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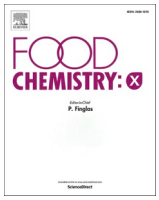

The role of respiration metabolism in dicyclohexylcarbodiimide and disodium succinate regulating the pulp breakdown occurrence of fresh longan (*Dimocarpus longan* Lour.) during storage

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ABSTRACT

Compared to control longan, on day 6, DCC-treated longan presented 18 %, 2 %, 15 %, 34 %, 21 %, 26 % and 10 % higher levels of pulp breakdown index, fruit respiration rate, PGI, SDH, NAD, NADH and QH_2, respectively. Meanwhile, DCC-treated longan displayed 25 %, 5 %, 8 % and 12 % higher activities of respiratory terminal oxidases including CCO, AOX, PPO and AAO, respectively. However, DCC-treated longan presented 9 %, 27 %, 24 %, 25 % and 16 % lower levels of NADK, G-6-PDH + 6-PGDH, NADP, NADPH and Q, respectively. These data indicate that DCC-hastened longan pulp breakdown occurrence was owing to the diminished PPP, and the elevated EMP, TCA cycle and CCP, thereby leading to an increased respiration rate. However, DS treatment displayed contrary effects, indicating DS restrained longan pulp breakdown occurrence through diminishing the respiration metabolism and depressing the respiration rate of fresh longan.

Introduction

Longan (*Dimocarpus longan* Lour.) is a well-known characteristic fruit in tropical and subtropical regions of the world. Longan fruit has a unique flavor and rich bioactive components, as well as has a long history of application in traditional Chinese medicine formulas for soothing nerves, preventing forgetfulness, and curing neuropathic pain (Lin et al., 2022; Zeng et al., 2024). However, longans ripen during the high temperature and humidity season, resulting in vigorous metabolic activities, and are being highly susceptible to quality deterioration after harvest (Lin et al., 2016; Sun et al., 2022). Pulp breakdown is the main problem leading to postharvest quality deterioration of longans, manifested by pulp softening, juice exudation, and pulp rot (Lin et al., 2021; Lin et al., 2022; Lin, Lin, Chen, et al., 2020; Zheng et al., 2025). This affects the quality characteristics, reduces the market value, and shortens the storage period of postharvest longan. Accordingly, it is crucial to investigate the mechanism of pulp breakdown occurrence in longans and explore its prevention and control methods.

Respiration metabolism, the core of plant physiological processes, involves multiple respiratory pathways, which is regulated by respiratory enzymes and electron donors (Sun et al., 2023; Tan et al., 2021). Duan et al. (2019) suggested that adenosine triphosphate (ATP) treatment effectually diminished the respiration rate of 'Nanguo' pears, thereby delaying the loss of pulp firmness and preserving fruit quality.

Abbreviations: AAO, ascorbic acid oxidase; AOX, alternate oxidase; ATP, adenosine triphosphate; CCO, cytochrome C oxidase; CCP, cytochrome pathway; DCC, dicyclohexylcarbodiimide; DS, disodium succinate; ELISA, enzyme linked immunosorbent assay; EMP, Embden-Meyerhof-Parnas pathway; G-6-PDH, glucose-6-phosphate dehydrogenase; HPLC, high performance liquid chromatography; H_2S, hydrogen sulfide; mM, mmol L^{-1}; NAD, nicotinamide adenine dinucleotide; NADH, the reduced form of NAD; NADK, NAD kinase; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, the reduced form of NADP; 6-PGDH, 6-phosphogluconate dehydrogenase; PGI, phosphohexose isomerase; PPO, polyphenol oxidase; PPP, pentose phosphate pathway; Q, ubiquinone; QH_2, panthenol; SDH, succinate dehydrogenase; TCA cycle, tricarboxylic acid cycle; TSS, total soluble solids..

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Additionally, Wang et al. (2023) found that the treatment of hydrogen sulfide (H_2S) could maintain energy supply, reduce respiration metabolism, and alleviate the quality decline of peach fruit. Thus, respiration metabolism interacts closely with energy level in fresh produces after harvest.

Dicyclohexylcarbodiimide (DCC), as a proton channel blocker, can lead to a decrease in ATP synthesis, thereby resulting in a deficiency of energy (Blbulyan & Trchounian, 2015; Toei & Noji, 2013). In contrast, disodium succinate (DS), as an exogenous proton channel enhancer, can enhance the ATP synthesis, thus sustaining a higher energy level (Sun et al., 2019). Our prior study has found that the treatments of DCC and DS could regulate the process of quality deterioration in postharvest longans, such as pulp breakdown (Zheng, Lin, Sang, Chen, et al., 2024; Zheng, Lin, Sang, Lin, et al., 2024) and pericarp browning (Chen et al., 2023; Lin et al., 2024). Whereas, the influences of DCC and DS treatments on the respiration metabolism and their relationships with longan pulp breakdown occurrence remain unclear. Accordingly, in present work, the influences of DCC and DS treatments on the pulp breakdown occurrence and respiration metabolism in fresh longan during storage, including the pulp breakdown index, fruit respiration rate, the activities of phosphohexose isomerase (PGI), succinate dehydrogenase (SDH), glucose-6-phosphate dehydrogenase + 6-phosphogluconate dehydrogenase (G-6-PDH + 6-PGDH), NAD kinase (NADK), and respiratory terminal oxidases such as cytochrome C oxidase (CCO), alternate oxidase (AOX), polyphenol oxidase (PPO) and ascorbic acid oxidase (AAO), as well as the contents of nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), the reduced form of NAD (NADH), the reduced form of NADP (NADPH), ubiquinone (Q) and panthenol (QH_2), were investigated. The purpose of this research is to discover the influences of DCC and DS treatments on the respiration metabolism and the process of longan pulp breakdown, so as to elucidate the mechanism of longan pulp breakdown occurrence. Our findings can provide new insights into the mechanism of longan pulp breakdown and help propose new approaches to delay the pulp breakdown occurrence in fresh longan, thereby maintaining the commercial value of postharvest longans and promoting the high-quality development of the longan industry.

Material and methods

Material and treatment

The fruit of 'Fuyan' longan (*Dimocarpus longan* Lour. cv. Fuyan), at commercial maturity with 15.43 % ± 0.38 % of pulp TSS content, and a light yellowish-brown fruit appearance, were harvested from Nan'an orchard, Fujian, China. Then, the harvested longans with undamaging and uniform size were selected, and treated with 0.4 mM of DCC and 40 mM of DS according to the detailed procedures described in our prior works (Zheng, Lin, Sang, Chen, et al., 2024; Zheng, Lin, Sang, Lin, et al., 2024). More specifically, 300 longans were taken for trait analysis on the day of harvest, while the remaining fruit were divided into three groups, each containing 1800 longans. One group was used as the control and soaked in distilled water for 15 min, while the other two groups were soaked in 0.4 mM DCC and 40 mM DS solutions for 15 min, respectively.

After treatment, the longans were dried in air and packed in polyethylene film bag (50 fruit per bag, 36 bags in each group), then stored at 25 °C and 85 % relative humidity for 6 d. During storage, 6 bags (300 longans) were sampled daily from each group for assessing the occurrence of longan pulp breakdown, and for detecting fruit respiration rate, then the pulps were smashed with liquid nitrogen to achieve the powder of longan pulp, after that the longan pulp powder was stored at −80 °C for quantifying the following indicators.

Assessment of longan pulp breakdown

Referring to our prior study (Chen et al., 2015), pulp breakdown was evaluated by estimating the extent of the total breakdown area in pulp of 50 individual longan fruit based on the following appearance grades: 0, no breakdown; 1, breakdown area < 1/4; 2, 1/4 ≤ breakdown area < 1/2; 3, 1/2 ≤ breakdown area < 3/4; 4, breakdown area ≥ 3/4. The calculation formula ∑ (breakdown grade × proportions of corresponding longans in each grade) was adopted for calculating longan pulp breakdown index.

Quantification of fruit respiration rate

Following our prior studies (Chen et al., 2015; Lin et al., 2024), 10 longans were used for detecting the respiration rate by Fruit and Vegetable Respiration Tester (GXH-3051H, Beijing Junfang Physics & Chemical Science and Technology Research Institute, Beijing, China), and mg CO_2 kg^{-1} h^{-1} was adopted for denoting the unit of respiration rate.

Quantification of enzymatic activity in respiration metabolism

The extraction and activity analyses of respiration metabolism-related enzymes followed the slightly modified procedures of our prior studies (Li et al., 2024; Lin, Lin, Chen, et al., 2020; Zhang et al., 2025; Supplementary material: Assaying the activities of respiration metabolism-related enzymes). Frozen powder of longan pulp (5 g) was added to corresponding extraction reagents to serve as enzyme solution for detecting the corresponding activity of respiration metabolism-related enzyme (PGI, SDH, G-6-PDH + 6-PGDH, CCO, PPO, AAO, NADK). Moreover, AOX activity was detected according to the instruction of ELISA kit (Shanghai Enzyme-linked Biotech. Co., Ltd., China).

Protein quantitative analyses of the solutions of above-mentioned enzymes were performed, following the procedure outlined by Bradford (1976). U kg^{-1} protein was adopted for denoting the unit of enzyme activity.

Quantification of pyridine nucleotide amount

The extraction and content analyses of pyridine nucleotides followed the slightly modified procedures from our previous paper (Lin et al., 2024; Supplementary material: Assaying the contents of pyridine nucleotides). Frozen powder of longan pulp (5 g) was added to the extract solution (100 mM HCl solution for assay of NAD and NADP, and 100 mM NaOH solution for assay of NADH and NADPH). The pyridine nucleotide amount was denoted in mmol kg^{-1}.

Quantification of Q and QH_2 contents

The extraction and content analyses of Q and QH_2 followed the slightly modified procedures of our prior study (Lin et al., 2024; Supplementary material: Assaying the contents of Q and QH_2). Frozen powder of longan pulp (10 g) was added to perchloric acid (200 mM) and petroleum ether for extraction. The extraction steps were repeated, then the petroleum ether phase was collected and dried with nitrogen. The dried residues were dissolved in methanol/ethanol (v/v = 1/9). After filtration, the sample was analyzed by HPLC-2030C 3D System (Shimadzu Corporation, Kyoto, Japan). The result was denoted in mmol kg^{-1}.

Statistical analyses

All parameters were measured in triplicate, and the data in the figures were presented as the mean ± standard error (n = 3). The experimental data was analyzed using IBM SPSS version 22.0. The symbol * or ** or * or ** (DCC-treated group), and □ or ▲ (DS-treated group) in the figures separately represented a clear difference (P < 0.05) or a highly clear difference (P < 0.01) compared to the control group at the same storage time. In addition, Pearson's correlation tests were adopted

for analyze the correlation between the various variables, and correlation heatmap was plotted with the R package ggcorrplot.

Results

Longan pulp breakdown index

Fig. 1A indicated that, during storage, the pulp breakdown index in three groups of longans sustained enhancement. Further comparison showed that pulp breakdown index in DCC-treated group consistently exceeded that of the control group during days 1 to 6, with clear discrepancies between days 2 and 6. However, DS-treated group exhibited a lower pulp breakdown index within storage (except day 0), with clear difference than the control between days 2 and 5. Additionally, the pulp breakdown index in DCC- and DS-treated group were 18 % higher and 24 % lower than the control group on day 6, separately. Consequently, DCC treatment could exacerbate the breakdown development in longan pulp, whereas DS treatment could retard the breakdown process in the longan pulp.

Respiration rate

Fig. 1B showed that respiration rates in the control and DS-treated groups enhanced during days 0–6. Whereas respiration rate in DCC-treated group increased during the initial five days, and then slightly declined between days 5 and 6. Further comparison revealed that respiration rate in DCC-treated group consistently exceeded that of the control group between days 1 and 6, with clear discrepancies on days 2, 4, and 5. While DS-treated group exhibited a lower respiration rate within days 1–6, with clearly lower rate of respiration than the control group between days 3 and 6. For example, the respiration rate in DCC and DS-treated longans were 2 % higher and 7 % lower than the control group on day 6, separately. Thus, DCC treatment could lead to an accelerated increment of respiration rate, while the DS treatment could retard the increment in the respiration rate of longan fruit.

Activities of PGI, SDH, G-6-PDH + 6-PGDH, CCO, AOX, PPO, and AAO

Fig. 2A illustrated that the pulp PGI levels in the control and DCC-treated group enhanced from days 0 to 1, and then dropped between days 1 and 6. Whereas, pulp PGI activity in DS-treated group showed a continuous decline within storage. Compared to the control group, DCC treatment clearly enhanced the PGI level, but DS treatment lowered PGI activity throughout the storage. For example, PGI activity in DCC- and DS-treated group were 15 % higher and 11 % lower than the control group on day 6, separately.

The changes in SDH levels (Fig. 2B) were similar in the control and DS-treated group, showing a quick ascension during days 0–1, and a gradual decline between days 1 and 6. The SDH activity in DCC-treated group manifested a sharp increment from day 0 to 1, but then decreased from day 1 to 6, except for day 3. Additionally, from days 1 to 6, compared to the control group, DCC-treated group exhibited higher SDH activities, whereas the DS-treated group showed lower levels of SDH activities. For instance, on day 6, the SDH activity in the DCC-treated group elevated by 34 % than the control group, whereas in DS-treated group, it was reduced by 33 % compared to the control group.

Fig. 2C showed that, during storage, the control and the DCC-treated group roughly showed a downward trend in G-6-PDH + 6-PGDH activity. The DS-treated group displayed a slight increment from days 0 to 1, and then a decline trend between days 1 and 6. Additionally, between days 1 and 6, DCC-treated group manifested a lower value of G-6-PDH + 6-PGDH, while the DS-treated group displayed a higher G-6-PDH + 6-PGDH activity, as compared to the control group. For example, G-6-PDH + 6-PGDH activity in DCC- and DS-treated group were 27 % lower and 26 % higher than the control group on day 6, separately.

Fig. 2D-2G illustrated the variation tendency of CCO, AOX, PPO, and AAO activities during storage. For the control group, these activities initially enhanced and then dropped as storage proceeded, with peak values reached on days 4 or 5. Additionally, during days 1–6, compared to the control group, DCC-treated group manifested higher values of CCO, AOX, PPO, and AAO, while DS-treated group showed lower levels of these enzymatic activities. For instance, on day 6, the CCO, AOX, PPO, and AAO activity in DCC-treated group were 125 %, 105 %, 108 %, and 112 % of the control group, separately, while these activities of enzymes in DS-treated group were 83 %, 94 %, 83 %, and 96 % of the control group, separately.

These findings suggest that DCC treatment for longan fruit could elevate the activity of PGI, SDH, CCO, AOX, PPO, and AAO, but reduce G-6-PDH + 6-PGDH activity. However, DS treatment showed the opposite effects on these enzymatic activities.

NADK activity and pyridine nucleotide amount

NADK activity trends (Fig. 3A) were similar across all groups,

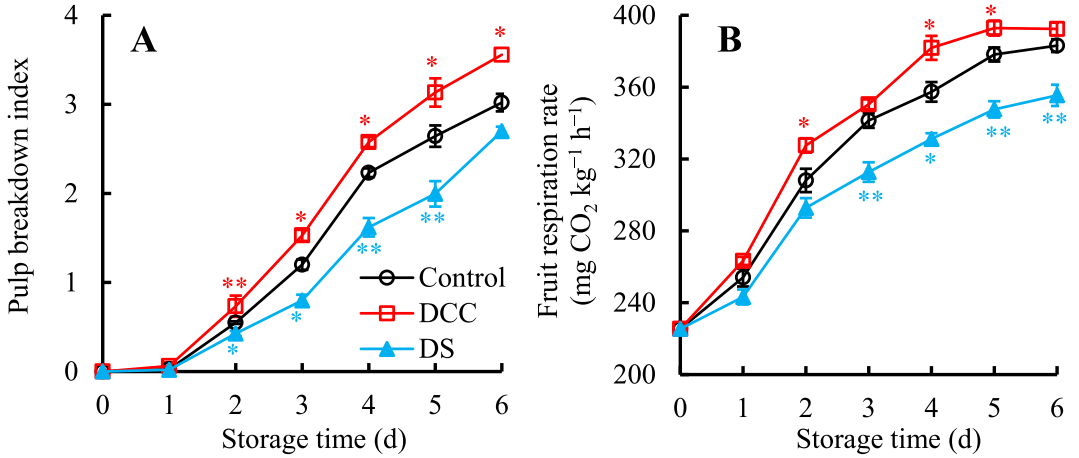

Fig. 1. The influences of DCC and DS treatment on the pulp breakdown index (A) and fruit respiration rate (B) in fresh longan during storage. Values presented in figure equal the mean ± standard error ($n = 3$). The asterisk * or ** (DCC-treated group), and * or ** (DS-treated group) in the figures separately represented a clear difference ($P < 0.05$) or a highly clear difference ($P < 0.01$) compared to the control group at the same storage time. ○, Control; □, 0.4 mM DCC; ▲, 40 mM DS. DCC, dicyclohexylcarbodiimide; DS, disodium succinate. The pulp breakdown index (Fig. 1A) was quoted from our work published in *Postharvest Biology and Technology* by Zheng, Lin, Sang, Lin, et al. (2024) (doi:https://doi.org/10.1016/j.postharvbio.2024.113041).

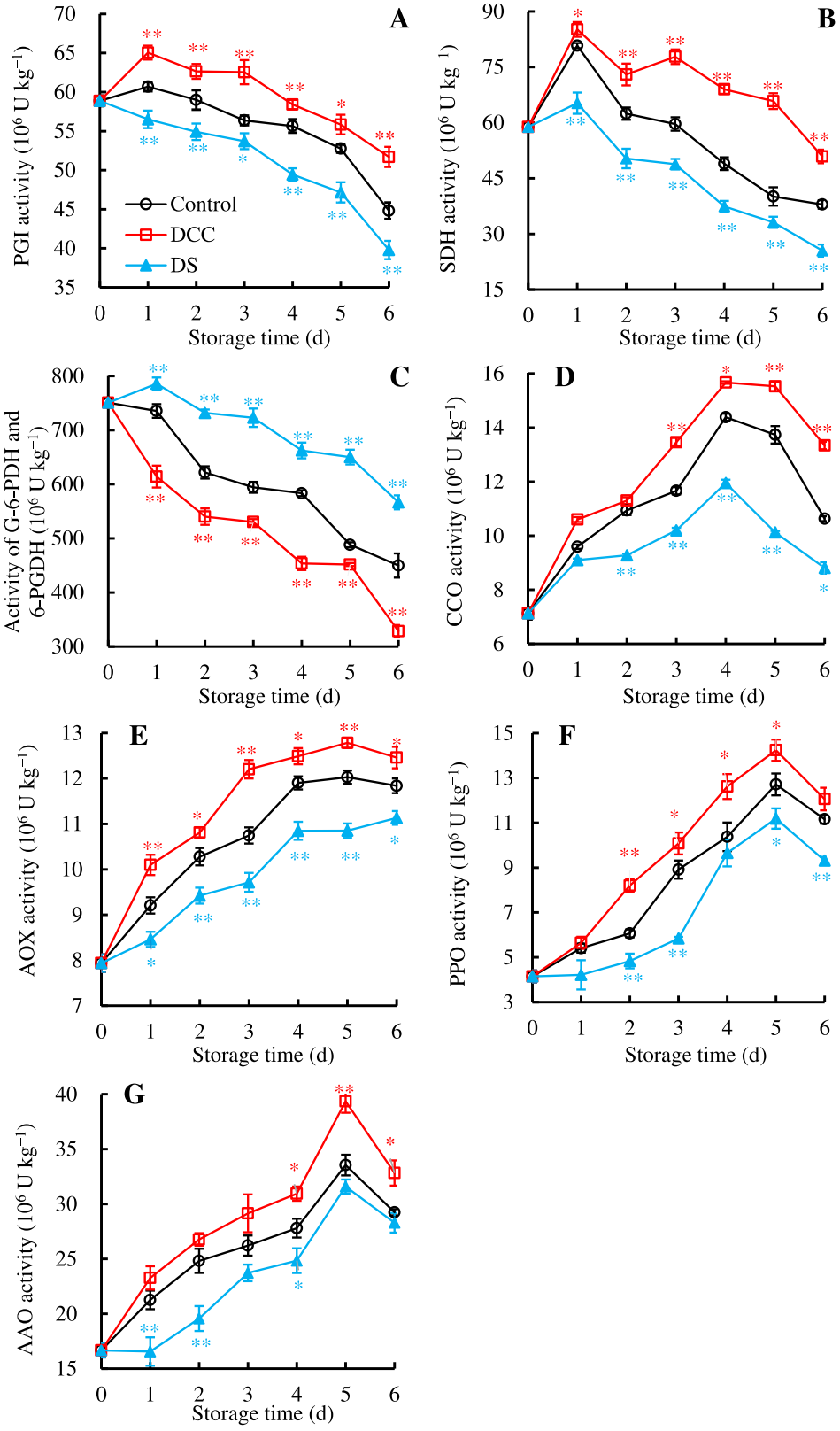

Fig. 2. The influences of DCC and DS treatment on the activities of PGI (A), SDH (B), G-6-PDH + 6-PGDH (C), CCO (D), AOX (E), PPO (F), and AAO (G) in pulp of fresh longan during storage. The asterisk * or ** (DCC-treated group), and * or ** (DS-treated group) in the figures separately represented a clear difference ($P < 0.05$) or a highly clear difference ($P < 0.01$) compared to the control group at the same storage time. ○, Control; □, 0.4 mM DCC; ▲, 40 mM DS. DCC, dicyclohexylcarbodiimide; DS, disodium succinate; PGI, phosphoglucose isomerase; SDH, succinate dehydrogenase; G-6-PDH, glucose-6-phosphate dehydrogenase; 6-PGDH, 6-phosphogluconate dehydrogenase; CCO, cytochrome C oxidase; AOX, alternative oxidase; PPO, polyphenol oxidase; AAO, ascorbic acid oxidase.

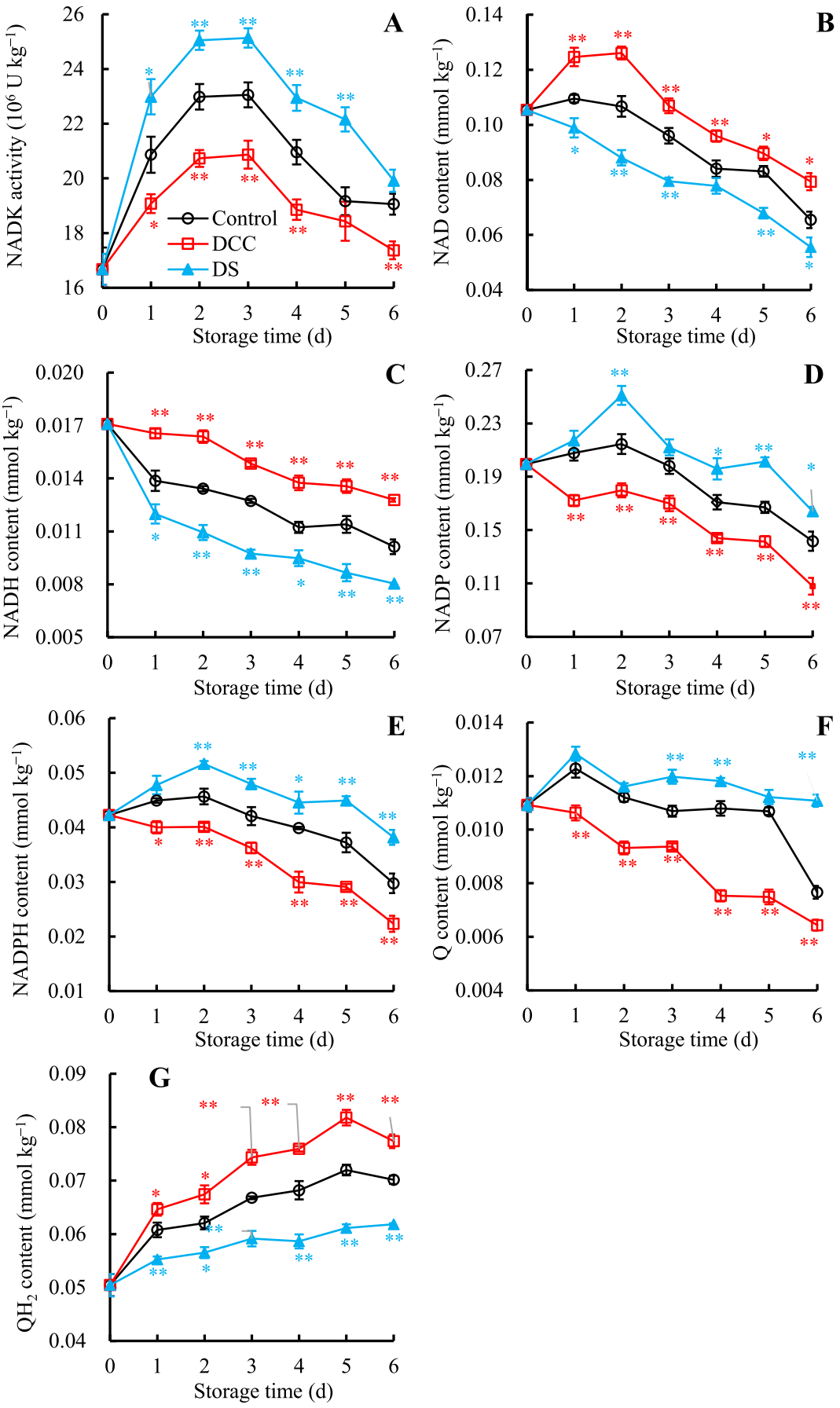

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Fig. 3. The influences of DCC and DS treatment on the NADK activity (A), and the levels of NAD (B), NADH (C), NADP (D), NADPH (E), Q (F) and QH_2 (G) in pulp of fresh longan during storage. The asterisk * or ** (DCC-treated group), and * or ** (DS-treated group) in the figures separately represented a clear difference ($P < 0.05$) or a highly clear difference ($P < 0.01$) compared to the control group at the same storage time. ○, Control; □, 0.4 mM DCC; ▲, 40 mM DS. DCC, dicyclohexylcarbodiimide; DS, disodium succinate; PGI, phosphoglucose isomerase; NADK, NAD kinase; NAD, nicotinamide adenine dinucleotide; NADH, the reduced form of NAD; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, the reduced form of NADP; Q, ubiquinone; QH_2, panthenol.

showing an upward trajectory from days 0 to 3, peaking on day 3, and then declining during days 3 to 6. In addition, compared to the control group, between days 1 and 6, DCC-treated group displayed lower NADK activity, whereas DS-treated group presented higher NADK activity. For example, on day 3, the NADK activity in DCC-treated and DS-treated group was 10 % lower and 9 % higher than the control group, respectively.

As manifested in Fig. 3B-3C, the NAD levels in the control group slightly elevated, and the NADH content sharply reduced within days 0–1. Subsequently, both NAD and NADH levels gradually declined during days 1–6. Additionally, compared to the control group, during 1–6 d, DCC-treated group exhibited higher values of NAD and NADH, while the DS-treated group exhibited lower levels of these pyridine nucleotides. For instance, on day 6, compared to the control group, DCC-treated group manifested a 21 % increment in NAD and a 26 % increase in NADH, but the DS-treated group showed a 15 % decrease in NAD and a 21 % decrease in NADH.

NADP and NADPH values in the control group showed a slight increment between days 0 and 2, and then a gradual decline trend between days 2 and 6 (Fig. 3D-E). Additionally, compared to the control group, during 1–6 d, DCC-treated group exhibited lower values of NADP and NADPH, whereas DS-treated group presented higher levels of these pyridine nucleotides. For example, on day 6, compared to the control group, DCC-treated group manifested a 24 % decrease in NADP and a 25 % decrease in NADPH, but DS-treated group presented a 16 % increment in NADP and a 28 % increase in NADPH.

These findings indicate that DCC treatment for longan fruit could reduce NADK activity, lower the values of NADP(H), and elevate the levels of NAD(H). Whereas DS treatment showed the opposite effects.

Q and QH_2 contents

As displayed in Fig. 3F, Q level in the control longan pulp displayed a quick increment from days 0 to 1, followed by a gradual declining trend during days 1–5, and a sharp decline from days 5 to 6. Additionally, compared to the control group, within days 1–6, DCC-treated group manifested a lower Q content, whereas DS-treated longan manifested a higher level of Q. For instance, on day 6, Q content in DCC-treated group was 84 % of the control group. However, Q content in DS-treated group was 145 % of the control group.

Fig. 3G indicated that the QH_2 level in the control group presented a gradual enhancement from days 0 to 5, followed by a mild decline. Additionally, within days 1–6, compared to the control group, DCC-treated fruit manifested a higher QH_2 content, while the DS-treated fruit exhibited a lower QH_2 level.

These findings suggest that DCC treatment for longan fruit could reduce the Q content, but elevate the level of QH_2. Whereas DS treatment showed the opposite effects.

Discussion

DCC and DS regulated longan pulp breakdown by influencing respiration rate

In addition to pericarp browning, pulp breakdown is another important factor affecting storage quality in fresh longan, manifesting as rapid softening, juice exudation and rotting. Moreover, the quality degradation of postharvest fruit was closely associated with the respiration rate. Prior work indicated that application of acid electrolytic water could effectively restrain the respiration rate of longan fruit, and preserves the fruit quality (Chen et al., 2020). Moreover, it has been found that the composite coating of guar gum with salicylic acid or the application of H_2S also could maintain the postharvest quality of 'Huangguan' pears or peaches via inhibiting respiration rate (Wang et al., 2023). In contrast, a high respiration rate rapidly depletes metabolic substrate, which can accelerate senescence, and reduce storage-life in fresh products (Li, Lv, Guo, & Wang, 2016). It has been found that the quality decline in postharvest pears (Sun et al., 2023) or apples (Shu et al., 2022) was related to increased respiration rate.

In this study, as storage time progressed, the pulp breakdown index (Fig. 1A) gradually increased across all treated longans, and the respiration rate (Fig. 1B) also continuously climbed. Correlation analysis in these treatments revealed that the pulp breakdown index had positive correlation with respiration rate (Fig. 4). The correlation results confirm that the increased respiration rate contributed to pulp breakdown occurrence. It could be inferred that, as storage proceeded, the enhanced respiration in longan pulp led to an increased nutrient depletion, which in turn accelerated tissue softening and collapse. Furthermore, compared to the control group, DCC-treated group manifested a higher index of pulp breakdown (Fig. 1A) and a higher rate of respiration (Fig. 1B). This suggests that exacerbation of pulp breakdown in DCC-treated group was due to an enhanced respiration rate. However, DS-treated group showed opposite trends, suggesting that DS-delayed longan pulp breakdown occurrence was because DS treatment maintained a lower respiration rate.

DCC and DS regulated longan pulp breakdown by affecting respiratory metabolic pathway-related enzyme and substance

The respiratory process involves a comprehensive collaboration among various respiratory pathways, including Embden-Meyerhof-Parnas pathway (EMP), tricarboxylic acid (TCA) cycle, pentose phosphate pathway (PPP), and cytochrome pathway (CCP) (Zhang et al., 2025). Previous studies showed that treatments with melatonin, H_2S, and riboflavin led to the reduced EMP, TCA cycle, and CCP, and an elevated PPP, thus delaying the quality decline in Chinese cabbage leaf (Tan et al., 2021), peaches (Wang et al., 2023), and strawberries (Zhang et al., 2023), respectively. Additionally, the application of 80 % O_2 + 20 % CO_2 for white mushrooms could reduce the decrease in CCP, and thus alleviating its quality decline (Li et al., 2019). However, infestation by *Alternaria alternata* accelerated the deterioration of pears, which connected with the raised EMP, TCA cycle, and CCP (Sun et al., 2023). Moreover, Luo et al. (2021) also discovered that the elevated EMP and TCA cycle could lead to the fruit deterioration rapidly. Therefore, the quality decline in fresh produce is strongly linked to variations in respiratory pathways.

Changes in the respiratory pathway are regulated by various respiratory metabolic enzymes. EMP is the most fundamental pathway in the entire respiratory chain (Wang et al., 2023). PGI is considered a marker to assess EMP strength (Sun et al., 2023). TCA cycle is a crucial biochemical process which generates energy, involving metabolite consumption and affecting fruit senescence (Zhang et al., 2025). SDH, a metric for evaluating TCA cycle efficiency, can catalyze succinic acid to form fumaric acid, and to produce ATP. In addition, SDH can also reduce Q to QH_2 in CCP (Tan et al., 2021). PPP is a biochemical pathway for glucose oxidative catabolism, as well as the primary source of oxidation-reduction potential to resist stress; while G-6-PDH + 6-PGDH is the rate-limiting enzyme in PPP (Sun et al., 2023).

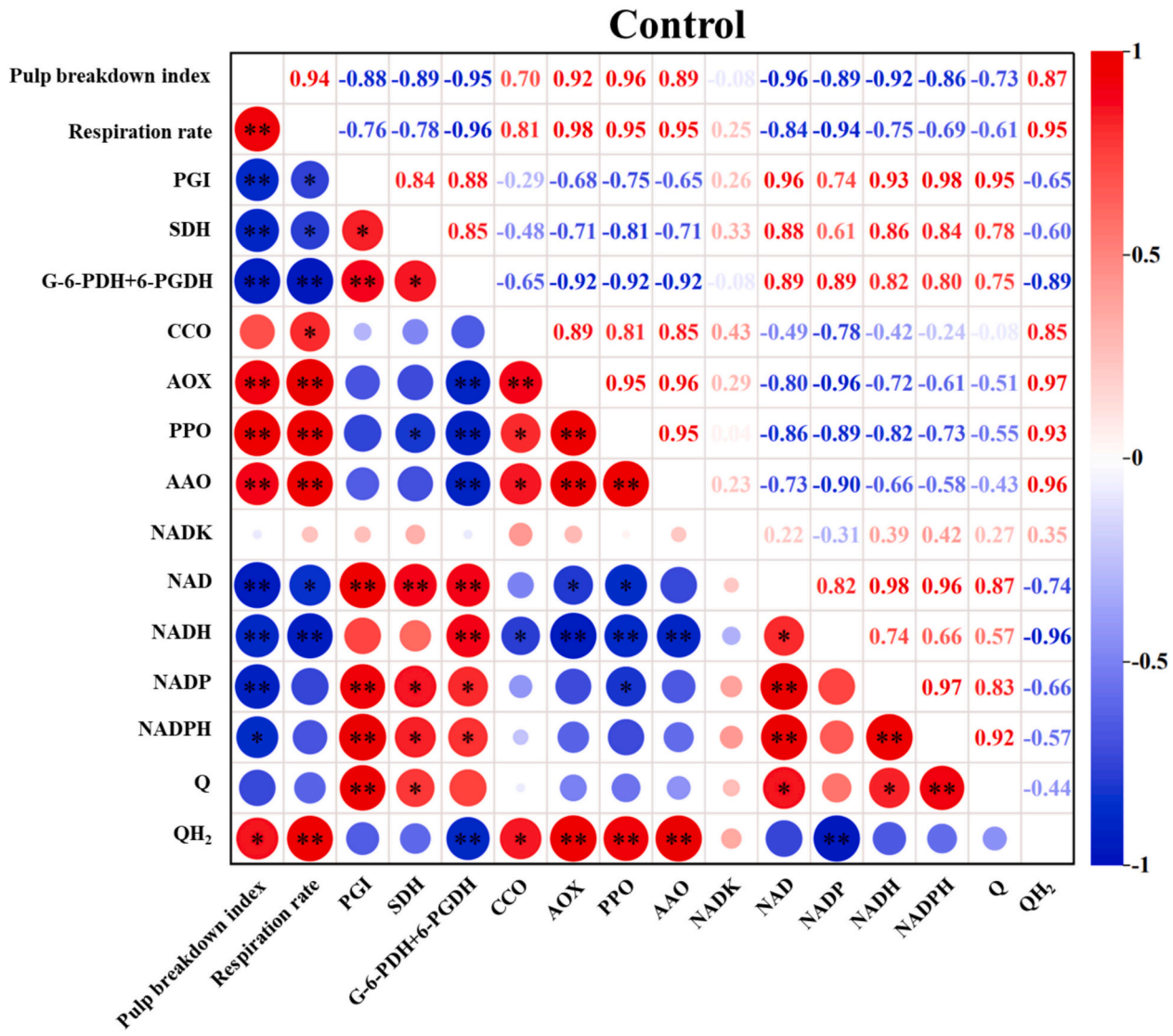

Fig. 4. Heatmaps of Pearson's correlation analysis between the estimated indicators related to the respiration metabolism and the pulp breakdown in the control longan, DCC-treated longan, and DS-treated longan during storage (*$P < 0.05$, **$P < 0.01$). Red represented a positive correlation, whereas blue represented an inverse correlation. DCC, dicyclohexylcarbodiimide; DS, disodium succinate; PGI, phosphoglucose isomerase; SDH, succinate dehydrogenase; G-6-PDH, glucose-6-phosphate dehydrogenase; 6-PGDH, 6-phosphogluconate dehydrogenase; CCO, cytochrome C oxidase; AOX, alternative oxidase; PPO, polyphenol oxidase; AAO, ascorbic acid oxidase; NADK, NAD kinase; NAD, nicotinamide adenine dinucleotide; NADH, the reduced form of NAD; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, the reduced form of NADP; Q, ubiquinone; QH_2, panthenol. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

During storage, as the pulp breakdown index (Fig. 1A) and respiration rate (Fig. 1B) continuously increased in the control group, G-6-PDH + 6-PGDH (Fig. 2C) continued to decrease, but PGI activity (Fig. 2A), SDH activity (Fig. 2B), and the contents of Q (Fig. 3F) and QH_2 (Fig. 3G) initially increased and then decreased. Correlation analyses reveal that pulp breakdown index had negative correlations with G-6-PDH + 6-PGDH level and Q amount, but positive correlation with QH_2 content (Fig. 4). Meanwhile, the respiration rate showed negative correlations with the values of G-6-PDH + 6-PGDH and Q, whereas positive correlation with QH_2 level (Fig. 4). It could be inferred that the increased QH_2 content, and the decreased G-6-PDH + 6-PGDH activity played crucial roles in pulp breakdown occurrence of longan. Compared to the control group, DCC-treated group showed higher values of pulp breakdown index (Fig. 1A), respiration rate (Fig. 1B), PGI, SDH, and QH_2 content (Fig. 2A, B, Fig. 3G), but lower values of G-6-PDH + 6-PGDH and Q within storage period (Fig. 2C, Fig. 3F). These outcomes suggest that DCC treatment promoted respiration rate and exacerbated longan pulp breakdown, which was due to DCC treatment enhancing EMP-TCA cycle and CCP, whereas diminishing PPP. Conversely, DS treatment manifested the lower longan pulp breakdown index and respiration rate, lower PGI and SDH activity, and a lower QH_2 content, but higher values of G-6-PDH + 6-PGDH and Q, suggesting that DS treatment decreased respiration rate, and then delayed longan pulp breakdown development, which was because DS treatment sustained higher activity of PPP and restrained EMP-TCA cycle and CCP.

Alterations in respiration pathways are also regulated by electron donors. Critically, the key electron donors in plant cells are NADP(H) and NAD(H). In EMP-TCA cycle, NAD obtains electrons to form NADH (Zhang et al., 2023). Moreover, NAD is catalyzed by NADK to produce NADP, which can be reduced to NADPH in PPP (Zhang et al., 2023). Hence, the variations of NADP(H) and NAD(H) contents can serve as a metric to appraise the intensity of PPP and EMP-TCA cycle, respectively.

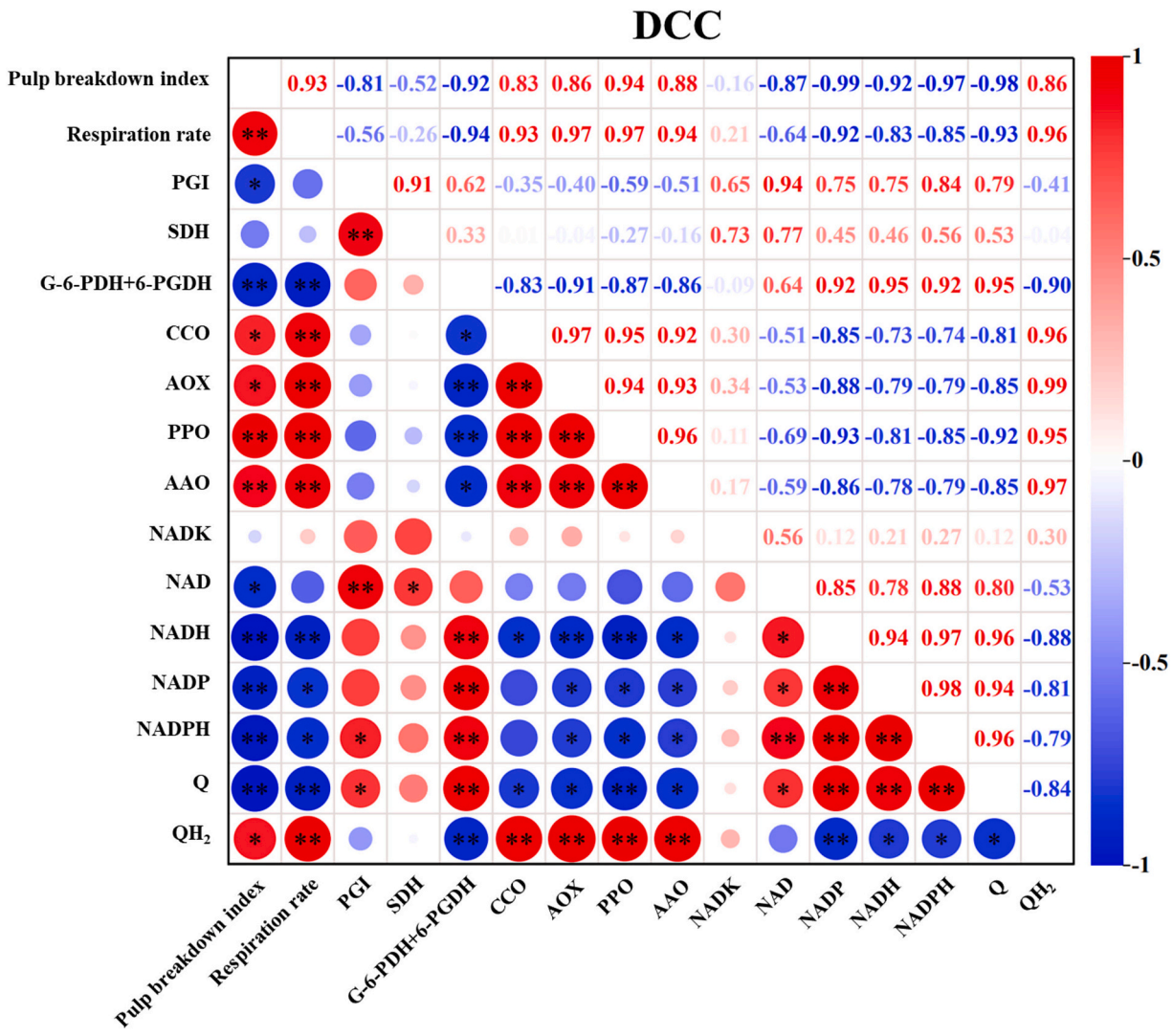

Fig. 4. (*continued*).

During storage, when the pulp breakdown index (Fig. 1A) of all groups continued to increase, NADK (Fig. 3A) activity first increased and then decreased, but the values of NAD (Fig. 2B), NADP (Fig. 2D), NADH (Fig. 2C), and NADPH (Fig. 2E) continued to decrease. Compared to the control group, DCC-treated group showed higher values of pulp breakdown index (Fig. 1A), higher values of NAD (Fig. 2B) and NADH (Fig. 2C), but lower levels of NADK (Fig. 2A), NADP (Fig. 2D), and NADPH (Fig. 2E). These data suggest that DCC treatment exacerbated longan pulp breakdown, which was because DCC treatment elevated the EMP-TCA cycle, but repressed PPP. However, DS-treated pulp showed opposite results, implying that DS treatment delayed longan pulp breakdown, which was because DS treatment maintained higher PPP levels and restrained EMP-TCA cycle.

These results demonstrate that DCC treatment exacerbated longan pulp breakdown occurrence, which was owing to the elevated EMP-TCA cycle and CCP, and attenuated PPP, and then promoted respiration rate. This is evidenced by the increased PGI and SDH activities, higher values of QH_2, NAD and NADH, reduced activity of G-6-PDH + 6-PGDH and NADK, and lower values of Q, NADP and NADPH. However, the delay in longan pulp breakdown occurrence due to DS treatment was because of the reverse regulation of DS treatment.

DCC and DS regulated longan pulp breakdown by affecting respiratory terminal oxidase

Respiratory terminal oxidase plays a key part in regulating the respiration rate of postharvest produce. Respiration rate was closely linked to the activity of respiratory terminal oxidase (CCO, AOX, PPO, AAO) (Zhang et al., 2025). To some extent, it reflects the efficiency of electron transfer and the level of respiration metabolism (Liu et al., 2023). Our work indicates that *Pestalotiopsis microspore* stimulated disease development and spoilage in postharvest Chinese olive, which was strongly associated with the elevated activities of CCO, AOX, PPO, and AAO, as well as with an increased rate of respiration (Lin et al., 2023). Meanwhile, Sun et al. (2023) also found that the promoted activities of CCO and AOX could decrease the quality of 'Korla' fragrant pear. Additionally, Zhang et al. (2017) suggested that 2,4-dinitrophenol treatment for *Lasiodiplodia theobromae*-infected longan could elevate CCO, PPO, and AAO activities, enhance the respiration rate, thereby accelerate fruit quality decline and disease development. However, ATP treatment could reduce CCO, PPO and AAO activities, lower respiration rate, and consequently attenuate disease development and retain the quality in postharvest longan (Zhang et al., 2017). Moreover, Hu et al. (2021) indicated that the irradiation of ^{60}Co γ-ray for walnuts could lower respiration rate and mitigate quality decline, which was associated with lower levels of CCO, PPO, and AAO.

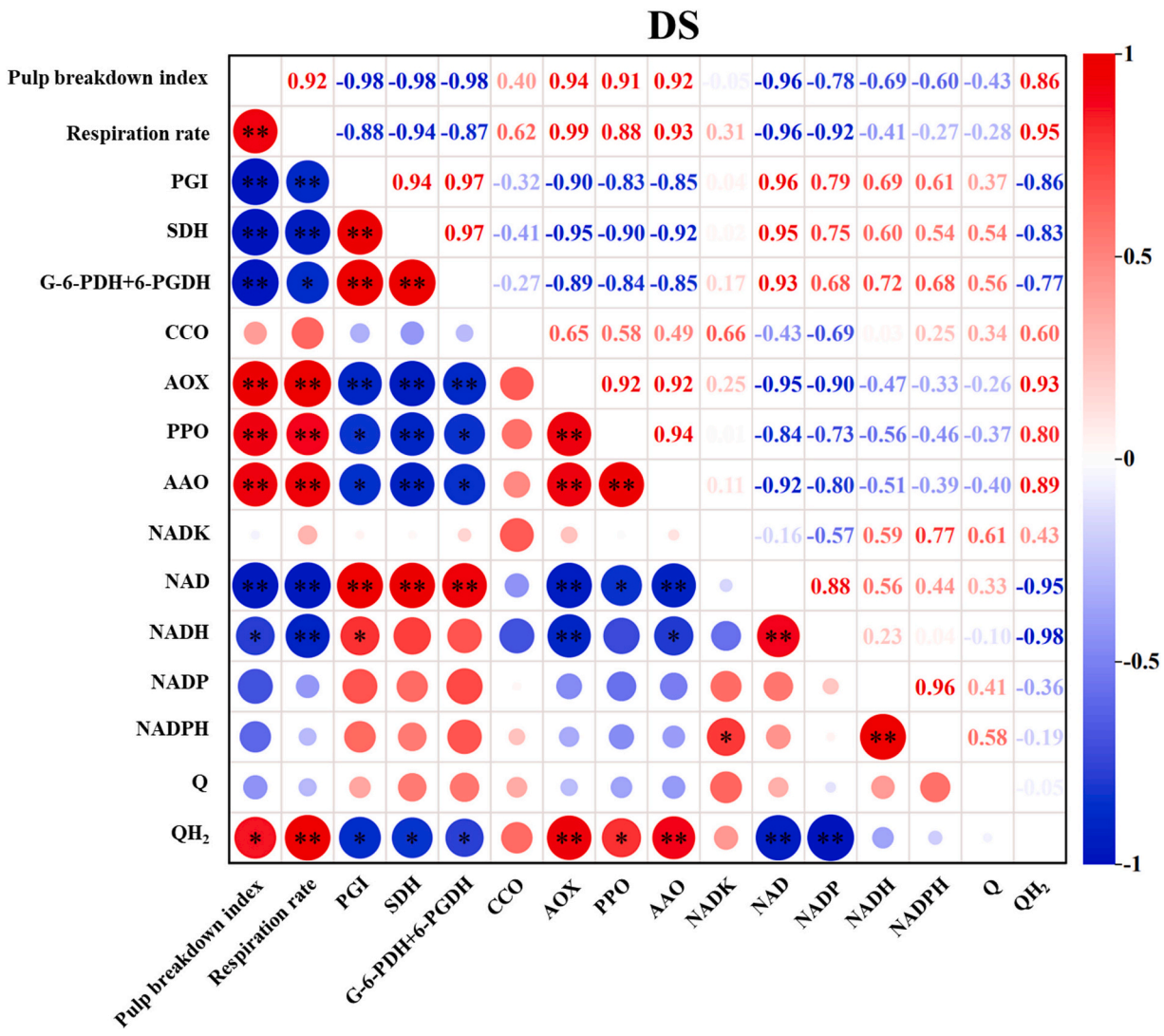

Fig. 4. (*continued*).

During storage, along with the continuous increase of the pulp breakdown index (Fig. 1A) and respiration rate (Fig. 1B) in all treated-groups, CCO, AOX, PPO, and AAO activities first increased and then decreased (Fig. 2D, E, F, G). Analysis of the correlation data reveals that pulp breakdown index and respiration rate in all treated groups were positively correlated with the activities of CCO, AOX, PPO and AAO (Fig. 4). It can be inferred that the increased activities of CCO, AOX, PPO, and AAO takes crucial roles in an enhanced respiration rate and longan pulp breakdown occurrence. Compared to the control group, within storage, DCC-treated group manifested a higher pulp breakdown index (Fig. 1A), and higher CCO, AOX, PPO and AAO activities (Fig. 2D, E, F, G).

These data suggest that the aggravation of longan pulp breakdown occurrence by DCC treatment was due to an increase in respiratory terminal oxidase (CCO, AOX, PPO, AAO) activity, thus increasing the respiration rate. However, DS treatment exhibited the opposite effects, suggesting that the delay in longan pulp breakdown occurrence was because DS treatment decreased the activity of respiratory terminal oxidase (CCO, AOX, PPO, AAO), consequently lowering the respiration rate.

Conclusions

Collectively, this study revealed that DCC treatment accelerated the pulp breakdown occurrence in postharvest longans. Conversely, DS treatment delayed this deterioration process. In detail, DCC treatment enhanced PGI, SDH, CCO, AOX, PPO, and AAO activities, and lowered the activity of G-6-PDH + 6-PGDH. Moreover, DCC treatment also decreased NADK activity, reduced the values of NADP, NADPH and Q, while promoted NAD, NADH and QH_2 levels. These changes led to an enhancing EMP-TCA cycle and CCP, a diminishing PPP, and thus, an increased respiration rate. Together, these outcomes suggest that DCC treatment elevated respiration metabolism and led to a rapid consumption of respiratory substrates, thereby accelerating the longan pulp breakdown. However, DS treatment helped to suppress respiration metabolism and the onset of longan pulp breakdown. The presumable mechanisms of DCC and DS modulating longan pulp breakdown occurrence through influencing on the respiration metabolism was proposed and shown in Fig. 5. However, the molecular mechanisms of DCC and DS modulating longan pulp breakdown occurrence remain unclear. Thus, the molecular mechanisms of DCC and DS modulating longan pulp breakdown occurrence need further exploration.

Additionally, DS has been applied in the food industry due to its advantages of safety, good chemical stability, and low cost. Present work also indicates that DS treatment could efficaciously delay longan pulp breakdown occurrence, suggesting DS is a potential approach to retard longan pulp breakdown occurrence, extend the storage-life and maintain the commercial value of postharvest longans.

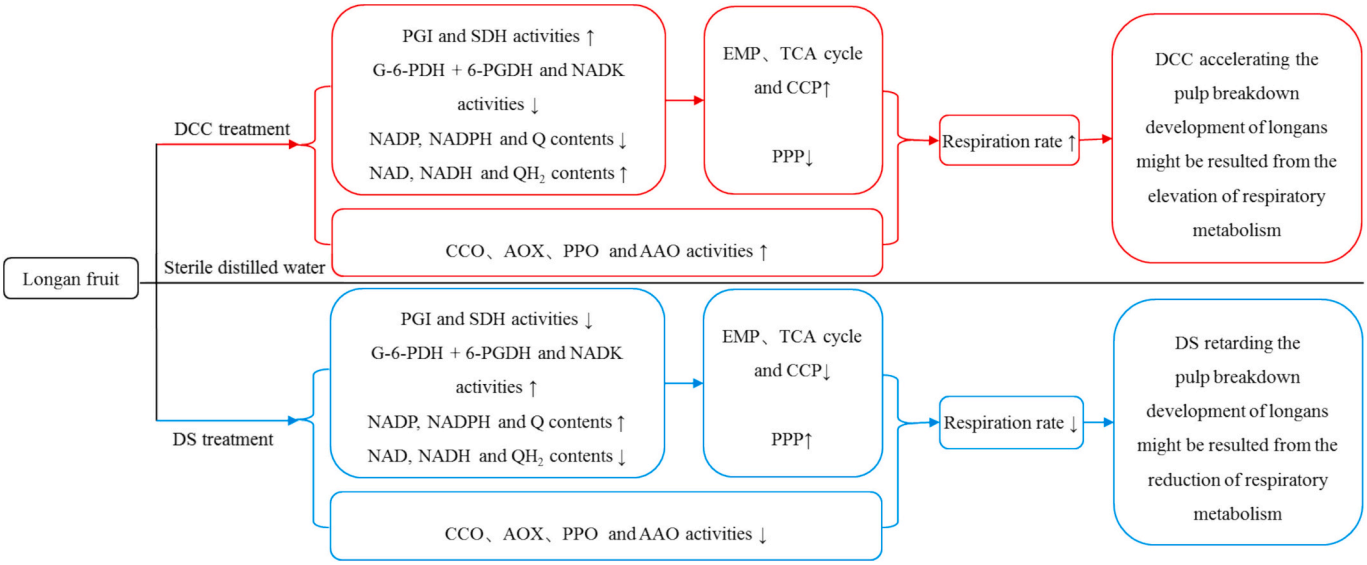

Fig. 5. The presumable mechanisms of DCC and DS modulating longan pulp breakdown occurrence through influencing on the respiration metabolism. The up arrow (↑) in the figure represented the higher levels of indicators in the DCC-treated fruit or in the DS-treated fruit as compared to the control fruit; whereas the down arrow (↓) in the figure represented the lower levels of indicators in the DCC-treated fruit or in the DS-treated fruit as compared to the control fruit. DCC, dicyclohexylcarbodiimide; DS, disodium succinate; PGI, phosphoglucose isomerase; SDH, succinate dehydrogenase; G-6-PDH, glucose-6-phosphate dehydrogenase; 6-PGDH, 6-phosphogluconate dehydrogenase; NADK, NAD kinase; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, the reduced form of NADP; Q, ubiquinone; NAD, nicotinamide adenine dinucleotide; NADH, the reduced form of NAD; QH_2, panthenol; CCO, cytochrome C oxidase; AOX, alternative oxidase; PPO, polyphenol oxidase; AAO, ascorbic acid oxidase; EMP, Embden-Meyerhof-Parnas pathway; TCA cycle, tricarboxylic acid cycle; CCP, cytochrome pathway; PPP, pentose phosphate pathway.

CRediT authorship contribution statement

Yi Zheng: Writing – original draft, Investigation, Formal analysis, Data curation. **Yixiong Lin:** Project administration, Investigation, Funding acquisition. **Hongyi Wen:** Investigation. **Yueying Sang:** Writing – original draft. **Mengshi Lin:** Writing – review & editing. **Zhongqi Fan:** Investigation. **Hui Wang:** Investigation. **Yihui Chen:** Investigation. **Yifen Lin:** Writing – review & editing, Project administration, Funding acquisition. **Hetong Lin:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102385.

Data availability

The authors do not have permission to share data.

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