Nitrite accumulation during storage of tomato fruit as prevented by hydrogen gas

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ABSTRACT
The adverse effects of intake nitrite upon human health are well known. However, eating fruits and vegetables is one of the main pathways to absorb nitrite because of nitrogen assimilation in plants. This study demonstrated that during storage of tomato fruit, the production of endogenous hydrogen (H₂) was decreased, in parallel with nitrite accumulation and the senescence rate. Furthermore, exogenously applied H₂ could delay the decreased fruit H₂ production and senescence, but importantly, nitrite accumulation was blocked. Consistently, the activities and transcripts of nitrate reductase (NR; catalyzing the synthesis of nitrite) and nitrite reductase (NiR; responsible for the reduction of nitrite to ammonium), were either inhibited or increased, respectively, by 0.585 mM H₂. Decreased or increased nitrite synthesis was observed when tungstate (an inhibitor of NR) or 2,6-dichloroindophenol sodium salt (a putative inhibitor of H₂ synthesis) were applied separately. Time-course analysis revealed that the decrease in vitamin C, a well-known nitrite scavenger, was blocked by H₂. Overall, this study strongly revealed that nitrite accumulation during storage of tomato fruit was prevented by H₂. This study describes potential applications for H₂ in agriculture and food industry, especially in the preservation of fruit and vegetable products.

Abbreviations: Ar, argon; DCPIP, 2,6-dichloroindophenol sodium salt; GC, gas chromatograph; H₂, hydrogen gas; HPLC, high-performance liquid chromatography; HRW, hydrogen-rich water; N, Newton; N₂, nitrogen; NO, nitric oxide; NiR, nitrite reductase; NO₂‾, sodium nitrite; NO₃‾, sodium nitrate; NR, nitrate reductase; ONOO−, peroxynitrite anion; qPCR, Real-time quantitative reverse transcription-PCR; RNS, reactive nitrogen species; ROS, reactive oxygen species.

Introduction
Tomato (Solanum lycopersicum cv.) is one of the most important vegetable crops and is consumed widely throughout the world. It is a good source of folate, potassium, and vitamins A, C and E, as well as lycopene and phenolic compounds. There is ample evidence to show that the consumption of tomato fruit and related food products play positive roles in preventing human diseases, such as cardiovascular and chronic degeneration diseases, and age-related macular degeneration. Despite these valuable characteristics, tomato is a climacteric fruit, so that nitrite content may increase...

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Compared to leafy vegetables, the concentration of NO$_2^-$ in tomato is typically too low to be problematic for adult health. However, taking into account the large-scale application of nitrogenous fertilizers in China (accounted for >30% of world’s total and exceeded the combined usage in both North America and the European Union in 2009), there has been an increasing threat of nitrate and nitrite accumulation in vegetable foods.\[3,4\] Additionally, since tomato can be normally stored in local open market or kitchen, and its genome has been sequenced,\[5\] it has become a suitable plant model to investigate the molecular mechanism(s) relating to NO$_2^-$ accumulation during storage and retail display.

Sodium and potassium nitrite, which are usually referred to as nitrite salts, have been widely used in meat production industry as preservatives for many centuries.\[6\] However, nitrite has adverse effects upon human health. Firstly, nitrite can increase methemoglobin content by reacting with hemoglobin, transforming the ferrous iron to the ferric state and then arresting or decreasing the oxygenation of blood. This dangerous disease, which is called methemoglobinemia, is common in infants these days.\[7\] Nitrite can also generate reactive nitrogen species (RNS), including nitric oxide (NO) and peroxynitrite anion (ONOO$^-$), in both animals and plants.\[8,9\] Besides the signaling role of NO in the human body, these RNS can also react with body tissues and trigger enzyme inactivation, DNA lesions, lipid peroxidation, damage different organs, and can be considered as one of the most important human dietary carcinogenic factors.\[10–12\] Nitrite, in particular, can easily react with amides and amines to produce carcinogenic N-nitroso compounds.\[13–17\] According to some epidemiological and toxicological studies, N-nitroso compounds may potentially increase the risk of esophageal and stomach cancer.\[10,18–21\] In fact, stomach cancer is globally the third most common cause of death in men, following lung and prostate cancer, and is fourth for women.\[18,22\]

Fruit and vegetables play an important role in human diet with respect to their provision of minerals, vitamins, protein, carbohydrate and other biologically active compounds.\[23\] Furthermore, some antioxidant compounds that are found in food, for example, vitamin C, could inhibit nitrite accumulation.\[6,24,25\] Because of nitrogen assimilation by plants due to nitrogen fertilization, eating fruit and vegetables is one of the main pathways to ingest nitrite.\[18,20,26\] In addition, during storage after harvesting, nitrite content has been found to increase constantly.\[26,27\]

In plants, nitrogen assimilation is a basic physiological process, which is necessary for plant growth and development.\[28,29\] Nitrite is normally generated from nitrate catalyzed by nitrate reductase (NR), a cytosolic enzyme. In this process, NR is a key rate-limiting enzyme of nitrate-to-nitrite reduction process in the plant assimilation pathway, and it catalyzes the NAD(P)H-dependent reduction of nitrate to nitrite. As a result, the regulation of NR activity and its expression plays an important role in the formation of nitrite.\[30\] After it is transferred to the chloroplast by specific systems, nitrite is reduced to ammonium catalyzed by nitrite reductase (NiR), which later becomes a part of plant organic components including proteins.\[31\]

Hydrogen gas (H$_2$), mainly catalyzed by hydrogenase and nitrogenase in plants, has recently been identified as a gasotransmitter.\[32\] A large number of studies have shown that exogenous application of H$_2$ (hydrogen-rich water; HRW) can influence multiple plant responses, including the alleviation of various abiotic stress and the promotion of root development.\[33–36\] In addition, the application of H$_2$ has been observed to prolong the storage life of fruits and flowers.\[37–39\] Importantly, the positive effects of H$_2$ involvement in these responses were partially mediated by modulating antioxidant enzyme gene expression and in turn their activities.\[34,37\] The interplay of H$_2$ and other gasotransmitters has also been observed. For instance, exogenously applied H$_2$-alleviated alfalfa root elongation inhibiton, caused by aluminum stress, was associated with the inhibition of NO generation, with the involvement of NR-mediated NO production being suggested.\[33\] However, there is yet no information regarding the effect of endogenous H$_2$ on nitrite accumulation in vegetables during storage.

To date, no information regarding the influence of H$_2$ on the preservation of food products during storage by delaying the nitrite accumulation has been reported. The aim of this research was to investigate if and how endogenous H$_2$ could help to prevent nitrite accumulation in tomato during storage which leads to some health adverse problems after human consumption.\[1,2\]
during storage. In this study, the effect of H₂ on preventing nitrite accumulation was investigated by physicochemical, biochemical and molecular methodology. The results observed in our investigations provided some insights into the molecular mechanisms of H₂ in food storage, which permitted the assessment of the potential application of HRW and H₂ gas, as suitable approaches to preventing nitrite accumulation during vegetable fruit storage.

**Materials and methods**

**Plant materials**

Commercial tomato fruits (*Solanum lycopersicum* cv. Jiafen No. 2) were purchased from the Suguo market in Nanjing (Jiangsu Province, China) with uniform size and shape without diseases, defects and physical damages. Tomatoes at commercial maturity were transported to the laboratory within 1 h. Before various treatments were applied, the tomato fruit were surface-sterilized by sodium hypochlorite for 3 min, then thoroughly washed with double-distilled water and dried at ambient temperature (25°C). In order to verify H₂ function, six other fruits and vegetables (eggplant, lettuce, daylily flower, spinach, apple and carrot) were also stored with the experimental conditions described below.

**Preparation of HRW**

Purified H₂ produced by a H₂ generator (SHC-300, Saikesaisi Hydrogen Energy Co., Ltd., Shandong, China) was bubbled into 4000 mL distilled water at a rate of 160 mL min⁻¹ for at least 90 min at ambient temperature, to reach a saturation level (0.78 mM, as determined by gas chromatography, GC).

Subsequently, the hydrogen-rich water (HRW) was immediately diluted to 0.195 mM and 0.585 mM to be used in further experiments. The half-life period (concentration) of HRW is 12 h as determined by GC.

**Storage condition and phenotypic analysis**

Fruits were immersed in a plastic box containing 4000 mL distilled water with or without 0.195, 0.585 and 0.78 mM H₂, 200 μM vitamin C, 500 μM tungstate (an inhibitor of NR), 100 μM DCPIP (an artificial electron acceptor, which can interrupt the electron transfer to hydrogenase, then inhibits H₂ production), 1 mM sodium nitrite (NO₂⁻), 1 mM sodium nitrate (NO₃⁻), either alone or in combination with other components for 20 min at ambient temperature, and then air-dried for 30 min.

Afterwards, to simulate a constant normal or microthermal storage condition, the fruits were placed at 25.0 ± 0.2°C (room temperature; mimicking the temperature in the open market or kitchen) or 4.0 ± 0.2°C (mimicking the temperature in refrigerator) in the plastic container (21 L, Lock & Lock) for 16 d. During storage, samples were taken at the indicated time points for phenotypic, physiological and biochemical analyses.

In order to verify that the inhibition of nitrite production was caused by H₂ gas rather than hypoxia, nitrogen (N₂)- and argon (Ar)-rich water controls were used. According to the method, as described by the previous report, a H₂ fumigation test was also conducted. The tomato fruit were fumigated with 4.5 μL L⁻¹ H₂ in a plastic container. The containers were placed in the same treatment condition for the indicated time points at room temperature (25.0 ± 0.2°C). Control group was placed in the same condition without H₂.

**Flesh firmness measurements**

Flesh firmness was determined using a TA-XT2i texture analyzer (Stable Micro System Ltd., U.K.) with a 5 mm probe at a speed of 1 mm s⁻¹. Measurements were performed on the opposite sides of each individual fruit tested. Flesh firmness of fruit was expressed as Newton (N). The higher firmness values meant that samples were more firm.
**Determination of H₂ content**

According to the previous methods, about 0.5 g fresh weight of fruit tissues were homogenized, and then placed in a clean vial, followed by the addition of 5 mL distilled water and 0.2 mL of 5 mM sulphuric acid. Afterward, the inside air of vial was displaced with pure N₂, and the vial was immediately capped. H₂ content was then analyzed by using an Agilent 7820 model gas chromatograph (GC; Agilent Technologies Inc., USA) equipped with a column containing the Molecular Sieve 5 Å stationary phase and a thermal conductivity detector. The column was held isothermally at 60°C, and the detector and injection temperature regulated at 150°C and 70°C N₂, respectively, was used as carrier gas, with the gas pressure being 0.2 MPa.

**Detection of nitrite and nitrate contents by high-performance liquid chromatography (HPLC)**

According to the method described in the previous report, the concentrations of nitrite and nitrate of fruit tissues were analyzed by HPLC (D-2000, Hitachi Ltd., Tokyo, Japan) with a ZORBAX Eclipse XDB-C₁₈ (250 × 4.6 mm; 5 μm particle size; Fortis Technologies Ltd., Cheshire, UK). The mobile phase was KH₂PO₄-H₃PO₄ (0.03 M, pH 3.3), and flow rate was 1.0 mL min⁻¹. After filtration through a 0.22 μm filter (Puradisc 25 NYL; Whatman, Maidstone, UK), all samples were injected into HPLC. The detection wavelength was 214 nm, and the volume of each sample injected into the HPLC was 20 μL. Total run time was 10 min, and detection time of nitrate and nitrite were 3.85 min and 5.95 min, respectively.

**NR and NiR activity assay**

According to the method described in a previous report, NR activity was assayed with some method modifications. About 5 g of fruit tissues were grinded with extraction buffer containing 50 mM HEPES-KOH (pH 7.5), 20 mM FAD, 2 mM EDTA, 1 mM leupeptin, 5 mM Na₂MoO₄, and 1% polyvinylpyrrolidone (v/v). After centrifuging at 10,000 g for 20 min at 4°C, the supernatant was obtained. To analyze NR activity, 1 mL of the supernatant was blended with 5 mL of reaction buffer (prewarmed at 25°C, containing 50 mM HEPES-KOH, pH 7.6, 5 mM KNO₃ and 0.2 mM NADH), and the mixture was incubated at 25°C for 30 min. The reaction was stopped by adding 0.1 M zinc acetate. After 10 min, the mixture was centrifuged at 13,000 g for 5 min at 4°C. Afterward, supernatant was added with 1 mL of reaction reagent containing 58.1 mM sulfenilamide in 3 M HCl and 1 mL of 1.07 mM N-(1-naphthyl)-ethylenediamine. Finally, the nitrite production was analyzed by colorimetry at 540 nm.

The NiR activity was analyzed by following previous methods with some modifications. The extract was added to a reagent including 0.5 M Tris-HCl buffer (pH 8.0), 20 mM sodium nitrite, 5 mM methyl viologen, sodium dithionite solution (25 mg sodium dithionite dissolved in 1 mL of 0.29 M NaHCO₃), and Griess reagent. The mixture was incubated at 30°C for 30 min, and the reaction was stopped by strenuous vortexing. The remaining nitrite content was analyzed by colorimetry at 540 nm.

**RNA extraction and analysis of gene transcription**

Total RNA was extracted from fruit tissues by homogenizing with pestle and mortar in liquid nitrogen until a fine powder appeared. The powder was transferred to clean tube with TransZol Up Kit (TransGen Biotech, Beijing, China), and then digested with RNase-free DNase. The quantification of RNA was performed by using a NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, USA).

Total first-strand cDNA synthesis was conducted in a 20 μL reaction volume containing 1 μL oligo(dT) primers and 1 μL SuperScript™ reverse transcriptase (Invitrogen, Carlsbad, USA). Real-
time quantitative reverse transcription-PCR (qPCR) was carried out by using Mastercycler ep realplex real-time PCR system (Eppendorf, Hamburg, Germany) with TransStart Top Green qPCR SuperMix (TransGen Biotech, Beijing, China). The genes expression levels of relevant genes were presented as values compared to the control samples, after normalization with two reference genes SlActin and SlGAPDH. The sequences of the primers were given in Supplementary Table S1. The quantification of expression levels of the relative genes was examined by using the $2^{-\Delta\Delta C_{T}}$ method.

**Evaluation of vitamin c content**

The determination of vitamin C content was carried out by using a previous method. Tomato fruit tissues were sampled and extracted with trichloroacetic acid solution (0.3 M). After derivatization with 1,2-o-phenylenediamine, quantification of vitamin C was performed by HPLC (D-2000, Hitachi Ltd., Tokyo, Japan). The mobile phase was 0.1 mol L$^{-1}$ K$_2$HPO$_4$-0.08 mol L$^{-1}$ KH$_2$PO$_4$-CH$_3$OH (55/25/20, v/v/v), and flow rate was 1.5 mL min$^{-1}$. Detection was performed by $\lambda$ (excitation) = 350 nm and $\lambda$ (emission) = 430 nm. The volume of each sample injected to HPLC was 20 μL. Total run time was 10 min. Quantification was carried out by external calibration with L-ascorbic acid.

**Experimental design**

According to previous methods with some minor modifications, all experiments were arranged in a randomized complete block design. The experiment was designed with three replicates for each. For the determination of NO$_2^-$ and NO$_3^-$ content, 10 tomato fruits per replicate were used, and the total tomato fruits in triplicate was 30 (10 × 3). For the other parameters, including firmness, H$_2$ concentration, enzyme (NR and NiR) activity and gene (SlNR and Slnii1) expression level, and vitamin C content, five tomato fruits per replicate were selected and/or sampled for the analyses, and the total tomato fruits in triplicate was 15 (5 × 3). To avoid decayed or overripe zone, all samples were from healthy tissues.

**Statistical analysis**

The data is presented as the means ± standard error (SE). Statistical analysis was performed using SPSS 18.0 software. The statistical analyses of two groups or multiple groups were performed using t-test or Duncan’s multiple range tests. In all cases, the confidence coefficient was set at 0.05.

**Results and discussion**

**H$_2$ not only delays senescence but also prevents nitrite accumulation during storage**

Previous results have shown that a delay in kiwifruit ripening can be achieved by HRW and H$_2$ gas individually. In order to evaluate the role of H$_2$ in nitrite accumulation, the contents of nitrite and nitrate in tomato fruit were analyzed by HPLC. Here, our results revealed that exogenous application with HRW not only delayed senescence of tomato fruit but also prevented nitrite accumulation during storage.

Initially, a pilot experiment revealed that pretreatment with HRW containing 0.195, 0.585 and 0.78 mM H$_2$ of tomatoes differentially blocked nitrite accumulation, with contrasting reductions in nitrate contents (Supplementary Figure S1). Given that the responses to 0.585 mM H$_2$ were maximal, HRW containing this concentration of H$_2$ was used in the following experiments.

Since the concentration of H$_2$ in HRW used can reduce the dissolved oxygen content in water (hypoxia), two controls with proportional saturation either argon- or nitrogen-rich water were assessed. As expected, hypoxia failed to alter endogenous nitrite contents during storage of tomato
fruit (Supplementary Figure S2), confirming that the responses observed above to HRW were H2-specific. Above conclusion was also supported by the direct H2 fumigation test (Supplementary Figure 3a-b).

Time-course analysis further showed that the symptoms of fruit quality deterioration appeared after 12 d of storage, which was reduced by H2 treatment (Figure 1a). Meanwhile, the decreased firmness during tomato fruit storage was arrested by H2 (Figure 1b, Supplementary Figure S3c). Similar results were obtained when postharvest kiwifruit was treated with H2 gas. During the

**Figure 1.** Exogenously applied H2 not only delayed senescence (a, b), but also slowed down H2 production (c) and nitrate content (d), and nitrite accumulation (e) during storage of tomato fruit. Bar = 5 cm. Asterisk denotes significant difference at P < .05 according to t-test.
storage period, endogenous H₂ production was progressively decreased (Figure 1c, Supplementary Figure S3d), indicating that H₂ homeostasis was disrupted by senescence. Interestingly, the reestablishment of H₂ homeostasis was observed when exogenous H₂ was applied, indicating that maintenance of homeostatic H₂ concentration was critical for delaying fruit ripening during storage.³⁸

Normally, nitrite and nitrate contents in fruit and vegetables are changed during storage,²⁶ and both are regarded as standards of freshness.²⁷ In addition, nitrite has a potential clinical risk.¹¹,¹²,²³ Importantly, it was observed that H₂-induced nitrate production and prevented nitrite accumulation in tomato fruit was in a time-dependent fashion (Figure 1d–e). With eggplant, lettuce, daylily flower, spinach, apple and carrot as experimental materials, further time-course experiments showed that H₂-prevented nitrite accumulation in these plant materials during storage, demonstrating that H₂ was a universal preservation agent (Supplementary Figure S4).

Changes in NR and nir activities and gene expression in response to H₂

In the process of nitrite metabolism, NR (catalyzing the synthesis of nitrite) and NiR (responsible for the reduction of nitrite to ammonium) were two of the key enzymes.²⁸ To investigate whether NR and NiR were regulated by H₂, corresponding enzymatic activities were assessed. The results showed that H₂ treatment reduced relative NR activity by 42.4% in H₂-treated fruit for 8 d, compared to the control fruit (Figure 2a). Conversely, H₂ treatment for 4 d significantly increased NiR activity in tomato fruit (Figure 2b). The changes reported above were consistent with the changes of SlNR and

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Figure 2. Changes of NR (a) and NiR (b) activities and corresponding transcripts (c and d). Asterisk denotes significant difference at P < .05 according to t-test.
Slnii1 transcripts, as analyzed by qPCR (Figure 2c-d). Combined with the changes in nitrite and nitrate contents (Figure 1d-e), the nitrite accumulation prevented by H₂ was at least partially, associated with the decreased NR activity.

**The inhibition of NR is involved in H₂-prevented nitrite accumulation**

To better characterize the contribution of NR-generated nitrite production in above H₂ response, the effects of the inhibitor of NR (tungstate) on nitrite content and NR activity in the presence and absence of H₂, were studied and compared.[28,33,35] Meanwhile, NO₃⁻, a substrate of NR enzyme and an inducer of its synthesis,[30,46] was used as a positive control. As shown in Figure 3, mimicking the response of H₂, nitrite content during storage was markedly reduced by the inhibition of NR activity with tungstate. Consistently, the inhibition of NR activity by H₂ was previously reported in aluminum-treated alfalfa seedlings.[33] An additive response was further observed when H₂ was added together with tungstate. By contrast, the increased nitrite content triggered by the addition of

![Figure 3](image)

*Figure 3.* Changes of nitrite level (a) and NR activity (b) during storage of tomato fruit for 12 d. Different letters denote significant difference at $P < .05$ according to Duncan’s multiple tests.
sodium nitrate, which can increase NR activity and its synthesis,[30] was impaired by H$_2$ (Figure 3a). Similarly, these findings paralleled the changes in NR activity (Figure 3b). Above findings suggested that the inhibition of NR might be involved in H$_2$-prevented nitrite accumulation. Certainly, the role of NiR activity might not be easily ruled out.

**Contrasting responses were observed when DCPIP and H$_2$ were applied individually**

In order to investigate the possible role of endogenous H$_2$ in preventing nitrite accumulation, a putative synthetic inhibitor of H$_2$, DCPIP was applied.[40] As shown in Figure 4a,b, delayed or accelerated senescence were observed in tomato fruit when supplemented with H$_2$ and DCPIP, respectively. Consistent with previous reports,[36,40] it was observed that exogenous H$_2$ application could increase endogenous H$_2$ content in tomato fruits, whereas DCPIP inhibited H$_2$ accumulation (Figure 4c). It was also noted that nitrite contents were decreased by H$_2$, but increased by DCPIP, in comparison with the chemical-free tomato fruit (Figure 4d), suggesting the critical role of endogenous H$_2$ homeostasis. This conclusion was further supported by the subsequent results, showing that above H$_2$ responses were sensitive to the addition of DCPIP. Collectively, it was concluded that alterations of endogenous H$_2$ in fruit were closely associated with the senescence process.

![Figure 4](image-url). Effects of H$_2$ and DCPIP on senescence (a and b), H$_2$ contents (c), and nitrite accumulation (d) during storage of tomato fruit for 12 d. Bar = 5 cm. Different letters denote significant difference at $P < .05$ according to Duncan’s multiple tests.
Participation of vitamin c in H₂-prevented nitrite accumulation

As a major source of antioxidant, vitamin C can react with many clinical health-risk factors, including nitrite. To investigate the possible role of vitamin C in the above observed H₂-prevention of nitrite accumulation, a time-course analysis of vitamin C level during tomato fruit storage, was determined by HPLC after treatment in the presence or absence of H₂. Similar to the reduction of endogenous H₂ production (Figure 1c), decreased vitamin C levels were observed. In contrast, these changes were partially blocked by the administration of H₂ (in particularly at 8 and 12 d; Figure 5). These findings were consistent with those reported previously, showing that the decreased vitamin C contents in mercury-stressed alfalfa seedlings were rescued by H₂. Importantly, these changes were also negatively correlated to the biological response of nitrite content (Figure 1e).

Previous studies suggested that intake of fresh fruit and vegetables containing vitamin C was negatively correlated with cancer of the stomach. Several approaches, including the addition of vitamin C or other antioxidants, have been applied to reduce nitrite content and nitrosamines formation. Accordingly, our results imply a possible interaction between H₂ and vitamin C governing NR-mediated nitrite accumulation. It has been reported that H₂ could also be an antioxidant, not only selectively scavenging reactive oxygen species (ROS), but also interacting with or modulating vitamin C metabolism. Therefore, the interaction among H₂, vitamin C and ROS with respect to nitrite metabolism during storage requires further verification.

During conditions of cold storage, nitrite level was observed to be lower because low temperature inhibits NR. To confirm the role of vitamin C in H₂-prevented nitrite accumulation, nitrite contents were determined in both room temperature (25°C) and cold storage conditions (4°C; mimicking the temperature in refrigerator). As shown in Figure 6, tomato fruit stored at 4°C showed a significant decrease in nitrite content compared to room temperature, suggesting that low temperature decreased nitrite formation by inhibiting NR activity. A subsequent experiment showed that, nitrite accumulation was prevented by vitamin C as well as H₂, but increased by exogenously applied NO₂⁻, at either 25°C or 4°C. H₂ inhibition of NO₂⁻-induced nitrite accumulation was also observed, regardless of the storage temperature. Combined with the previous results that the decreased vitamin C was partially rescued by H₂ administration (Figure 5), we further suggest that

Figure 5. Time-course analysis revealed that the decreases in vitamin C were slowed down by H₂ administration. Asterisk denotes significant difference at \( P < .05 \) according to t-test.
H$_2$ may play a role in reducing nitrite accumulation by regulating the endogenous vitamin C level. We advocate that future studies should investigate the extent to which level of vitamin C synthetic gene expression during storage is dependent on H$_2$ signaling pathway.

**Conclusion**

In summary, this research showed that endogenous H$_2$-prevented nitrite accumulation was due to decreased NR activity and the maintenance of vitamin C content in stored tomato fruit. Considering the other advantages of H$_2$, such as nontoxicity, absence of side effects and easy diffusion, these results identify potential uses of H$_2$ in the agricultural and food industries with respect to the preservation and storage of fruit and vegetable products.

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