., 2017).

The relative expression of the *NnPPOA* gene increased under AP (Fig. 6), whereas the expression level in MAP was about one third that of AP. This indicates that a high concentration of MAP inhibited the expression of *NnPPOA*. Compared with *NnPPOA*, the expression level of *NnPPOC* transcribed mRNA in AP was similar to that of the MAP group, but the expression level was very low (one tenth that of *NnPPOA*). In addition, the results showed that the mRNA expression level of *NnPPOA* was consistent with the trend of PPO enzyme activity and browning degree. Therefore, *NnPPOA* was very likely to participate in lotus root browning, as shown elsewhere (Min et al., 2017, 2019).

Moreover, the relative expression of *NnPOD2/3/4* was reduced significantly compared with that under AP during the storage period (Fig. 7), but the *NnPOD4* transcript abundance was much less than that of others. In addition, *NnPOD1/5/6* was inhibited during early storage and was upregulated by MAP during late storage. There was no significant difference in *NnPOD7* expression between the two packaging methods; the transcript abundance remained at a low level. The results show that the expression of *NnPOD2/3* was reduced significantly by MAP during the whole storage period. This phenomenon indicates that MAP inhibits *NnPOD2/3* expression, which is closely related to changes in POD enzyme activity and browning degree, which may be the key gene for lotus root browning.

A previous study found that the down-regulation of *NnPAL1*, *NnPPOA*, and *NnPOD2-5* was consistent with a decrease in the browning of fresh lotus root at low temperatures, combining two different varieties (Min et al., 2017). In our study, the changes in *NnPAL1*, *NnPPOA*, and *NnPOD2/3/4* expression occurred in parallel with changes in PAL, PPO, and POD enzyme activities and browning degree in MAP and AP. Based on our results and our previous report, *NnPAL1*, *NnPPOA*, and

Fig. 6. Messenger RNA from polyphenol oxidase (PPO) genes in response to different temperature treatments. Fresh-cut lotus root was separately stored modified atmosphere packaging (MAP; black) and atmospheric packing (AP; white). Error bars represent SEM from three biological replicates. LSD = least significant difference.

NnPOD2/3/4 are the key genes affecting the browning of fresh-cut lotus root, although further functional analyses need to be performed.

ERF gene expression. Seven *ERF* genes had different expression patterns (Fig. 8). The expression of *NnERF2/5* was inhibited significantly under MAP (high CO_2 storage) in comparison with the AP samples. The inhibition multiples were 16-fold and 3.4-fold. In addition, the expression of *ERF1* and *ERF4* was induced by MAP (high CO_2) in comparison with the AP samples during the early stage of storage, but there was no significant difference during the late-storage stage. The relative expression of *ERF4* was clearly the lowest. *ERF3* was inhibited by MAP during the early stage of storage, and induced by MAP during the later stage of storage in comparison with the AP samples. *ERF6* and *ERF7* were not significantly different during early storage in the two samples, but were induced by MAP during later stages of storage in comparison with the AP samples, and their content increased gradually.

Seven *ERF* genes were isolated in our previous work (Min et al., 2014, 2019). *NnERF2/5* expression was suppressed

significantly by MAP, which is consistent with *NnPAL1*, *NnPPOA*, and *NnPOD2/3/4* changes and browning. This indicates that, in lotus root, decreased expression of *NnERF2/5* is concurrent with a decrease in browning.

Reactive oxygen species (ROS) produced in mitochondria are involved in the postharvest senescence of fresh-cut produce under normal respiratory conditions (You et al., 2012). POD can scavenge ROS efficiently, whereas CO_2 levels can affect these enzyme activities (Li et al., 2013). In addition, high CO_2 treatment can cause changes in the quality of fruits and vegetables, such as increasing the content of ethanol and acetaldehyde (Hu et al., 2017), which may be related to the induction of *ERF1* and *ERF4* with high concentrations of CO_2 during the early stages of storage. Previous studies have shown that the *DkERF9* and *DkERF10* genes were involved in acetaldehyde and ethanol synthesis in persimmon deastringence (Min et al., 2014). In addition, high CO_2 levels can also inhibit the proliferation of microorganisms in fresh-cut produce (Oliveira et al., 2015). *ERF6* and *ERF7* were induced by high concentrations of CO_2 during the

later stages of storage. Whether *ERF6* and *ERF7* are involved in microbial inhibition needs further study.

In a previous study, we found that *NnERF4/5* is downregulated continuously by vacuum packaging. we propose that *NnERF4/5* could be an important candidate as a regulator of fresh-cut lotus root browning under vacuum packaging (Min et al., 2019). In the current study, we examined further the effects of MAP on the browning and *ERF* gene expression in lotus root. *NnERF2/5* was found to correlate highly with the browning process of lotus root, which further supports our previous findings (Min et al., 2019). Therefore, *ERF5* is likely to be an important browning-related gene, although the specific relationship between *ERFs* and browning remains unclear, and the relationships of *NnERFs* and *NnPAL1*, *NnPPOA*, and *NnPOD2/3* during lotus root browning should be the subject of further research. Although, in the current study, *ERF4* was induced at day 7 in samples stored under MAP conditions that induced a lower browning degree, its relative expression is very low (one tenth for *ERF5* and one one-hundredth for *ERF2*). In addition, lotus root could activate the synthesis of ethylene in the event of mechanical damage. Consequently, the high expression of *ERF1/4/7* during the first 7 d may be related to this activation (Razavi et al., 2018), whereas high CO_2 treatment also affects the expression of signal genes (Simpson et al., 2009). *ERF2* remained stable under MAP conditions (105.1 ± 20.5), but the AP group changed greatly, which may have been caused by respiration of the high levels of CO_2 (Qian et al., 2016). Similar results have been reported elsewhere. *AdERF* genes respond differentially to abiotic stresses during postharvest storage. *AdERF4* and *AdERF6* showed an increase in high CO_2 treatment which transcription factors may have a similar function in hypoxia response (Yin et al., 2012). In addition, the study by Romero et al. (2016) showed that high CO_2 treatment maintained table grape quality, which seems to be mediated by the regulation of *ERFs* and, in particular, *VviERF2-c* might play an important role by modulating the expression of pathogenesis-related genes.

Conclusions

The browning; PAL, PPO, and POD enzyme activity; and gene expression assay results in this study show that high CO_2 packaging is effective in delaying lotus root browning. The downregulation of *NnPAL1*, *NnPPOA*, and *NnPOD2/3* by MAP coincided with an increase in related enzyme activity and the browning degree of fresh-cut lotus root. Moreover, the expression pattern of *NnERF2/5* was consistent with the change in *NnPAL1*, *NnPPOA*, and *NnPOD2/3* gene expression. It is proposed that *NnERF2/5*, especially *NnERF5*, could be an influential

Fig. 7. Messenger RNA from peroxidase (POD) genes in response to different temperature treatments. Fresh-cut lotus root was stored separately in modified atmosphere packaging (MAP; black) and atmospheric packing (AP; white). Error bars represent SEs from three biological replicates. LSD = least significant difference.

candidate as a regulator for fresh-cut lotus root browning.

Literature Cited

Abohater, M., J. Zouine, and I.E. Hadrami. 2011. Low concentrations of BAP and high rate of subcultures improve the establishment and multiplication of somatic embryos in date palm

suspension cultures by limiting oxidative browning associated with high levels of total phenols and peroxidase activities. *Scientia Hort.* 130:344–348.

Artés, F., P.A. Gómez, and F. Artés-Hernández. 2006. Modified atmosphere packaging of fruits and vegetables. *Stewart Postharvest Rev.* 2:1–13.

Christudass, M.D., K. Sood, and V. Robert. 2012. Taxol resistance in prostate cancer: Rescue of

resistance and expression of prostate cancer-associated genes upon treatment with HDAC inhibitors. *J. Urol.* 187:E323.

Conway, W.S., B. Leverenz, R.A. Saftner, W.J. Janisiewicz, C.E. Sams, and E. Leblanc. 2000. Survival and growth of *Listeria monocytogenes* on fresh-cut apple slices and its interaction with *Glomerella cingulata* and *Penicillium expansum*. *Plant Dis.* 84:177–181.

Fig. 8. Transcriptional analysis of *NnERF* genes. Transcripts of *NnERF* genes were measured by real-time polymerase chain reaction. Fresh-cut lotus root was stored separately in modified atmosphere packaging (MAP; black) and atmospheric packaging (AP; white). Day 0 values were set as 1. Error bars indicate SEM from three biological replicates. LSD = least significant difference.

Dong, T.T., J.Y. Shi, C.Z. Jiang, Y.Y. Feng, Y. Cao, and Y.Q. Wang. 2015. A short-term carbon dioxide treatment inhibits the browning of fresh-cut burdock. *Postharvest Biol. Technol.* 110:96–102.

Francesco, L., O.T. Masaru, and P. Pierdomenico. 2013. *Ap2/ERF* transcription factors: Mediators of stress responses and developmental programs. *New Phytol.* 199:639–649.

Giordano, F.J. 2005. Oxygen, oxidative stress, hypoxia, and heart failure. *Eur. J. Clin. Invest.* 115:500–508.

Gunes, G. and Y.L. Chang. 2010. Color of minimally processed potatoes as affected by modified atmosphere packaging and anti-browning agents. *J. Sci. Food Agr.* 62:572–575.

Hu, H., L. Zhang, and P. Li. 2017. Effects of elevated CO₂ on aldehyde and ethanol contents

and the activities of related enzymes in post-harvest *Pleurotus eryngii*. *J. Agr. Sci.* 2:432–437.

Karlova, R.L., F.M. Rosin, J. Busscher-Lange, V. Parapunova, P.T. Do, A.R. Fernie, P.D. Fraser, C. Baxter, G.C. Angenent, and R.A. de Maagd. 2011. Transcriptome and metabolite profiling show that *APETALA2a* is a major regulator of tomato fruit ripening. *Plant Cell* 23:923–941.

- Kubo, Y., A. Inaba, and R. Nakamura. 1990. Respiration and C₂H₄ production in various harvested crops held in CO₂-enriched atmospheres. *J. Amer. Soc. Hort. Sci.* 115: 975–978.
- Kwon, S.Y. and H.H. Baek. 2014. Effects of temperature, pH, organic acids, and sulfites on tagatose browning in solutions during processing and storage. *Food Sci. Biotechnol.* 23: 677–684.
- Li, P., Z. Xuan, H. Hu, Y. Sun, Y. Wang, and Y. Zhao. 2013. High carbon dioxide and low oxygen storage effects on reactive oxygen species metabolism in *Pleurotus eryngii*. *Postharvest Biol. Technol.* 85:141–146.
- Liu, S., E.C. Liu, B.Q. Zhu, B.W. Chai, R.J. Liu, Q. Gao, and B.L. Zhang. 2018. Impact of maceration time on colour-related phenolics, sensory characteristics and volatile composition of mulberry wine. *J. Inst. Brewing* 124:45–56.
- Min, T., F. Fang, H. Ge, Y.N. Shi, Z.R. Luo, Y.C. Yao, D. Grierson, X.R. Yin, and K.S. Chen. 2014. Two novel anoxia-induced ethylene response factors that interact with promoters of deastringency-related genes from persimmon. *PLoS One* 9:1–8.
- Min, T., E.C. Liu, J. Xie, Y. Yi, L.M. Wang, Y.W. Ai, and H.X. Wang. 2019. Effects of vacuum packaging on enzymatic browning and ethylene response factor (*ERF*) gene expression of fresh-cut lotus root. *Hort-Science* 54:331–336.
- Min, T., M.M. Wang, H. Wang, X. Liu, F. Fang, D. Grierson, X.R. Yin, and K.S. Chen. 2015. Isolation and expression of *NAC* genes during persimmon fruit postharvest astringency removal. *Intl. J. Mol. Sci.* 16:1894–1906.
- Min, T., J. Xie, M. Zheng, Y. Yi, W.F. Hou, L.M. Wang, Y.W. Ai, and H.X. Wang. 2017. The effect of different temperatures on browning incidence and phenol compound metabolism in fresh-cut lotus (*Nelumbo nucifera* G.) root. *Postharvest Biol. Technol.* 123:69–76.
- Oliveira, M., M. Abadias, J. Usall, R. Torres, N. Teixidó, and I. Viñas. 2015. Application of modified atmosphere packaging as a safety approach to fresh-cut fruits and vegetables: A review. *Trends Food Sci. Technol.* 46: 13–26.
- Phukan, U.J., G.S. Jeena, V. Tripathi, and R.K. Shukla. 2017. Regulation of apetala2/ethylene response factors in plants. *Front. Plant Sci.* 8:150, doi: 10.3389/fpls.2017.00150.
- Qian, Y.F., J. Xie, S.P. Yang, W.H. Wu, Q. Xiong, Z.L. Gao, and J.B. Shi. 2016. Effect of CO₂ on chemical and microbial changes of pacific white shrimp during modified atmosphere packaging. *J. Aquat. Food Prod. Technol.* 25: 644–655.
- Razavi, F., R. Mahmoudi, V. Rabiei, M.S. Aghdam, and A. Soleimani. 2018. Glycine betaine treatment attenuates chilling injury and maintains nutritional quality of hawthorn fruit during storage at low temperature. *Scientia Hort.* 233:188–194.
- Romero, I., V.H. Maria, M.I. Escribano, M. Carmen, and M.T. Sanchez-Ballesta. 2016. Expression profiles and DNA-binding affinity of five *ERF* genes in bunches of *Vitis vinifera* cv. cardinal treated with high levels of CO₂ at low temperature. *Front. Plant Sci.* 7:1748, doi: 10.3389/fpls.2016.01748.
- Simpson, R., C. Acevedo, and S. Almonacid. 2009. Mass transfer of CO₂ in MAP systems: Advances for non-respiring foods. *J. Food Eng.* 92:233–239.
- Vigneault, C., D.I. Leblanc, B. Goyette, and S. Jenni. 2017. Invited review: Engineering aspects of physical treatments to increase fruit and vegetable phytochemical content. *Can. J. Plant Sci.* 92:373–397.
- Waghmare, R.B. and U.S. Annapure. 2018. Integrated effect of radiation processing and modified atmosphere packaging (MAP) on shelf life of fresh fig. *J. Food Sci. Tech. Mys.* 55:1–10.
- Wilson, D.M.D., R.A. Stanley, D.A. Eyles, and D.T. Ross. 2017. Innovative processes and technologies for modified atmosphere packaging of fresh and fresh-cut fruits and vegetables: A review. *Crit. Rev. Food Sci. Nutr.* 59:411–422.
- Xie, J., Y. Dai, H.X. Wang, Y. Yi, W.F. Hou, Y.W. Ai, and T. Min. 2018. High concentration CO₂ modified-atmosphere packaging inhibits enzymatic browning of fresh-cut lotus root slices by regulating phenolic metabolism. *Modern Food Sci. Technol.* 34:168–174.
- Yang, R., J. Liu, Z. Lin, W. Sun, Z. Wu, H. Hu, and Y. Zhang. 2018. *ERF* transcription factors involved in salt response in tomato. *Plant Growth Regulat.* 84:573–582.
- Yin, X.R., A.C. Allan, Q. Xu, J. Burdon, S. Dejnopratt, K.S. Chen, and I.B. Ferguson. 2012. Differential expression of kiwifruit *ERF* genes in response to postharvest abiotic stress. *Postharvest Biol. Technol.* 66:1–7.
- You, Y., Y. Jiang, J. Sun, H. Liu, L. Song, and X. Duanb. 2012. Effects of short-term anoxia treatment on browning of fresh-cut chinese water chestnut in relation to antioxidant activity. *Food Chem.* 132:1191–1196.
- Zhou, D., L. Li, Y.W. Wu, J.F. Fan, and J. Ouyang. 2015. Salicylic acid inhibits enzymatic browning of fresh-cut chinese chestnut (*Castanea mollissima*) by competitively inhibiting polyphenol oxidase. *Food Chem.* 171:19–25.