Optimized precooling combined with $\text{SO}_2$-released paper treatment improves the storability of longan (\textit{Dimocarpus longan} Lour.) fruits stored at room temperature

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\textbf{Abstract}
Precooling and sulfur dioxide fumigation were proved as effective methods for the preservation of longan (\textit{Dimocarpus longan} Lour.) fruits. However, inadequate precooling and sulfur dioxide fumigation resulted in unexpected losses and short shelf life. A $L_9(3^4)$ orthogonal test was conducted to screen out ideal dosage of sodium metabisulfite (factor A), precooling method (factor B), and precooling duration (factor C) to improve the storability of longan fruit stored for 48 hr at room temperature (RT) (25°C). The overall qualities of all of the treated longan fruits after a 48-hr storage (OQST) and during the 5-day shelf at 25°C (OQSF) were better than those of the control fruits. The treated fruits showed brighter fresh color (higher L*, b*, C*, and h° values but lower a* value), higher flavonoid, and chlorophyll contents. Moreover, the SO$_2$ residue was concentrated in pericarp but little in aril for any of the 12 treatments. The multivariate variance analysis showed that factor A was dominant to determine both of the OQST and OQSF, while factor B affected the OQST, and factor C affected the OQSF. In total, “0.22% sodium metabisulfite + 4 hr precooling + uncovered precooling” was considered to be an ideal treatment. These results would contribute to improving longan postharvest preservation technology.

\textbf{KEYWORDS}
Longan (\textit{Dimocarpus longan} Lour.) fruit, orthogonal test, postharvest performance, precooling, SO$_2$-released paper
INTRODUCTION

Longan (Dimocarpus longan Lour.), one of the typical tropical and subtropical fruits, is extremely unresistant to a long-term postharvest storage and transportation. The biggest problem during postharvest for longan is pericarp browning and aril breakdown (Han et al., 2014). The harvested longan fruit might be able to extend its life about 15–30 days when stored at suitable low temperature but only 3–5 days at ambient temperature (ABT) (nearly 32–35°C). The typical deterioration of longan fruits was usually featured with a serious pericarp browning or aril juicy (Han et al., 2015; Luo, Li, et al., 2019). In China’s domestic markets, longan fruit is usually stored and transported at ABT, sealed in a foam box with ice bags to keep it cool. Obviously, unfavorable internal environmental conditions, such as higher temperature and more condensate water in the package, resulted in serious postharvest losses. Therefore, it is essential to develop appropriate and effective technologies serviced for ambient temperature logistic and storage of longan fruits.

Precooling not only rapidly reduces the temperature of horticulture products, but also helps the products quickly slowdown its metabolic activity, enter a low energy consumption state and prolongs its shelf life (Brosnan & Sun, 2001; Ladaniya, 2008). As it was known, the precooling method is very important for the efficiency of product cooling. A previous work reported that a forced air precooling combined with suitable packaging can quickly reduce the internal temperature of citrus fruits and shorten the precooling duration (Defraeye et al., 2014). It was worthy to note that the cold-sensitive fruit was suggested to be gradually cooled for avoiding cold injury (Defraeye, Verboven, Opara, Nicolai, & Cronjé, 2015). More importantly, the precooling practice should be promptly implemented after harvest. A 6-hr delay of precooling at 30°C resulted in increased water loss rate of strawberry by 50%, decreased hardness by 14%-22%, and significantly declined quality Reichel (Nunes, Brecht, Morais, & Sargent, 1995).

In harvesting season, the respiration rate of longan fruit is very high due to the high orchard temperature (usually 35°C or higher) and strong sun exposure. Therefore, a precooling practice is necessary to be performed on the longan fruit before its storage. However, longan fruits are usually packaged in cartons or baskets after being air-cooled in a shade and covered with some leaves to keep moisturizing, which does not achieve the purpose of extending the shelf life. In addition, the conventional and direct precooling methods usually resulted in a high water loss from pericarp of longan fruits without covering. Precooling using ice-water mixture is also found to be not suitable for longan, which probably aggravates the pericarp browning and rottenness (Lin, Chen, Kong, & Xi, 2007). Previous results indicated that packaging of longan fruits in the films with stronger water permeability leads to more serious pericarp browning (Han et al., 2017). Therefore, the existing precooling methods need to be optimized depending on different storage and transportation purposes.

SO$_2$ and its donors (such as sodium metabisulfite) have been widely used for long-term storage of fresh or processed agricultural products due to its remarkable bleaching action on organic pigments, strong capacity to inhibit bacterial, and act as an antioxidant and inhibitor of enzyme-catalyzed or nonenzymatic oxidative discoloration and of browning during storage (Li, Yang, Chen, Li, & Liu, 2015; Prabhakar & Mallika, 2014; Wang, Cui, & Fang, 2007). Therefore, it has been widely applied in the processing of food to protect or improve the apparent color and prevent rottenness (Chen, 2010; Hu et al., 2017). Its excellent preservation effect was observed on Chinese herbal medicines (Ji, Li, Peng, & Dong, 2015; Wang et al., 2015), the exported fresh litchi fruits (Kumar, Teotia, Prasad, Varma, & Kumar, 2017), and the table grapes (Carter, Chapman, Gabler, & Brandl, 2015; Liu et al., 2015) during a long-term storage. SO$_2$ fumigation was proved as a key technology for postharvest treatment of international traded fresh longan fruits (Chen, 2013; He et al., 2006). After the fumigation, SO$_2$ gas can fully penetrate into the skin and flesh tissue of longan fruit, rapidly bleach the skin by reducing the brown pigments (Pang, Zhang, Gong, & Zhang, 2007; Wu, Han, Ji, & Chen, 1999), reduce the respiration rate, and significantly extend its storage life (Han, Wu, Ji, & Han, 1999). However, the SO$_2$ fumigation technology requires particular facilities and fixed sites, as well as a large investment, which inhibited its widely application in domestic market.

In view of its excellent effect on color protection, SO$_2$ gas combined with the appropriate precooling was expected to reduce the condensate water in the package, the mildew, and rot of fruit. Based on this thesis, our self-made SO$_2$-released paper capable of releasing SO$_2$ gas (patent application no. 201610227848.7 in China, based on sodium metabisulfite as the main ingredient) has been applied in longan fruit storage experiment. Our previous trial (“SO$_2$-released paper + 5°C precooling”) was applied on longan fruits, which were packed in foam box with ice bags for 44 hr plus 3-day shelf at 25°C. This treatment significantly reduced the condensate water in package, resulted in a higher shelf marketability rate (up to 91.92%) when compared to the control fruits (up to 80.69%). However, the precooling method and the duration of precooling still need to be investigated and optimized. A L$_9$$(3^4)$ orthogonal test was conducted to investigate the effects of three factors, including the dosage of compound preservative (factor A), precooling method (factor B), and precooling duration (factor C) on the overall qualities of longan fruits after a 48-hr storage (OQST) within foam boxes and during the shelf (OQSF) at 25°C and screen out an ideal treatment. The results from this work were expected to help improve postharvest technology of longan fruits and the similar researches on other fruits and vegetables.

MATERIALS AND METHODS

Fruits, SO$_2$-released paper, and reagents

Longan fruits cv. “Chuliang” with 85%-95% maturity degree were harvested on 26 July 2017 from Huaxiang Orchard in Yangxi, Guangdong Province, China. The environmental temperature of the
The orchard was 36–37°C. The fruits were harvested with long branches, which were cut short to be 15–20 cm. Fruits with uniformed size and appearance were selected and immediately transported to the laboratory within 3 hr in an air-conditioned car (about 23°C).

2.2 | Chemicals

Food-grade sodium metabisulfite for making SO\textsubscript{2}–released paper was obtained from Shandong Weifang Lujiu Chemical Co., Ltd. The prochloraz and iprodione were, respectively, supplied by Jiangsu Huifeng Agrochemical Co., Ltd. and Jiangsu Kuaida Agrochemical Co., Ltd. Ethanol, acetone, methanol, zirconium oxychloride (ZrOCl\textsubscript{2}·8H\textsubscript{2}O), sodium chloride, mercuric chloride, zinc sulfate, borax, and para-rose aniline hydrochloride were all analytical reagents and supplied by Sinopharm Chemical Reagent Co., Ltd.

2.3 | Sterilization

Fruits with no disease and no damage were selected and dipped into solution containing 500 mg/L prochloraz and 1,000 mg/L iprodione for 2 min. The fruits were then taken out and dried at an air-conditioned room about 25°C.

2.4 | Package, precooling, storage, and sampling

Sterilized fruits of 2.5 kg were put into a foam box (inner size 36 cm × 26 cm × 10 cm) with a 0.02-mm thickness polyethylene (PE) bag liner. The foam boxes without sealing were then moved into a refrigeration room. The fruits with or without coverage (see Table 1, factor B) were precooled at 5 ± 0.5°C for 0, 4, 12, or 20 hr (see Table 1, factor C).

After the precooling, one bag of 800 g ice was placed under the liner (upon the bottom of the box). A sheet of SO\textsubscript{2}–released paper containing 0.12%, 0.14%, 0.18%, or 0.22% (w/w) sodium metabisulfite (see Table 1, factor A) was laid upon the top of fruits with the SO\textsubscript{2} releasing side faced down. After the lid was covered, the foam boxes were sealed with tape and stored in a storage room with constant temperature at 25 ± 1°C for 48 hr. After the room temperature storage, the foam boxes were opened. Thirty fruits from each treatment were sampled: Half of the pericarp and half of the aril were separated from each fruit and immediately frozen in liquid nitrogen, ground, wrapped in a piece of aluminum foil paper, and stored at 80°C until used. Three replicates were performed for each treatment.

2.5 | Shelf experiment and sampling

After the foam boxes were opened, the fruits with long branches from each treatment were trimmed into single fruit with 3- to 5-cm branch. The trimmed fruits were placed into a transparent plastic box with two holes (diameter 8 mm) in each face. Each box of fruits was weighted about 500 g and stored on the shelf at 25 ± 1°C. Three replicates were performed for each treatment. After the 4-day shelf experiment, 30 fruits from each treatment were sampled as described above.

2.6 | Orthogonal test design

A L\textsuperscript{9} (3\textsuperscript{4}) orthogonal test of three factors including factor A (dosage of sodium metabisulfite), factor B (different coverage methods during precooling), and factor C (precooling duration) was designed (Table 1).

Twelve treatments designed from the combinations of factors and levels were, respectively, labeled as S1–S12 (Table 2). S9–S12 were derived from the additional level 4 of factor A, and only one repeat for each treatment. The four levels of factor A were set according to the effective dosages of sodium metabisulfite to control

| Treatment | Factors & levels |
|-----------|-----------------|
|           | A: sodium metabisulfite (%) | B: precooling method | C: precooling duration (h) | Error |
| S1        | 1 | 1 | 1 | 1 |
| S2        | 1 | 2 | 2 | 2 |
| S3        | 1 | 3 | 3 | 3 |
| S4        | 2 | 1 | 2 | 3 |
| S5        | 2 | 2 | 3 | 1 |
| S6        | 2 | 3 | 1 | 2 |
| S7        | 3 | 1 | 3 | 2 |
| S8        | 3 | 2 | 1 | 3 |
| S9        | 3 | 3 | 2 | 1 |
| S10       | 4 | 1 | 3 | 2 |
| S11       | 4 | 2 | 1 | 3 |
| S12       | 4 | 3 | 2 | 1 |
| CK        | – | – | – | – |

Abbreviations: PP, precooling with paper, covered with a single layer of newsprint; UP, Uncovered precooling, with no covering; WP, wet precooling, covered with a piece of wet cloth after squeezing.
decrease and result in a SO$_2$ residue in longan aril below the national standard limit (30 mg/kg) (Yang et al., 2007, NY 1440–2007, maximum limit of sulfur dioxide residue in tropical fruits in China). The levels of factor B were set according to their gradient moisturizing ability. The levels of factor C were considered according to the fact that the fruit inner temperature might be closed to 5°C after being precooled for about 12 hr at 5°C. In addition, the fruits with no SO$_2$-released paper and no precooling treatment were marked as control (CK) and repeated three times. The cooled fruits were packed in batches according to the planned precooling duration of different treatments. All of the treatments and CK were unpacked after stored for 48 hr at 25°C.

2.7 | Indicators related to commodity

After a 48-hr storage within foam box at 25°C, no obvious pericarp browning and mildew were observed on the pericarp of the treated and control longan fruits. The differences among 12 treatments were mainly manifested during the period of shelf life. Thus, the evaluation of the performances indicated by edible fruit rate (EFR) and marketability rate (MR), which were checked once a day during a 5-day shelf. The fruits with no moldy and rotten appearance but completely browning pericarp were considered as edible fruits, and the fruits with no moldy, rotten, and browning appearance were considered as commodity fruits. During the shelf period, the MR and EFR of each treatment were the same, but the MR of CK-48 hr (CK after 48-hr storage) was 0 from the second day because of its severe pericarp browning.

$$\text{ERF} \% = \frac{\text{number of no moldy and rotten fruits}}{\text{total fruit number}} \times 100\%$$ (1)

$$\text{MR} \% = \frac{\text{number of commodity fruit}}{\text{total fruit number}} \times 100\%$$ (2)

2.8 | Determination of content of total soluble solids (TSS %)

The TSS contents (% w/w) of fruits after a 48-hr storage within foam box at 25°C and the fruits after a 4-day shelf were determined. The aril of 30 fruits from each sample was used for juicing and determination of TSS by a Brix refractometer (PR-32α; ATAGO Co.). Each analysis was subjected to three repeats.

2.9 | Color measurement

According to our previously reported method (Han et al., 2015), the chroma parameters including L*, a*, b*, C*, and h° of fruits after a 48-hr storage within foam box at 25°C and the fruits after a 4-day shelf were determined using an automatic color analyzer (KONICA MINOLTA CR-300). L* value represents lightness (+) to darkness (−), a* value represents red (+) to green (−), and b* value represents yellowness (+) to blueness (−), and the h° and C* values mean hue angle and saturation. Fifteen fruits from each treatment were measured. All treatments were performed in three biological repeats.

2.10 | Measurement of contents of chlorophyll a, chlorophyll b, and carotenoid

According to the method of Ye and Chen (2004), 1.0 g frozen pericarp powder was weighed and mixed with 2 ml extracting solution (ethanol: acetone: water = 4.5:4.5:1.0 (V:V:V)). The sample was ground, and the upper solution was transferred and filtered into a 25-ml tube. The residue was ground again and washed with four times with total 8 ml extracting solution, and the upper solution was collected and filtered into a 25-ml tube. The volume of collected filtrate was set to 10 ml with extracting solution. Then, the extract was used for determining the absorbance values at 663, 646, and 470 nm, respectively. The concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid (Car) in pericarp were calculated according to equations (3-5):

$$\text{Chl} \text{a content (µg/g)} = \left( 12.21 \times A_{663nm} - 2.81 \times A_{646nm} \right) \times 10$$ (3)

$$\text{Chl} \text{b content (µg/g)} = \left( 20.13 \times A_{646nm} - 5.03 \times A_{663nm} \right) \times 10$$ (4)

$$\text{Car content (µg/g)} = \left( 10^3 \times A_{470nm} - 3.27 \times \text{Chl} \text{ a} - 104 \times \text{Chl} \text{ b} \right) / 229 \times 10$$ (5)

2.11 | Measurement of flavonoid content

One gram of frozen pericarp was ground, extracted, and washed by 80% (v/v) methanol. The extract was filtered, and its volume was set to 10 ml by 80% methanol. The content of flavonoid (Fla) was measured by the differential spectrophotometry method (Kang, 2005). 2% (w/v) zirconium dichloride (ZrOCl$_2$·8H$_2$O) in methanol solution was used as the complexant of flavonoid. 1.0 ml extract was transferred into a 15-ml tube and then fully mixed with 7.0 ml methanol (solution A). 1.0 ml 2% ZrOCl$_2$·8H$_2$O and 6.0 ml methanol were added into another 15-ml tube containing 1.0 ml extract and fully mixed (solution B). After a standing for 1 hr, the absorbance at 420 nm of solution A (A$_{420\text{nm}}$
A) and solution B (A$_{420\text{nm}}$
B) was measured by a UV-Vis spectrophotometer (Mapada UV-1800). A calibration curve was prepared for total flavonoid using rutin standard solution (52 µg/ml). A regression equation was obtained with a linear correlation coefficient r = .9999 and a linear range within 3.25 to 19.50 µg/ml. The total flavonoid content was calculated according to equation (6):

$$\text{Total flavonoid content (µg/g)} = \left( A_{420\text{nm}} \text{B} - A_{420\text{nm}} \text{A} - 0.0051 \right) / 0.0065 \times 10$$ (6)

2.12 | Determination of sulfite residue

The sulfite residue (content, expressed as SO$_2^2-$) was measured using a previously reported method (Luo, Niu, et al., 2019). 1.0 g
pericarp or 5.0 g aril was ground into powder with liquid nitrogen and extracted using 2.0 ml saturated ZnSO₄ solution and 5.0 ml saturated Na₂B₄O₇·10H₂O solution. The filtrate of the sample was transferred into a 100-ml volumetric flask. Then, 4.0 ml 0.5 M NaOH solution was added and shaken. After a reaction for 5 min, 4.0 ml acid solution (H₂SO₄: deionized water = 1:71) was added and shaken. After a reaction for 2 min, 20 ml tetrachloromercurate sodium solution (absorbent, comprised of 1.36% (w/v) HgCl₂ and 0.6% (w/v) NaCl) was added and fully shaken, and then, the volume was made up to 100 ml by deionized water. After be filtrated, the solution was used for the determination of sulfite residue. 5.0 ml tetrachloromercurate sodium solution, 1.0 ml formaldehyde (2.0 g/L), and 1.0 ml pararosaniline solution (0.02%, w/v, pararosaniline hydrochloride in 7.2% [v/v] HCl) were fully mixed and a linear range within 0.11–0.78 µg/ml. The sulfite residue was calculated based on equations (7) and (8):

\[
\text{SO}_2 \text{ in pericarp (SO}_2 - P, \ \mu g/g FW) = \left(\frac{A_{550 nm} - 0.0035}{0.0356 \times 2 \times 1}\right) \times 100\]

(7)

\[
\text{SO}_2 \text{ in aril (SO}_2 - A, \ \mu g/g FW) = \left(\frac{A_{550 nm} - 0.0035}{0.0356 \times 2 \times 5}\right) \times 100\]

(8)

### 2.13 Statistical analysis

Compared with the harvest day of CK (marked as CK-0 day), changes in chroma values, TSS%, pigment contents after 48-hr storage, and 4-day shelf were all expressed as the differences (Δ) between the measured day and CK-0 day, that was, Δvalue = measured value - average value of CK-0 day. Among them, the “STΔchroma value” meant “after 48-hr storage,” “SFΔchroma value” meant “after 4-day shelf.” The excel software was used to calculate the Δvalue’s standard errors (SE), which are equal to those of measured values.

Multiple comparisons were performed using Duncan’s new complex-difference method in SPSS 19.0. To comprehensively and intuitively assess the fruit storability, the factor analysis method in SPSS 19.0 was used to not only reduce the dimensionality of EFR and MR data, but also evaluate the overall quality of fruits after a 48-hr storage (OQST) and the fruits after a 4-day shelf (OQSF). Based on the common factors and their contributions calculated by variables (the EFR and MR data of 3–5-day shelf), the evaluation scores of EFR (or MR) for all of 12 treatments and CK-48 hr were obtained by \(\sum\) (common factor \(\times\) contribution). In the same way, the evaluation scores of OQST were calculated by Δchroma values and ΔTSS after 48-hr storage; those of OQSF were calculated by EFR score, Δchroma value, and ΔTSS after 4-day shelf.

The orthogonal experiment data were analyzed by the general linear model in SPSS 19.0, and the descriptive statistics and multifactorial multivariate analysis of variance were performed on the trial results.

### 3 RESULTS

#### 3.1 Changes in TSS contents and chroma values in longan fruit after a 48-hr RT storage

As shown in Figure 1, after a 48-hr storage, the TSS % showed no difference between CK-48 hr and CK-0 day (p > .01), and the TSS % of S1, S3, S4, S5, S6, S7, S8, S11, and S12 was decreased with no significant difference (p > .01) (Figure 1c). The \(L^*\), \(b^*\), \(C^*\), and \(h^*\) values of S1 to S12 were significantly higher than those of CK-48 hr and CK-0 day (p < .01), but the a* values of most of the treatments were significantly lower than those of CK-48 hr and CK-0 day (p > .01) except for S8 and S10 (p > .01) (Figure 1a-b). Thus, the deeper greenish-brown pericarp of CK-48-hr fruits compared with the pericarp of CK-0 day fruits might be resulted from the lower \(L^*\), \(b^*\), \(C^*\), and \(h^*\) values and higher a* value (p > .01). More importantly, compared to CK-48-hr fruits, better appearance quality (bright yellowish-green pericarp) of the fruits from 12 treatment groups was consistent with their higher overall quality scores after a 48-hr storage (OQST) (Figure 1c).

#### 3.2 Changes in pigment contents in pericarp of longan fruit after a 48-hr RT storage

As shown in Figure 2a, compared with those in CK-0-d pericarp, except the Chl a content of S3 decreased significantly (p < .01), S1 and S9–S11 increased significantly (p < .01), the others had no significant differences (p > .01). The contents of Chl b were all increased, and most of them were significantly higher than those of CK-0 day and CK-48 hr (p < .01). But the Car content showed no significant difference except for S3. Besides, CK-48 hr showed higher chl a and chl b contents than CK-0 day (p > .01). In Figure 2b, the Fla contents in CK-48-hr pericarp decreased with no significance (p > .01). But the Fla contents of 4 treatments (S1, S2, S4, and S5) were significantly higher than that of CK-0 day (p < .01). The above results indicated that most of the treatments (especially S1) helped maintain chlorophyll (mainly Chl b) and Fla contents in the longan pericarp during a 48-hr storage in foam box at 25°C.

#### 3.3 SO₂ residues in longan fruit after a 48-hr RT storage

The SO₂ residues in the pericarp (SO₂-P) of treated longan fruits ranged from 615.23 ± 30.96 to 2,841.81 ± 173.62 mg Kg⁻¹, but the SO₂ residues in the pulp (SO₂-A) ranged from 1.73 ± 0.05 to 18.47 ± 0.79 mg Kg⁻¹. Significant differences were also observed between the treatments and CK-48 hr (p < .01) (Figure 3). These results indicated that SO₂ residue was mainly accumulated in
pericarp and little in aril during the 48-hr storage, and the SO$_2$-A residues of treatments were all below the national limit ($\leq$30 mg Kg$^{-1}$).

3.4 | Performance and evaluation of the apparent and internal quality in longan fruits during the shelf life

As shown in Figure 4a, compared to CK-0 day, CK-48 hr showed a significant decrease in L* and h° values but increase in a* value after a 4-day shelf. All of the 12 treatments showed higher L* values than CK-0 day and CK-48 hr, and lower a* values and higher h° were observed in S1, S2, S3, S6, and S7. The above results explained that the treated fruits after a 4-day shelf life still maintained a better exterior quality than CK-48 hr and CK-0 day. Figure 4b showed that TSS% in fruits of CK-48 hr and 12 treatments was significantly lower than that in fruits of CK-0 day ($p < .01$). Moreover, TSS% in fruits of S2, S3, S7, S8, S9, and S10 (but not other 6 treatments) was significantly lower than that of CK-48 hr ($p < .01$). Because CK-48-hr pericarp was severely browed, its 4th-day MR was 0 without commercial value any more. Among S1–S12, except for S2, S3, S5, and S11, there was no significant difference in 4th-day MR ($p > .01$).

In Figure 4c, CK-48 hr had a higher EFR score than 4 of 12 treatments, but it had much lower MR than 12 treatments. So, it could be concluded that the storability difference between CK-48 hr and 12 treatments was mainly due to the apparent quality, CK-48 hr had a much lower OQSF score than 12 treatments because of its poor appearance, that is, all of its fruits were completely browed.
Results of multivariate test for 3-test factors in the orthogonal experiment

Using all the detected indicators as the dependent variables and using chroma values as dominant ones, four-test methods including Pillai’s track, Wilks’s $\lambda$, Hotelling’s track, and Roy’s maximum root were performed on the 3-test factors. Because Roy was the largest eigenvalue in the Hotelling test matrix, the significance values ($p$ value) of the two tests were the same.

As shown in Table 3, the results gave the effect of each factor on the whole trial, the $p$ values of the four tests for the three factors were all in the order of $A < C < B$. This suggested that FacA had the greatest effect on the storability difference between 12 treatments, reaching a significant level ($p < .05$ in 2-test methods), then followed by FacC ($p = .05–.10$ in two tests), and FacB had the least effect ($p > .10$).

Results of main effect test based on all the detected indicators (dependent variables)

As listed in Table 4, the results from corrected model shown that the combined three factors had certain statistical significance for $\Delta T \Delta b$, $\Delta T \Delta C^*$, $\Delta T \Delta h^*$, $SO_2-P$, and $SO_2-A$ ($p = .027–.097$).

For FacA, there were some significant differences between three levels of chroma values, Fla contents, and $SO_2$ contents.
after 48-hr storage \( p = .009–.075 \), but there were no significant differences in OQST and OQSF \( p = .150–.942 \). For FacB, OQST \( p = .632 \) had a more significant difference than OQSF \( p = .942 \). For FacC, there was a less significant difference between three levels of \( \Delta \text{ST} b^* \) and \( \Delta \text{ST} C^* \), and the OQST difference \( p = .913 \) was lower than OQSF \( p = .448 \). For all of the three factors, the level differences in OQST and OQSF were not significant \( p = .150–.942 \).

### 3.7 Average value comparisons of the effective indicators at different levels of each factor

Because OQST and OQSF are the primary indicators used for choosing the appropriate level of each factor, the relevant indicators to them were also screened out and analyzed on the basis of their average values of different levels of each factor (Table 5).

Firstly, the higher the level of FacA, the higher the \( \Delta \text{ST} b^* \), \( \Delta \text{ST} C^* \), \( \Delta \text{ST} h^* \), and \( \Delta \text{Fla} \); the lower the \( \Delta \text{ST} a^* \), the better the OQST and OQSF; and the higher the \( \text{SO}_2 \) residues in fruit, but those in aril were all under the national standard limit (30 mg/kg). So, A\(_1\) was selected.

Secondly, FacB mainly affected the OQST. Among three precooling methods, UP (uncovered precooling) gave the highest \( \Delta \text{ST} L^* \), \( \Delta \text{Fla} \), and \( \Delta \text{SO}_2^* \); lower \( \Delta \text{ST} b^* \) and \( \Delta \text{ST} C^* \); medium \( \Delta \text{ST} h^* \) and OQST, but the best OQSF. So, B\(_1\) was selected.

Finally, FacC mainly affected the pigment contents and OQSF. With the prolonging of precooling duration, although \( \Delta \text{ST} b^* \) and \( \Delta \text{ST} C^* \) showed an upward trend, \( \Delta \text{Fla} \), \( \text{SF} \Delta L^* \), and \( \text{SF} \Delta h^* \) were all the opposite, and \( \text{SF} \Delta a^* \) raised, this led to a worse fruit appearance. Meanwhile, the \( \text{SF} \Delta \text{TSS}\% \) declined, and OQSF also dropped.
So, C₁ was selected. Therefore, the reports in the above suggested that A₁B₁C₁ (0.22% compound preservative + uncovered precooling + 4 hr precooling) was a more reasonable treatment combination.

4 | DISCUSSION

During the storage at room temperature (25°C), the higher temperature of longan fruits can cause a lot of breathing heat let out which will be accumulated inside the package little by little, and more and more condensed water will gather on the surface of fruit, which can easily lead to the browning of pericarp after the fruit is taken out and dried. So, a timely precooling after harvest is essential to cool the fruits and remove the respiratory heat inside them, and reduce the dewing on the internal surface of package (Brosnan & Sun, 2001; Wang, Chen, Yu, & Ji, 2014). Although the treatments by environmentally friendly compounds such as microencapsulated essential oils (Alikhani & Daraei Garmakhany, 2012) were encouraged and the residue from SO₂ treatment might be harmful to human health, the sulfur dioxide fumigation was proved to be the most effective preservation method for longan in the export trade. Slow and continual release of SO₂ was an important improvement to reduce sulfur residue in the recent years. In this work, SO₂-released paper was used and combined with low-temperature precooling to enhance longan fruit storability which were stored in foam boxes at 25°C room temperature, and the desired effects were achieved as shown by not only drastically improving the OQST but also maintaining an excellent OQSF during 5-day shelf period. In spite of the fact that UP could lead to a more water loss and a darker appearance, the cooling was quicker and beneficial to protect the OQST and OQSF. Maybe NP and WP give a better chroma quality after 48-hr storage, but resulted in a worse OQSF, as a longer-term precooling would do. And the longer the precooling duration, the lower the TSS content in aril. So, the comprehensive analysis showed that the combination of A₁B₁C₁, that is, “0.22% compound preservative + uncovered precooling + 4 hr precooling” was a more appropriate treatment and worthy of further optimization research.

### TABLE 3  Multivariate test for the significance of three factors based on all the indicators (dependent variables) dominated by chroma values

| Effect                          | Value   | F     | Hypothetical df | Error df | Sig.  |
|---------------------------------|---------|-------|-----------------|----------|-------|
| Intercept                       | 0.999   | 473.497 | 4               | 1        | 0.034 |
| Pillai’s track                  | 2.46    | 3.42  | 12              | 9        | 0.037 |
| Wilks’s λ.                      | 0.001   | 3.309 | 12              | 2.937    | 0.181 |
| Hotelling’s track               | 31.532  | 23.649 | 4               | 3        | 0.013 |
| Roy’s maximum root              | 1.672   | 2.55  | 8               | 4        | 0.191 |
| Pillai’s track                  | 1.793   | 4.332 | 8               | 4        | 0.087 |
| Wilks’s λ.                      | 0.008   | 2.555 | 8               | 2        | 0.312 |
| Hotelling’s track               | 24.041  | 0     | 8               | 0        | -     |
| Roy’s maximum root              | 18.631  | 9.315b| 4               | 2        | 0.099 |

aAccurate statistics.
bThis statistic was the upper limit of F, which produced a lower bound on the level of significance.
cDesign: intercept + FacA + FacB + FacC.
The preservation effect of SO$_2$ on longan fruits lies in the protection of appearance and internal quality. Its mechanism of action includes the viewpoints of antioxidation and antibrowning (Wu et al., 1999; Pang, Zhang, Gong, & Zhang, 2007; Han, Wu, Ji, & Han, 1999), and delaying the TSS content decline to some extent (Pang, Zhang, Gong, & Zhang, 2007). However, the changes in the color-related pigment contents have not been understood in details before and after treatment with SO$_2$. It has been reported that appropriate SO$_2$ treatment could facilitate the accumulation of anthocyanins (Liu, Wang, Yi, & Yi, 2015), polyphenols, flavonoids, and lignin in Vitis vinifera during storage (Xue & Yi, 2017), but promoted the degradation of flavonoid glycosides in dried chrysanthemum morifolium cv. Hangju (Wang, Hao, et al., 2014), whereas the antioxidant activity of flavonoids before oxidation was higher than that after oxidation (Li, Long, Li, & Hao, 2012). As described in this paper, although all the fruits of 12 treatments had a significantly higher RECP than the control, their pericarp browning was instead inhibited. The Fla contents in the pericarp were significantly increased, even higher than CK-0 day. At the same time, the ST$\Delta a^*$ decreased, but the ST$\Delta b^*$, ST$\Delta c^*$, ST$\Delta h^\circ$, and SF$\Delta L^*$ all went

| Source | Dependent variable | Type III sum of square | df | Mean square | F    | Sig. |
|--------|-------------------|------------------------|----|-------------|------|------|
| Corrected model | ST$\Delta b^*$ | 47.125 | 7 | 6.732 | 4.534 | 0.081 |
| | ST$\Delta c^*$ | 44.471 | 7 | 6.353 | 4.408 | 0.085 |
| | ST$\Delta h^*$ | 16.603 | 7 | 2.372 | 4.068 | 0.097 |
| | SO$_2$-P | 5,669,433.298 | 7 | 809,919.043 | 5.525 | 0.059 |
| | SO$_2$-A | 344.404 | 7 | 49.201 | 8.758 | 0.027 |
| FacA | ST$\Delta b^*$ | 34.755 | 3 | 11.585 | 7.803 | 0.038 |
| | ST$\Delta c^*$ | 31.961 | 3 | 10.654 | 7.391 | 0.041 |
| | ST$\Delta h^*$ | 13.59 | 3 | 4.53 | 7.77 | 0.038 |
| | SO$_2$-P | 5,346,476.736 | 7 | 1,782,158.912 | 12.156 | 0.018 |
| | SO$_2$-A | 344.404 | 7 | 49.201 | 8.758 | 0.027 |

**TABLE 4** Effective indicators (dependent variables) plus OQST and OQSF with significant or less significant differences, which were screened out from the main effect test

| Source | Dependent variable | Type III sum of square | df | Mean square | F    | Sig. |
|--------|-------------------|------------------------|----|-------------|------|------|
| FacA | ST$\Delta b^*$ | 34.755 | 3 | 11.585 | 7.803 | 0.038 |
| | ST$\Delta c^*$ | 31.961 | 3 | 10.654 | 7.391 | 0.041 |
| | ST$\Delta h^*$ | 13.59 | 3 | 4.53 | 7.77 | 0.038 |
| | SO$_2$-P | 5,346,476.736 | 7 | 1,782,158.912 | 12.156 | 0.018 |
| | SO$_2$-A | 344.404 | 7 | 49.201 | 8.758 | 0.027 |

**TABLE 5** Comparisons of average values of the effective indicators (plus OQST and OQSF) at different levels of three factors

| Level | SO$_2$-A | SO$_2$-P | ST$\Delta a^*$ | ST$\Delta b^*$ | ST$\Delta c^*$ | ST$\Delta h^*$ | ΔFla | OQST | OQSF |
|-------|---------|---------|---------------|---------------|---------------|---------------|------|------|------|
| FacA  | 0.22%   | 16.22   | −2.27         | 8.29          | 7.87          | 5.53          | 127.41 | 0.59 | 0.63 |
|       | 0.18%   | 7.85    | −1.57         | 6.58          | 6.23          | 4.31          | 68.42  | 0.27 | 0.04 |
|       | 0.14%   | 3.22    | −1.27         | 4.34          | 4.06          | 3.36          | 19.74  | −0.24| 0.09 |
|       | 0.12%   | 4.64    | −0.88         | 4.16          | 3.94          | 2.70          | 24.41  | −0.62| −0.76|
up. Apart from the bleaching of pigments such as anthocyanins, it was presumed to be related to the reduction in oxidized flavonoid or polyphenols by SO$_2$ suitable low-temperature precooling also protected the pigments such as total chlorophyll and flavonoid from rapid degradation, and reduced the formation of carotenoid, so that the process of oxidative browning of longan fruit pericarp was delayed. In addition, the TSS content had no significant relationship with the chroma quality and MR, which indicated that the relationship between the TSS content and longan fruit storability is not notable.

In brief, among three factors, FacA is the most important to the OQST and OQSF of longan fruit, and FacB and FacC play an auxiliary role; FacB mainly affects the OQST, and FacC affects the OQSF. What’s more, the follow-up observations showed that the longest shelf life among the treatments reached 10 day, so the 4-day shelf life could not adequately demonstrate the differences between 12 treatments in OQST and OQSF, which explained the results of OQST and OQSF with no marked difference in this study, and provided a future reference for predicting the fruit shelf in the similar trials.

5 | CONCLUSION

From this study, it could be concluded that SO$_2$-released paper combined with low-temperature precooling was able to significantly improve the OQST and OQSF of longan fruits after 48-hr storage within foam box at 25°C, featured with a shelf life of more than 4 days. The combined treatment “0.22% compound preservative + uncovered precooling + 4 hr precooling” was a suitable treatment, which helped to increase the total chlorophyll and Fla content significantly, and consequently improve the chroma levels after 48-hr storage and during the shelf, but the changes in TSS content and RECP had no significant correlations with them. The results of this work would contribute to developing a new application of SO$_2$ in longan fruit preservation technology and making more progress in the postharvest technology of longan fruit.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

This study does not involve any human or animal testing.

REFERENCES

Alikhani, M., Daraei Garmakhany, A. (2012). Effect of microencapsulated essential oils on storage life and quality of strawberry (Fragaria ananassa cv. Camarosa). Quality Assurance and Safety of Crops & Foods, 4, 106–112. https://doi.org/10.1111/j.1757-837X.2012.00128.x

Brosnan, T., & Sun, D. W. (2001). Precooling techniques and applications for horticultural products—a review. International Journal of refrigeration-revue Internationale Du Froid, 24, 154–170. https://doi.org/10.1016/s0140-7007(00)00177-7

Carter, M. Q., Chapman, M. H., Gabler, F., & Brandl, M. T. (2015). Effect of sulfur dioxide fumigation on survival of foodborne pathogens on table grapes under standard storage temperature. Food Microbiology, 49, 189–196. https://doi.org/10.1016/j.fm.2015.02.002

Chen, B. J. (2013). Sulfur dioxide detected in Longan imported from Thailand by 5 times higher than the Standard. World Tropical Agriculture Information, 4, 21. (in Chinese).

Chen, Y. M. (2010). The use and residue control of sulfite in food. Journal of Inspection and Quarantine, 20, 22–24. https://doi.org/10.3969/j.issn.1674-5354.2010.03.007. (in Chinese)

Defraeye, T., Lambrecht, R., Deele, M. A., Tsige, A. A., Opara, U. L., Cronjé, P., ... Nicolai, B. (2014). Forced-convective cooling of citrus fruit: Cooling conditions and energy consumption in relation to package design. Journal of Food Engineering, 121, 118–127. https://doi.org/10.1016/j.jfoodeng.2013.06.021

Defraeye, T., Verboven, P., Opara, U. L., Nicolai, B., & Cronjé, P. (2015). Feasibility of ambient loading of citrus fruit into refrigerated containers for cooling during marine transport. Biosystems Engineering, 134, 20–30. https://doi.org/10.1016/jbiosystemseng.2015.03.012

Han, D. M., Gu, L. T., Li, S. S., Wu, Z. X., Li, J. G., Guo, D. L., ... Li, R. (2017). Effects of different packagings on the storability of Longan (Dimocarpus longan Lour.) fruits. Chinese Journal of Tropical Crops, 38, 2347–2354. (in Chinese) https://doi.org/10.3969/j.issn.1000-2561.2017.12.023

Han, D. M., Li, J. G., Pan, X. W., Li, R., Guo, D. L., Wu, Z. X., & Shuai, L. (2014). Recent advances in postharvest research on longan fruit. Acta Horticulturae, 1029, 325–330. https://doi.org/10.17660/ActaHortic.2014.1029.40

Han, D. M., Wu, Z. X., Ji, Z. L., & Han, M. (1999). Effects of SO$_2$ treatment on the oxidation and senescence of Longan fruit. Journal of Fruit Science, 16, 24–29. (in Chinese). https://doi.org/10.3969/j.issn.1009-9980.1999.01.006

Han, D. M., Yang, W., Wu, Z. X., Li, J. G., Shuai, L., Niu, J. J., ... Guo, D. L. (2015). Establishment of an evaluation system for longan fruit storability based on the physicochemical indices. Journal of South China Agricultural University, 36, 39–46. https://doi.org/10.7671/j.issn.1001-411X.2015.06.007. (in Chinese)

He, C. W., Yan, G. S., Tan, L., Ling, J. C., Yang, Y. F., Liang, K., ... Liao, Y. M. (2006). Analysis of sulfur dioxide residue in fresh Longan flesh from Vietnam. Chinese Journal of Health Laboratory Technology, 16, 1493. (in Chinese). https://doi.org/10.3969/j.issn.1009-8685.2006.12.041

Hu, G. X., Lai, A. P., Yuan, Y. W., Zhang, Z. H., Zhao, S. P., Zhu, J. H., & Wang, Q. (2017). Cumulative risk assessment of dietary sulfur dioxide residues of consumers. Scientia Agricultura Sinica, 50, 1317–1325. https://doi.org/10.3864/j.issn.0578-1752.2017.07.014. (in Chinese)

Ji, L., Li, D. X., Peng, Y., & Dong, L. (2015). Advances in study of sulfur dioxide residue in traditional Chinese medicine and its prepared slices. Modern Chinese Medicine, 17, 185–190. (in Chinese) https://doi.org/10.13313/j.issn.1673-4890.2015.2.022

Kang, X. Z. (2005). Determination of flavonoids in Mulberry folium by differential spectrophotometry. Chinese Journal of Spectroscopy
