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Controlled atmosphere storage with high CO_2 concentration extends storage life of fresh pomegranate fruit by regulating antioxidant capacity and respiration metabolism

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Abstract

Background Pomegranate is susceptible to low temperature. Controlled atmosphere (CA) with high CO_2 concentration can prolong storage life by affecting fruit quality. Our study probed into the effect of CA storage on the pomegranate fruit quality as well as antioxidant attributes at 5 ℃ for 30 d, plus at 18 ℃ for 9 d in simulate marketing conditions. The atmosphere compositions of 7% O_2 + 4% CO_2 (CA1) and 2% O_2 + 5% CO_2 (CA2) were chosen, the regular air as control (CK).

Results In arils, the highest contents of total phenols, total flavonoids, and anthocyanins were all found after CA2 treatment, significantly higher 0.04 g kg^{-1} FW, 0.19 g kg^{-1} FW, and 0.70 g L^{-1} FW than those in CA1, respectively. Also, the highest enzyme activity of superoxide dismutase (SOD), glutathione reductase (GR), and ascorbate peroxidase (APX) in husks were presented in CA2, 36.30%, 36.05%, and 4.36%, higher than in CA1, respectively. Thus, CA2 treatment maintained better husk appearance due to significantly reducing chilling injury, malondialdehyde content, electrolyte leakage, and two enzymes activity, including peroxidase (POD) and/or polyphenol oxidase (PPO), meanwhile improving phenylalanine ammonia-lyase (PAL) enzyme activity. Also, CA2 effectively maintained the postharvest quality and reduced nutrition loss for fresh pomegranate fruit by reducing respiration rate, maintaining suitable energy charge, increasing ROS scavenging ability.

Conclusions Totally, 2% O_2 + 5% CO_2 (relative higher CO_2 concentration) could prolong the storage life of pomegranate fruit during cold storage by regulating antioxidant capacity and respiration metabolism, both higher nutrition value and better appearance.

Keywords Pomegranate, Controlled atmosphere storage, Fruit quality, Antioxidant capacity

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Background

Pomegranate (*Punica granatum* L.) exerts biological functions due to rich polyphenols, such as ellagic acid glycosides, punicalagin, caffeic acid, and so on. Also, its husks, leaves, arils, and seeds have been made use for traditional herbal medicine for a long time. Therefore, pomegranate has various pharmacological effects, benefiting to human health [1–3]. However, the harvested pomegranate fruit is very sensitive to temperature and humidity, resulting in physiology disorders, including chilling injury, cracks, shrink, and husk scald, which greatly shortens the postharvest life and causes considerable economical loss [4, 5].

During storage, physiological damage of pomegranate fruit is still inevitable, e.g. the browning of husk and aril, directly affecting the marketability along with the loss of nutrient compounds and firmness [6]. And, chilling temperature causes cell membrane dysfunction in pomegranate, furthermore, the oxidation reaction of tannins or other phenol compounds with PPO, leading to shrivel and fade of husk and aril, even decay [7, 8]. Generally, respiratory metabolism, energy metabolism and reactive oxygen species (ROS) metabolism form a complex metabolic network and interact with each other to regulate the postharvest fruit quality [9, 10]. High intensity respiration accelerates the consumption of metabolic substances, leading to the senescence of horticultural products [11]. Whereas, high adenosine triphosphate (ATP) level effectively inhibited the development of decay and browning in some fruits, e.g. blueberry [12], flat peach [13], and apple [14]. ROS production destroys membrane integrity, and accelerates enzymatic browning caused by POD and/or PPO [15], which directly resulted in fruit quality degradation, even, enormous economic loss.

In view that low temperature can slow down the metabolism rate of plant cell, cold storage is widely applied in retarding senescence and prolonging the storage life of vegetables and fruits. It is reported that the combination of low temperature and the optimal atmospheric condition surrounding the horticultural products benefits for postharvest quality and decreases pathogen development of fruit [13, 16–18]. Currently, for CA-stored pomegranate fruit, 2 kPa O_2 + 5 kPa CO_2 was superior to the regular air for fruit appearance characteristics at 7 ℃ after 5 months storage, based on the comparison of three pomegranate cultivars [19]. Also, Sidhu et al. [20] thought that 3% O_2 + 5% CO_2 at 5 ℃ maintained better fruit quality than regular air storage, among 6 pomegranate cultivars. The previous studies discussed that the less scald development of 'Wonderful' pomegranate was founded in 5 kPa O_2 + 15 kPa CO_2 than 1 kPa O_2, and 1 kPa O_2 + 15 kPa CO_2 at 7 ℃ [21]. Similarly, the gray mold was greatly prevented by 5 kPa O_2 + 15 kPa CO_2 with potassium sorbate in pomegranate fruit [16]. In summary, the optimal O_2/CO_2 ratio was beneficial to postharvest pomegranate fruit, especially, relatively high CO_2 concentration. However, its effects varied from the storage temperature, gas conditions, and genetic backgrounds. At present, 'Tunisia' pomegranate has been widely cultivated in China. The plenty of fresh pomegranate fruit pile into the market around October every year, and, 'Tunisia' as a soft-seed pomegranate cultivar, is more sensitive to low temperature [22]. Thus, it is extremely necessary to develop an optimal atmosphere condition for perishable pomegranate fruit, especially short-term storage and shelf-life storage. Thus, fruit quality characteristics and the related enzymes involving in antioxidant system were investigated in stored pomegranate in the current study.

Materials and methods

Fruit and storage procedures

Pomegranate cv. 'Tunisia' fruit were harvested at the commercial mature stage in Xingyang, Henan, China (34°46'-N,113°21'-E) in October, 2022. To stagger the peak of sales and improve economic income, 5 ± 0.5 ℃ for 30 d and 18 ± 1 °C for 9 d were set in the present study. The fruit without visual symptoms of any disease, sunburn, decay, and cracks were selected. The single fruit weight was 380 ± 30 g. The fruit samples were precooled in the regular air at 5 ℃ and 85 ± 1% of relative humidity (RH) for 24 h, and immediately stored in the controlled atmosphere storehouse at 5 ± 0.5 ℃ and 85 ± 1% RH (Dehezi Artificial Environment Technology Co. Ltd, Beijing, China) for 30 d. Two CA conditions were set, 7% O_2 + 4% CO_2 (CA1) and 2% O_2 + 5% CO_2 (CA2), the regular air for the control (CK). Then, the fruit were transferred to 18 ± 1 °C and 98 ± 1% of RH for 9 d, to simulate the shelf life, and collected every 3 d during the shelf life. Each treatment included fifteen fruits in three technique replicates.

Chilling injury index (CI), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity, electrolyte leakage (EL), and malondialdehyde (MDA) content in husks

Husk CI was calculated for pomegranate fruit with the method from Sayyari et al. [23]. The damaged area of the husk surface included browning, pitting and dehydration, and scored based on no symptoms as 0, 1–25% as 1, 26–50% as 2, and > 51% as 3. The formula is: CI= ∑(value of hedonic scale)×(fruit number with the corresponding scale number)/(total fruit number in the sample×4) [23].

Free radical DPPH scavenging capacity was performed with the instruction of DPPH-1-D Kit (Suzhou Comin, Jiangsu, China), and the absorbance at 515 nm was assessed to be expressed as %. EL in husk was evaluated using the method reported by Mirdehghan et al. [24]. The husk tissues were autoclaved at 121 ℃ for 20 min, and tested after overnight, which was expressed as % for EL.

MDA concentration level (μmol kg^{-1} FW) was assessed in husks using the commercial Kit (MDA-2-Y Kit, Suzhou Comin, Jiangsu, China).

Color traits in husks

Color traits were conducted with a high-precision colorimeter (HP-C210, China), including L^* value (lightness), b^* value (blue to yellow), and a^* value (green to red). Color difference (ΔE) = $\sqrt{(L^*-L_0^*)^2+(a^*-a_0^*)^2+(b^*-b_0^*)^2}$, ($L^*$, b^* and a^* mean the sampling day. L_0^*, a_0^*, and b_0^* means the harvested day).

Aril tastes and nutraceutical properties

Pomegranate arils were wrapped using the sterile gauze and squeezed. The obtained juice was used for examining the content of anthocyanins and titratable acid (TA) as previously reported [8]. Briefly, for the determination of TA content, 5 mL pomegranate juice was titrated with 0.1 N NaOH, and calculated by the consumed volume, being given as %. The anthocyanins accumulation was quantified by the absorbance value at 510 and 700 nm in buffers at pH 1.0 and 4.5, respectively, and then measured as mg L^{-1} of cyanidin-3-glucoside.

To measure total soluble sugar (TSS) concentration in arils, the standard curve was drawn, and represented as g kg^{-1} FW [25]. The accumulation of ascorbic acid (AsA) was conducted in arils based on the previous procedure, being given as mg kg^{-1} FW [8].

The accumulation of total phenols and total flavonoids were performed as reported by Liu et al. [26]. The absorbance value was tested at 725 nm, and gallic acid equivalents (g kg^{-1} FW) was as total phenolic concentration. For the concentration level of total flavonoids, the absorbance at 506 nm was calculated, being expressed as mg kg^{-1} FW of rutin equivalents.

Enzyme activity of PPO, POD, and PAL in husks

To assay activity of PPO and PAL was in accordance with the procedure from Nguyen et al. [27]. The determination of PPO enzyme activity was calculated by the change of 0.01 absorbance per min at 420 nm. A change of 0.01 unit of the absorbance at 290 nm per hour was defined as a unit of PAL enzyme activity. For POD activity measurement, frozen 1.0 g husk tissues were powdered and homogenized with 5 mL, pH 8.5, 0.1 mol L^{-1} Tris-HCl buffer. After 1800-g centrifugation for 5 min, the supernatant was added pH 6.0, 0.2 mol L^{-1} buffer and Extraction solution. The amount of enzyme was as POD activity by 0.01 difference of the absorbance at 470 nm per min [8].

The results of PPO, POD and PAL activity were expressed as mkat kg^{-1} FW.

Biochemical parameters in ROS system in husks

Superoxide anion ($O_2^{-}\cdot$) content in husks was analyzed by hydroxylamine hydrochloride oxidation method (SA-1-G Kit, Suzhou Comin, China), being represented as μmol kg^{-1} (FW). The titanium sulfate method was applied to assay hydrogen peroxide (H_2O_2) content according to H_2O_2-1-Y Kit (Suzhou Comin, Jiangsu, China), being given as mmol kg^{-1} (FW). Nicotinamide adenine dinucleotide phosphate hydrogen oxidase (NOX) Assay Kit (Suzhou Comin, Jiangsu, China) was applied for the determination of NADPH oxidase (NOX) activity. The results were given as mkat kg^{-1} FW.

To measure SOD activity in husk samples was in accordance with the instruction of SOD-2-Y (Suzhou Comin, Jiangsu, China). Frozen 0.1 g pomegranate husk was grounded and homogenized with 1 mL extraction solution in ice bath. Then the solution was conducted the 8000-g centrifugation at 4 ℃ for 10 min. The absorbance value of the supernatant was tested and given as mkat kg^{-1} FW.

The reduction method of GR-1-W Kit was applied to determine the activity of (GR) (Suzhou Comin, Jiangsu, China). GR activity was given as μmol min^{-1} kg^{-1} FW at 340 nm. For assaying APX activity, the difference of 0.01 absorbance at 290 nm per min was calculated as mkat kg^{-1} FW [15].

Biochemical parameters in metabolism of energy and respiration in husks

The respiratory rate was expressed with CO_2 content (mg kg^{-1} h^{-1}), and evaluated with F-950 portable carbon dioxide analyzer (Beijing Sunshine Yishida, China). ATP content (mmol kg^{-1} FW) was conducted using the phosphomolybdic acid colorimetry by ATP-2-Y Kit (Suzhou Comin, Jiangsu, China). Frozen 0.1 g husk were mixed in 1 mL distilled water in an ice bath, and extracted in water bath of 100 ℃ for 5 min. At 4 ℃, the 8000-g centrifugation lasted for 15 min, and the absorbance value of collected supernatant was assayed at 700 nm.

To evaluate the activity of cytochrome C oxidase (CCO) enzyme, the method was referenced to Lin et al. [28]. One unit of CCO activity (mmol min^{-1} kg^{-1} FW) was defined by the enzyme amount that oxidized 1 μg cytochrome C in a minute.

For the assaying of nicotinamide adenine dinucleotide kinase (NADK) activity was performed using NADK assay Kit (Suzhou Comin, Jiangsu, China). The supernatant was calculated the absorbance value, being given as μmol min^{-1} kg^{-1} FW.

MTT colorimetric assay was applied to determine the concentrations of nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide hydrogen (NADH) using NAD Assay Kit (Suzhou Comin, Jiangsu, China). For determine the contents of nicotinamide

adenine dinucleotide phosphate ($NADP^+$) and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), NADP Assay Kit was used (Suzhou Comin, Jiangsu, China). The absorbance value at 570 nm was tested and given as μmol kg^{-1} FW.

Statistical analysis

Significant difference was assayed by one-way ANOVA and Duncan's multiple range test. Difference at $p < 0.05$ level was defined as significantly among treatments and sampling dates in technical triplicate. Origin 8.0 was used to draw the figures.

Results

Influence of CA treatments on husk appearance

The differences in husk and arils were exhibited in Fig. 1. As storage, husk browning appeared, however, little accidence frequence was found in CA2 treatment. Therefore, husk appearance in CA2 was better than CA1 and CK.

Chilling injury directly reflects husk damage of pomegranate fruit during storage. Compared with CK, CA treatments significantly inhibited the increase of husks CI during storage ($p < 0.05$), especially, during the cold storage, the fruit with CA2 treatment did not appear the symptoms of chilling injury (Fig. 1). Until the end of shelf life, CIs at each sampling dates were all significantly lower in CA2 than in CA1 and the CK ($p < 0.05$) (Fig. 2A). Electrolyte leakage is an important indicator to evaluate membrane integrity. In Fig. 2B, EL with CA2 storage always maintained the lowest level, with a significant difference compared to CA1 and the CK. Whereas, EL gradually increased in husk of CA1-stored fruit during storage. Figure 2C showed that the two CA treatments significantly alleviated the MDA accumulation in husks during the storage, especially, CA2 treatment. The DPPH scavenging capacity appeared the decreasing trend during the whole storage (Fig. 2D), and, CA2 treatment had significantly stronger DPPH scavenging capacity than

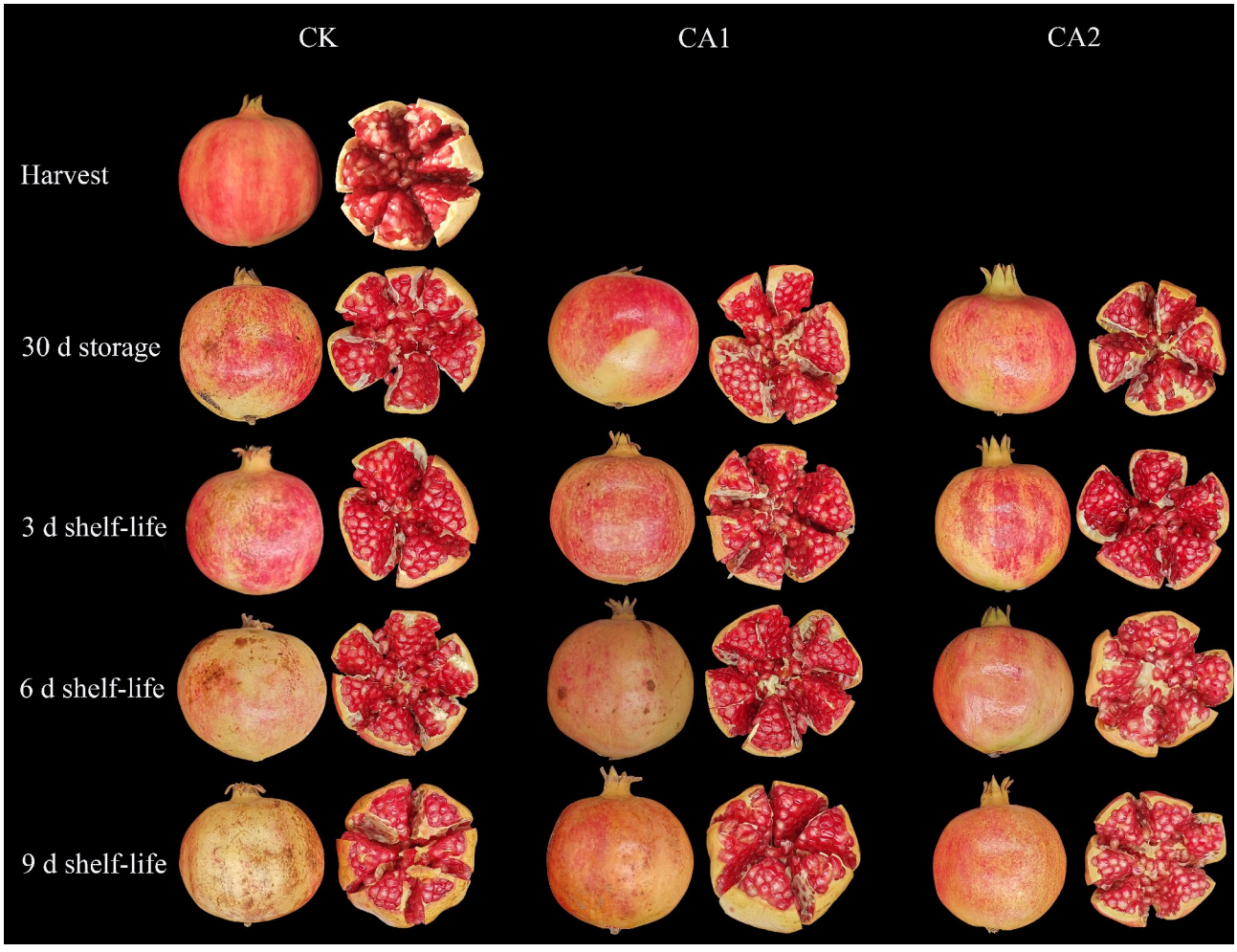

Fig. 1 Effect of CA treatments on husk appearance

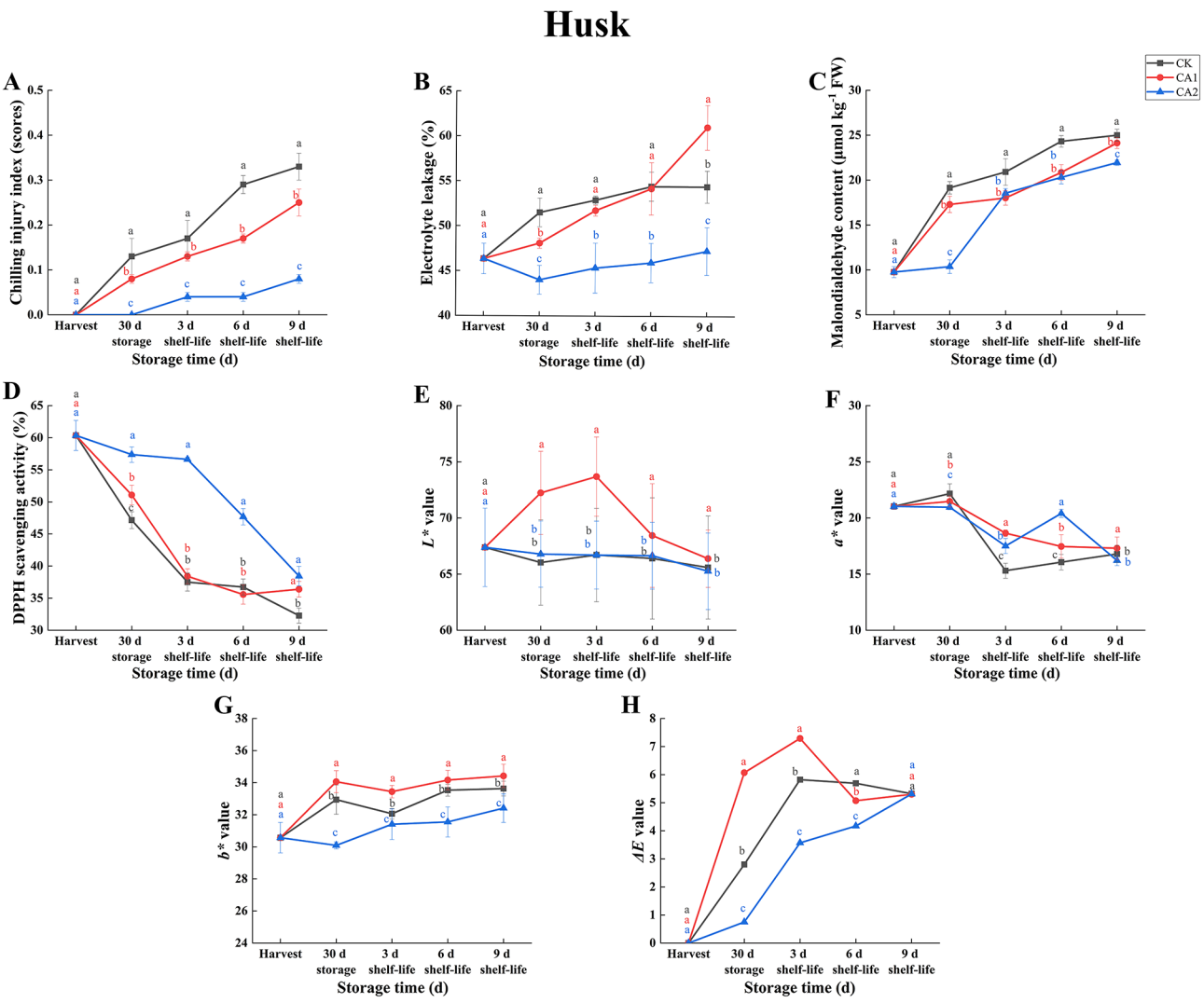

Fig. 2 Effect of CA treatments on chilling injury, and color traits of pomegranate husk during storage. **A**, chilling injury; **B**, electrolyte leakage; **C**, MDA content; **D**, DPPH scavenging activity; **E**, *L** value; **F**, *a** value; **G**, *b** value; **H**, *ΔE*. Vertical bars represent the standard deviations of means. Different letters present significant differences among treatments on sampling day during the storage at $p < 0.05$

CA1 and CK except at 9 d shelf-life. Therefore, CA2 treatment with high CO_2 concentration might be close relation to lighter symptom of chilling injury.

Husk color traits represent the first impression of pomegranate fruit for consumers, affecting the purchase intention of consumers. As shown in Fig. 2E-G, the color traits of pomegranate husk, including *L**, *a**, and *b**, were found distinct differences between the two CA treatments, and they were significantly higher in CA1 than in CA2 during storage ($p < 0.05$). However, from Fig. 2H, the *ΔE* was significantly lower in CA2 treatment than CA1 except the last day of shelf life ($p < 0.05$). The results indicated that the better husk color was found in CA2 atmosphere condition, which was partly proved by Fig. 1.

Influence of CA treatments on aril tastes and nutrition

In the present research, aril tastes and nutrition values were investigated to explore how to affect pomegranate fruit quality of CA storage. It was found from Fig. 3A and F, the contents of TA and anthocyanins in arils increased during the storage, while the accumulations of TSS, ascorbic acid, total phenols, and total flavonoids declined in stored arils. Furthermore, TSS, TA, anthocyanins, total phenols, and flavonoids were all observed significant accumulation in arils treated with the CA2 treatment during storage ($p < 0.05$). AsA accumulated the highest in CA1 at the end of storage, and more slowly declined in the two CA treatments than the CK (Fig. 3C). Thus, according to our results, it was inferred that CA2 might more greatly reduce loss of nutrient contents in arils.

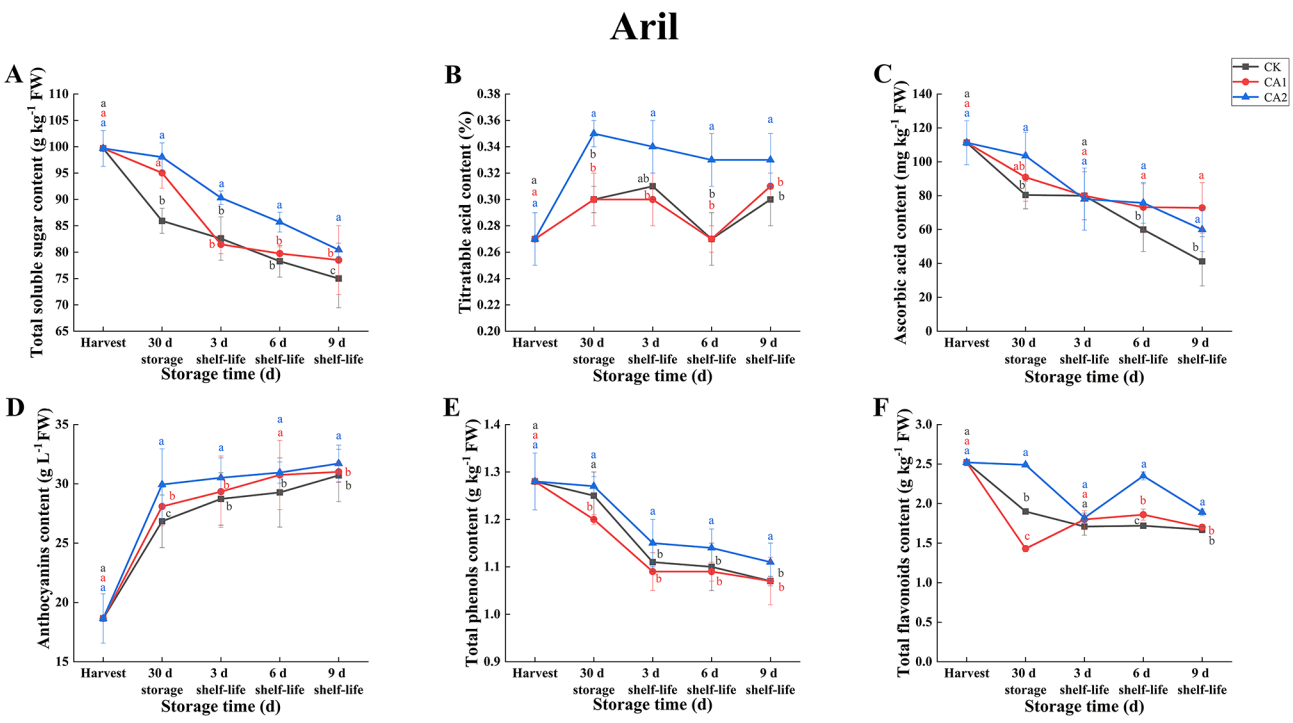

Fig. 3 Effect of CA treatments on aril parameters of pomegranate during storage. **A**, total soluble sugar content; **B**, titratable acid content; **C**, ascorbic acid content; **D**, anthocyanins content; **E**, total phenolics content; **F**, total flavonoid content. Vertical bars represent standard deviations of means. Different letters present the significant differences among treatments on sampling day during the storage at $p < 0.05$

Influence of CA treatments on ROS metabolism in husks

To furtherly clarify the effect of CA treatments on chilling injury, ROS production was measured. $O_2^{\cdot-}$ and H_2O_2 are two main indicators to evaluate the ROS production in fruit and vegetables. We found from Fig. 4A and B, that the $O_2^{\cdot-}$and H_2O_2 contents gradually rose in pomegranate husks with storage duration, and CA treatments had the lowest accumulation of $O_2^{\cdot-}$ and H_2O_2 during storage. Additionally, the CA1 treatment maintained lower $O_2^{\cdot-}$ content while higher H_2O_2 content than the CK. As the storage duration, the NOX activity increased (the lowest in CA2), while the activity of SOD, APX, and GR exhibited a declining trend (the highest in CA2) (Fig. 4C-F). Compared with the CK, the activities of SOD, GR, and APX were relatively higher in the two CA treatments, especially in CA2, though no significant difference during cold storage (Fig. 4D-F).

Influence of CA on activity of PPO, POD and PAL in husks

As we known, PPO and POD cause the damage of fresh fruit and vegetables by oxidating phenols. From Fig. 5A and B, PPO and POD appeared an increasing trend in husks during storage. Furthermore, the PPO activity was significantly lower in CA2 during the storage, compared with the CK (Fig. 5A). Meanwhile, the two CA treatments significantly inhibited an increasement of POD activity during the whole storage (Fig. 5B). As storage duration, the PAL enzyme activity decreased, and its maximum levels appeared in CA2 treatment at every time point (Fig. 5C). Totally, CA2 treatment with low O_2 content might be the most effective to reduce the oxidation browning for pomegranate during cold storage, which was modulated by PPO rather than POD.

Influence of CA on metabolism of respiration and energy in husks

Respiration rate is generally used to evaluate the senescence of horticultural products. From Fig. 6A-C, the respiration rate, ATP content and CCO activity in husks exhibited the lower level at 5 ℃ in comparison with at 18 ℃. CA2 with high CO_2 provided the significant lower respiration rates ($p < 0.05$) during the storage.

NADK as the only enzyme, catalyzes the conversion of NAD^+ to $NADP^+$. From Fig. 6D, the two CA treatments maintained a higher NADK activity in husks, especially CA2 significantly higher than the CK during the storage. Furthermore, the NADH/NAD^+ and NADPH/$NADP^+$ were measured to evaluate energy metabolism. Between the two CA treatments, NADH/NAD^+ and NADPH/$NADP^+$ in husks showed similar trend. It was shown in Fig. 6E and F that it was higher with CA2 treatment than with CA1treatment at the end of the storage.

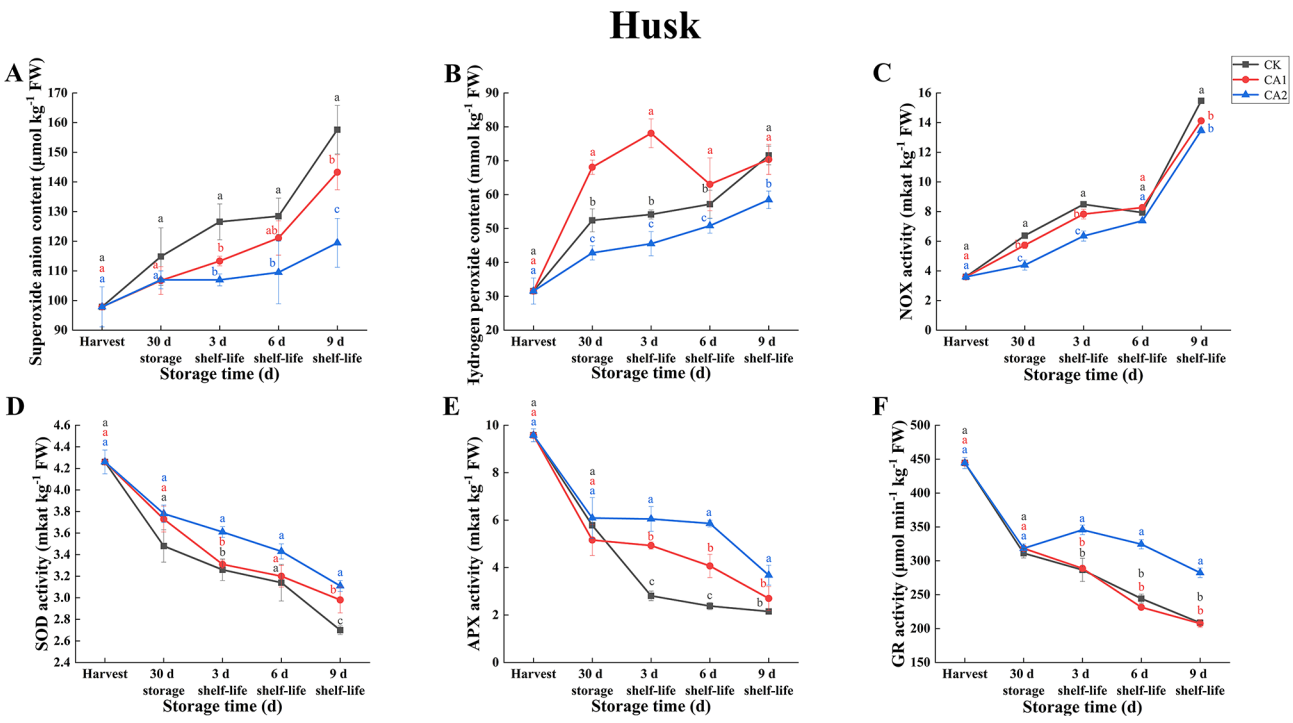

Fig. 4 Effect of CA treatments on ROS metabolism of pomegranate husk during storage. **A**, $O_2^{\cdot-}$ content; **B**, H_2O_2 content; **C**, NOX activity; **D**, SOD activity; **E**, APX activity; **F**, GR activity. Vertical bars represent the standard deviations of means. Different letters present significant differences among treatments on sampling day during the storage at $p < 0.05$

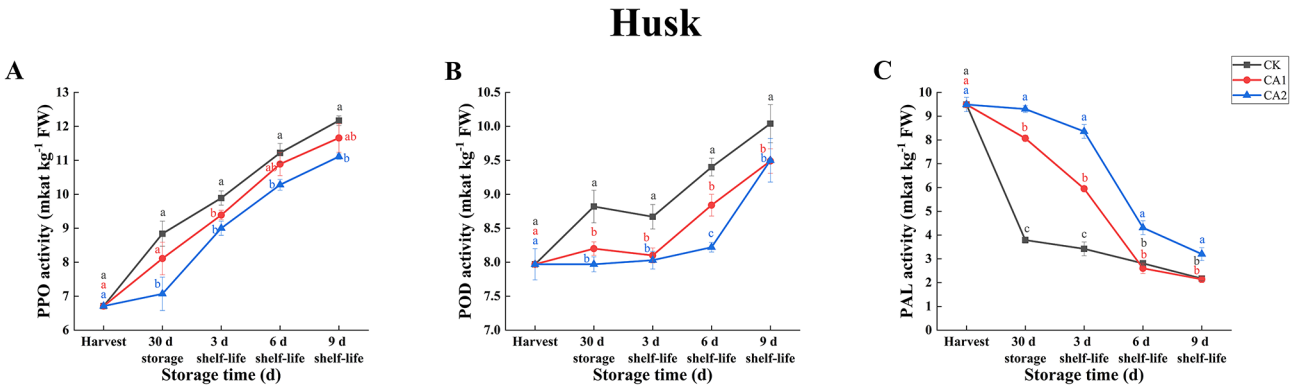

Fig. 5 Effect of CA treatments on PPO (**A**), POD (**B**) and PAL (**C**) activities in pomegranate husks during storage. Vertical bars represent standard deviations of means. Different letters present the significant differences among treatments on sampling day during the storage at $p < 0.05$

Discussion

For postharvest pomegranate fruit, chilling injury, water loss, and decay all can cause fruit quality degradation, directly limiting the transportation and marketability of pomegranate [6]. CA storage can alleviate internal browning of cold-stored flat peach [13], reduce decay of highbush blueberry [29], and benefit the visual quality of fresh pomegranate [19] and fig [18]. The current study showed that high CO_2 concentration treatment, CA2 (2% O_2 + 5% CO_2) effectively maintained high fruit quality via reducing respiration metabolism, and maintaining higher antioxidant enzymes activity and antioxidants content.

During the cold storage, cell membranes dysfunction of pomegranate husk first occurs [8, 30]. We found that CI, MDA content, and EL in husks increased during storage, however, they were delayed by CA2 storage, accompanied by lower NOX enzyme activity and lower contents of $O_2^{\cdot-}$ and H_2O_2. This was due to NOX which catalyzes the conversion of O_2 to $O_2^{\cdot-}$ in plant tissue [31], and $O_2^{\cdot-}$ and H_2O_2 as the main components in ROS, cause oxidative damage in cells [32]. Meanwhile, ROS production is accompanied by altered NAD(P)H redox status within the cell [9]. In comparison with CA1 and the CK, the ratio of $NADH/NAD^+$ and $NADPH/NADP^+$ in pomegranate husks was lower during CA2 storage. Hence,

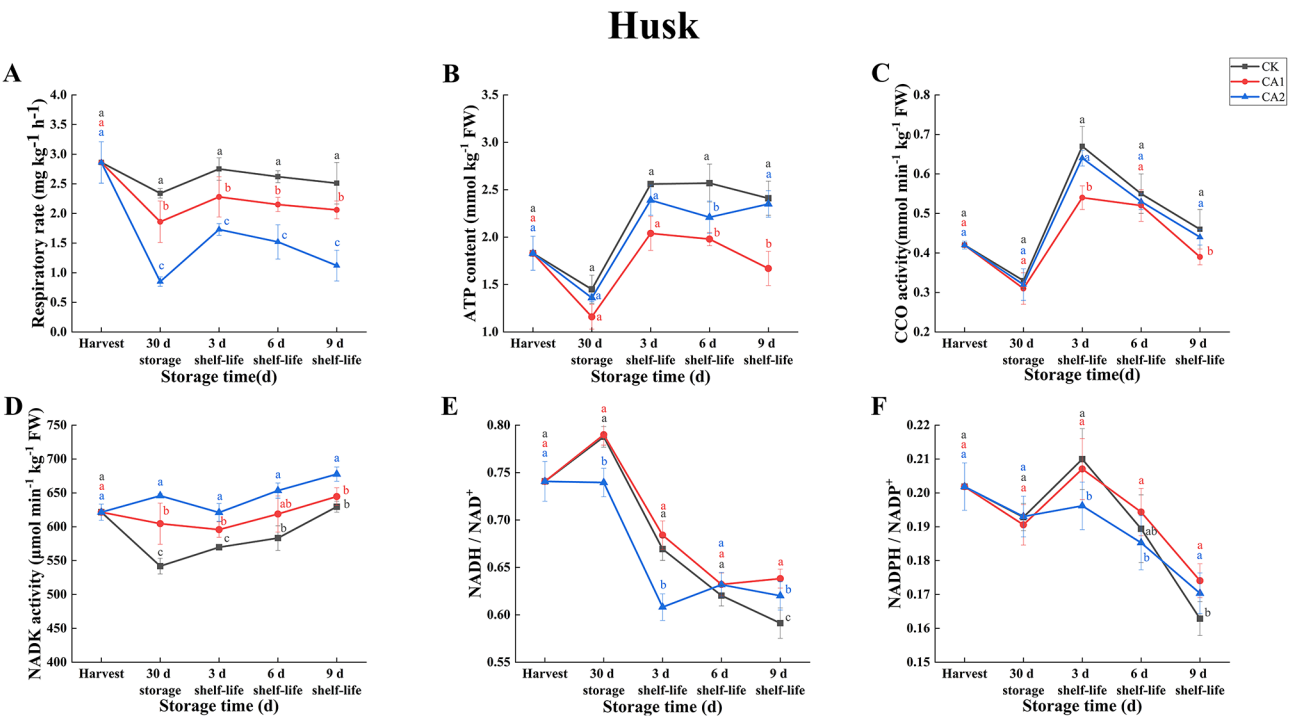

Fig. 6 Effect of CA treatments on respiratory pathway and energy metabolism in pomegranate husks during storage. **A**, respiratory rate; **B**, ATP content; **C**, CCO activity; **D**, NADK activity; **E**, NADH/NAD^+; **F**, NADPH/$NADP^+$. Vertical bars represent standard deviations of means. Different letters present the significant differences among treatments on sampling day during the storage at $p < 0.05$

relatively lower ROS production resulted in lower oxidative damage under CA2 with high CO_2, and fruit quality degradation had a little incidence. In summary, CA2 with lower O_2 content was helpful to alleviate husk injury for pomegranate fruit by the maintaining cell membrane integrity and reducing oxidative damage during storage, compared with CA1 and the CK.

The integrity of cell membrane is impaired, subsequently, the enzymatic oxidation with polyphenols as substrates is promoted by PPO in pomegranate fruit. In the present study, CA storage inhibited the growing activity of PPO and POD in husks in comparison with the regular air. Moreover, the decrease of PAL activity was greatly delayed by CA2 storage, meanwhile, in CA2-stored fruit, phenols and flavonoids were observed relatively higher accumulation. Hence, with CA2 storage, the higher PAL activity produced the more phenolic substances, meanwhile, the activity of PPO and POD were relatively lower, indicating lower enzymatic oxidation occurred in CA2 storage.

Respiration rate directly influences the ripening and senescence of postharvest horticultural products. Generally, maintaining low level of respiration is beneficial to slow down the physiological metabolism, delay senescence, and prolong storage life [33]. Moreover, Dogan et al. [18] found that high CO_2 concentration decreased respiration rate for fig fruit during storage. Similarly, our results demonstrated that two CA treatments decreased the respiration rate in stored pomegranate fruit, and high CO_2 concentration provided lower respiration rate. Otherwise, respiration rate has a close relationship with energy metabolism in horticultural products. Recent studies reported that high ATP level has the potential of retarding senescence of some fruit, such as enhancing chilling tolerance of blueberry [12], and flat peach [13], also, alleviating longan pericarp browning [28]. Notably, CCO is the last enzyme in the respiratory electron transport chain [10]. CCO activity during storage were higher in CA2 and the CK than CA1, whose trend was coincided with that of ATP level (Fig. 6B and C), indicating that the ATP level in CA2-stored pomegranate fruit might be more appropriate for 'Tunisia' pomegranate. Our results were in agreement with peach fruit [34]. Collectively, in the complex metabolic network composed of respiratory metabolism, ROS metabolism, and energy metabolism, the first two metabolisms may more crucial for improving fruit storability of pomegranate during CA2 treatment.

On the other hand, scavenging ROS is closely associated with antioxidant enzymes activity, which affects the ripening and senescence of horticultural products. Higher SOD activity contributes to scavenging O_2^- · into H_2O_2, and then converted into H_2O by APX [15]. CA2-stored pomegranate fruit possessed the higher antioxidant system comprised of higher activity of antioxidant enzymes (including SOD, APX, and GR) and

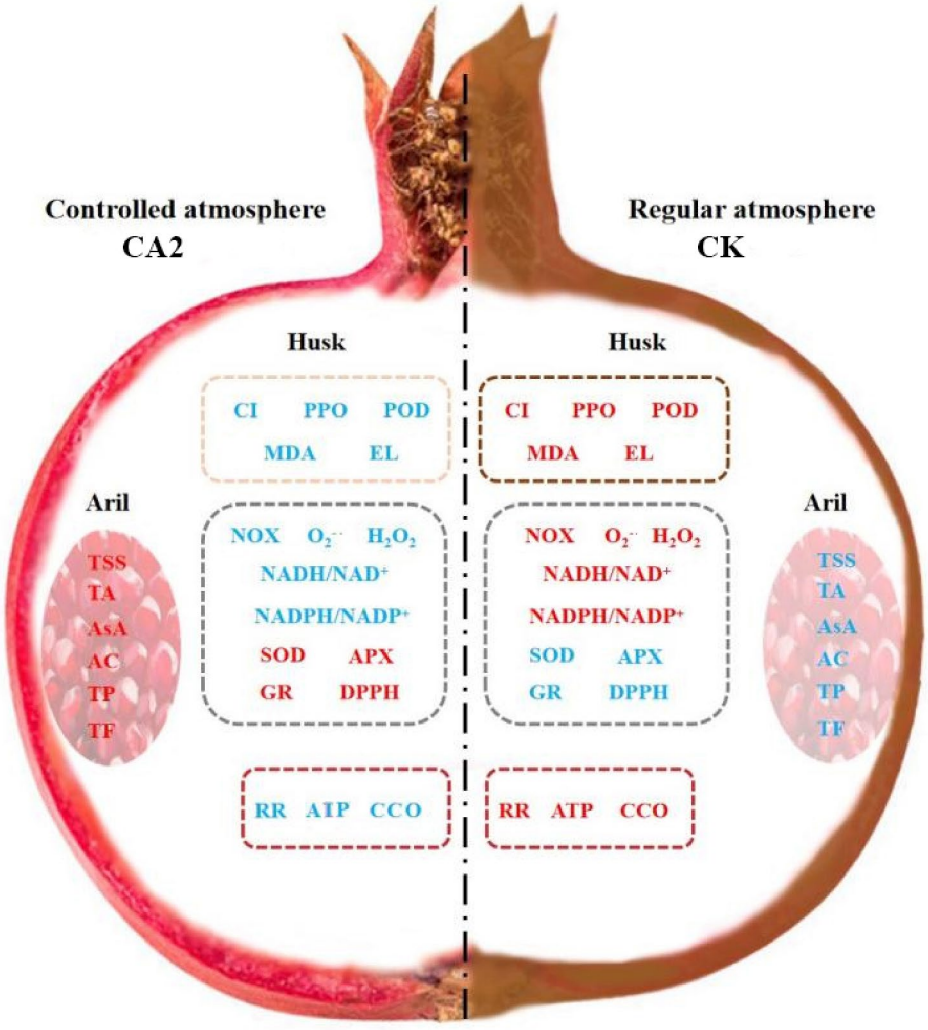

Fig. 7 Mechanism of pomegranate fruit response to the controlled atmosphere during storage. Notes: CI, chilling injury index; EL, electrolyte leakage; RR, respiratory rate; TA, titratable acid content; AsA, ascorbic acid content; AC, anthocyanins content; TP, total phenolics content, TF, total flavonoid content. The word in red represents higher while in blue for lower between CA2 and CK

higher antioxidants accumulation (including anthocyanins, total phenols and flavonoids). Interestingly, similar results were documented in the researches with exogenous applications including α-lipoic acid [15], and acetyl salicylic acid [30]. Also, the research on fresh-cut melon stored at 15 ℃ proved that lower antioxidant system activity was related to lower levels of AsA, POD, APX, GR, and glutathione peroxidase [9]. Additionally, the highest scavenging activity of DPPH in husks was presented in CA2 storage (Fig. 2D). Overall, CA2 storage maintained higher the antioxidant level, therefore, both preserving better nutritional values, and defensing against ROS-induced oxidative damage.

Conclusions

Fresh pomegranate fruit suffers from water loss and husk crack in natural condition. The present study demonstrated that the optimal atmosphere compositions on pomegranate fruit maintained higher fruit quality and improved storability. Compared with CA1 and CK, the optimal effects of CA2 storage on pomegranate fruit could be attributed to three aspects (Fig. 7): First, lower incidence of enzymatic browning. CA2-stored pomegranate fruit produced lower activity of PPO and POD, accompanied with lower level of MDA and electrolyte leakage, which maintained the cell member integrity. Second, alleviated oxidative damage by enhanced ROS scavenging capacity. The fruit treated with CA2 had $O_2^{\cdot-}$ and H_2O_2 at a lower level, while, DPPH, higher antioxidant activity (SOD, APX, and GR) and accumulation of

anthocyanins, total phenols and flavonoids at a higher level, which contributed to alleviating oxidative damage during storage. Third, lower respiration rate remained lower metabolism level, beneficial for delaying the senescent of pomegranate fruit. Additionally, the suitable ATP energy was attributed to the balance between CCO activity and ROS level under CA2 storage. Collectively, CA2 treatment with high CO_2 concentration effectively maintained husk color, cell membrane integrality, and nutritional compounds in arils.

Author contributions

Jiangli Shi: Conceptualization, Supervision, Writing, Editing. Ruiran Tong: Methodology, Writing, Editing. Jianan Yao, Sen Wang, and Sa Wang: Resources, Investigation, Methodology. Jing Li, Chunhui Song, Kunxi Zhang, and Jian Jiao: Software, Data curation. Miaomiao Wang, Pengbo Hao, and Yujie Zhao: Formal analysis, Investigation. Wanyu Xu and Yu Liu: Investigation. Ran Wan and Xianbo Zheng: Supervision, Funding acquisition. All authors reviewed the manuscript.

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Data availability

Data will be available on request to corresponding authors.

Declarations

Ethics approval and consent to participate

The experimental research on plants is comply with relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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