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Control of Unexpected *Mucor lusitanicus* in Litchi Fruit by Hydrocooling with Hypochlorous Acid and Cold Storage

I-Fang Liu, Huey-Ling Lin and Chang-Lin Chen *

Department of Horticulture, National Chung Hsing University, 145 Xingda Road, Taichung City 40227, Taiwan; g111032103@smail.nchu.edu.tw (I.-F.L.); hllin@dragon.nchu.edu.tw (H.-L.L.)
* Correspondence: changlinchen@dragon.nchu.edu.tw

Abstract: Litchi fruit (*Litchi chinensis* Sonn.) is highly perishable because its shelf life is significantly limited by pericarp browning and microbial spoilage. While sulfur dioxide (SO_2) fumigation has been traditionally used to preserve color and reduce spoilage, concerns over potential health risks have prompted the exploration of safer alternatives. This study investigated the application of hypochlorous acid (HClO) as an alternative treatment during postharvest processes to mitigate pathological decay, targeting *Mucor lusitanicus*, a fungus primarily responsible for litchi fruit rot in Taiwan. In vitro experiments demonstrated that *M. lusitanicus* growth was completely inhibited by HClO concentrations at 40 mg L^{-1} or higher, as well as by temperatures below 1 °C. In vivo experiments further revealed that disease symptoms in inoculated litchi fruit were fully suppressed at 25 °C for seven days after hydrocooling with HClO. When 40 mg L^{-1} HClO treatment was combined with hydrocooling and subsequent storage at 5 °C, the decay ratio of litchi fruit was reduced to below 3% after 21-day storage. The browning index and disease incidence of litchi fruit hydrocooled with an 8 h hydrocooling delay were significantly lower than those with a 12 h hydrocooling delay after 21 days at 5 °C, followed by 1 day at 26 °C. Therefore, hydrocooling within 8 h of harvest is recommended for commercial scales. This treatment effectively prevented pericarp browning and maintained total soluble solid levels, ensuring the quality. These findings suggest that integrating HClO with hydrocooling not only decreases spoilage and delays pericarp browning but also offers a viable alternative to traditional SO_2 fumigation, optimizing the postharvest process and enhancing food safety. This approach can extend the storage ability of litchi fruit while maintaining its quality, providing a safer method for local and international markets.

Keywords: browning; postharvest disease; storage quality; precooling

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1. Introduction

Litchi (*Litchi chinensis*) is a non-climacteric fruit with a red pericarp and milky white aril known for its high susceptibility to postharvest losses due to pericarp browning, flesh deterioration, and fruit rot [1,2]. The delicate nature of litchi results in a short storage duration and shelf life, primarily because of its high respiration, decay rates, vulnerability to mechanical injury, and environmental factors [3]. During late spring and summer in tropical or subtropical regions, high temperatures and humidity further exacerbate pericarp browning and fruit infection during postharvest processes [4–6].

Browning in litchis responds to various factors, including injury, infection, desiccation, and senescence. Previous studies have linked browning to the degradation of anthocyanins and the activity of polyphenol oxidase (PPO) [7,8]. Anthocyanins are hydrolyzed into

anthocyanidins by anthocyanase and then oxidized by PPO, leading to the formation of brown pigments and browning of the pericarp [9]. Desiccation causes membrane disintegration, releasing phenolic compounds and anthocyanins from vacuoles, which are then catalyzed by PPO in the pericarp [1,4,10]. To prevent browning, it is beneficial to reduce PPO activity through low temperatures or SO_2 treatment on the one hand, and to maintain intact membranes with high humidity by packaging on the other hand [2,11]. Addressing pericarp browning and microbial diseases is crucial to extend storage ability of litchis. Postharvest techniques such as SO_2 fumigation, precooling, sanitation, and modified atmosphere packaging (MAP) are commonly used to enhance the storage ability and extend shelf life [12–15]. While SO_2 fumigation effectively suppresses enzymatic browning and reduces postharvest diseases [14], recent studies have highlighted its drawbacks, including altered taste, pericarp bleaching, and potential health risks to consumers [16]. Consequently, the European Union has set a maximum sulfur residue level of 10 mg L^{-1} in the edible portion, promoting the exploration of alternative treatments for the global litchi industry [16].

Several significant postharvest fungal pathogens have been identified in litchi fruit, including *Gloeosporium*, *Colletotrichum*, *Penicillium*, *Botrytis*, *Galactomyces*, *Peronophythora*, and *Mucor* spp., which were isolated from 17 litchi packinghouses [5,17,18]. Recently, an unknown postharvest disease affecting litchi fruit was observed in Taiwan, particularly in fruit stored for several weeks at low temperatures during 2023 and 2024. The disease symptoms were highly similar to the mucor rot, so we tried to identify this postharvest disease and support disease control methods. In recent years, mucor rot has emerged as the dominant postharvest disease in mandarin fruit in California [19], and *Mucor* spp. contribute to substantial losses in fresh agricultural products [20]. To combat this issue, natamycin has been recommended for controlling *Mucor* spp. in citrus, and combination with salts and heat treatments has proven effective [21]. However, no official chemical treatments are currently recommended for *Mucor* spp. in litchi fruit during the postharvest process in Taiwan. Once the pathogen infects the fruit, either in the field or during the postharvest process, it can proliferate rapidly under favorable conditions. Precooling is a critical step in inhibiting pathogen growth and preserving fruit quality, as low temperatures not only suppress fungal growth but also reduce the respiration rate of horticultural produce [15,22]. Although numerous studies have documented postharvest diseases in litchis [5,17,18], effective treatments beyond SO_2 remain limited. Therefore, there is a need for a simple and effective method to control fungal diseases like mucor rot during postharvest handling.

To minimize environmental and health impacts, hypochlorous acid (HClO) has been widely adopted as a safe and effective sanitizer and disinfectant in agriculture, medicine, and the food industry [23,24]. Due to its high oxidation potential, HClO exhibits broad-spectrum antimicrobial activity against various bacteria and fungi [25]. Low concentrations of HClO have recently been applied to reduce postharvest pathogens, showing effectiveness against fungi such as *Colletotrichum*, *Botrytis*, *Monilinia*, *Penicillium*, *Fusarium*, and *Rhizopus* [13]. However, the effectiveness of HClO against *Mucor* in litchis remains unexplored. Combining hydrocooling with HClO may effectively inhibit disease and keep fruit quality during storage.

This study aimed to extend the storage ability of early-maturing 'Yu Her Pao' litchis through the application of hydrocooling and temperature management. The objectives were to (1) determine the critical concentration of HClO and the optimal temperature for inhibiting *M. lusitanicus*; (2) assess the ability of HClO and temperature treatments to maintain fruit quality; and (3) evaluate the effects of hydrocooling combined with HClO and delayed precooling on disease incidence and fruit quality during storage and shelf life.

2. Materials and Methods

2.1. Identification and Purification of Plant Pathogenic Fungi

This fungus was initially isolated from litchi fruit displaying symptoms of spoilage after storing at cold temperatures (from 5 to 10 °C) for a period in 2023 (Cishan District, Kaohsiung City, Taiwan) (Figure S1). Diseased pericarp tissues were cut into 0.5 cm pieces, placed on water agar, and cultured at 25 °C. After three subcultures on synthetic Mucor agar (40 g dextrose, 2 g asparagine, 0.5 g KH_2PO_4, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.5 g thiamine chloride, and 15 g agar in 1 L of deionized water) [26], a single mycelium was isolated and maintained at 25 °C for 2 days. DNA sequencing was used to identify the isolate, focusing on the internal transcribed spacer (ITS) region, which was amplified and sequenced. The ITS sequences were compared using a Mega BLAST search on the National Center for Biotechnology Information (NCBI) database, confirming the fungus as *Mucor lusitanicus*.

2.2. Effects of Temperature on Mycelial Growth

To assess how temperature influences mycelial growth, a 5 mm agar plug containing the mycelium was placed upside down at the center of a new synthetic Mucor agar (SMA) plate. The plates were incubated in darkness at various temperatures (1, 5, 10, 15, 20, 25, 30, and 35 °C). The radial growth of the mycelia was measured at 24 h intervals, with each condition replicated three times.

2.3. Effects of HClO on Mycelial Growth and Sporangiospore Germination

After *M. lusitanicus* had been cultured on SMA for 5 days, 5 mm-diameter plugs of mycelium-containing agar were transferred to new SMA plates with various concentrations of HClO (0, 5, 10, 20, 40, and 80 mg L^{-1}). The plates were incubated at 25 °C for 7 days, and mycelial growth was measured every 24 h. For spore germination analysis, plugs were rinsed and mixed with different HClO concentrations (0–80 mg L^{-1}) for 20 h at 25 °C. Three 2 μL suspensions were placed on a glass slide and observed under a light microscope at 40× magnification. The number of zoospores was estimated using a mechanical counter, with three replications of each treatment. The germination rate (%) was calculated by dividing the number of germinated sporangiospores by the total number of sporangiospores. The inhibition rate (%) was calculated by comparing the ungerminated sporangiospores in the treatment and total sporangiospores. Each treatment was replicated three times.

2.4. Suppression of M. lusitanicus by Both Low Temperature and HClO

Litchi pericarps were wounded with a sterile needle, and a sporangiospore suspension (10^6 mL^{-1}) was applied to the wounds. After inoculation for 1 h, the treated fruit was then immersed in tap water, 1 °C water, 40 mg L^{-1} HClO, or 1 °C water plus 40 mg L^{-1} HClO for 30 min, with inoculated fruit as the control group. The fruit was incubated in darkness at 25 °C for 7 days, with disease symptoms recorded daily. Each treatment included three replicates, and each fruit was considered a separate replication.

2.5. Measurement of Respiration Rate and Ethylene Production

Litchi fruit was placed in sealed chambers set at temperatures of 1, 5, 10, 15, 20, 25, 30, 35, and 40 °C for 2 h, with each temperature condition replicated three times, each replication containing 15–20 fruit. A 1 mL gas sample was collected from each chamber with a plastic syringe and analyzed for CO_2 concentration by an infrared CO_2 analyzer (Rosemount X-STREAM Enhanced XEGK, Emerson, MO, USA). For ethylene production, a 1 mL gas sample was taken from the same chamber and analyzed using a gas chromatography detector (GC-8A, Shimadzu Scientific Instruments Inc., Kyoto, Japan).

2.6. Delayed Hydrocooling of Litchi Fruit

'Yu Her Pao' litchi fruit at commercial maturity (1/3 to 1/2 rosy red color) were harvested and watered for 1 min to maintain moisture. They were transported to the lab within 1.5 h. Then, they were hydrocooled for 30 min, with precooling applied either immediately (0 h delay groups), after an 8 h delay (8 h delay groups), or after a 12 h delay (12 h delay groups). Different delay times (8 or 12 h) were assessed for the impact of delayed precooling on fruit quality. For hydrocooling, the fruit was immersed in 1 °C water with 40 mg L^{-1} HClO. Each batch of 110 fruit (approximately 3 kg) was soaked in 15 L of 1 °C water for 30 min. After hydrocooling to 1–2 °C, twenty fruit were placed in a polyethylene box (18.5 × 12 × 9 cm) at 90–95% RH, covered with 0.03 mm plastic bags, and stored in a walk-in cold room at 5 °C for three weeks.

2.7. Quality of Litchi Fruit

A portion of the fruit at 5 °C was taken out for analysis of quality and weight loss, whereas browning levels and decay incidence were recorded weekly. For the weight loss, each treatment contained 5 boxes as 5 replications. Before storage, every box was weighed (W1) using a digital balance (PB3002, Mettler, Columbus, OH, USA). After storage, every box was weighed again (W2), and the following formula was used to calculate the weight loss (%) = [(W1 − W2)/W1] × 100%. Disease incidence was calculated according to the percentage of lichis with microbial lesions or disease symptoms in 5 individual boxes. Disease incidence (%) = (number of litchi fruit with microbial lesions or disease symptoms/total number of litchi fruit) × 100%. At each sampling, the 5 litchis from different boxes (20 fruit in a box were considered 1 replication, and each treatment had 5 replications) were examined. The browning index was assessed by the total browned area on each fruit pericarp. The browning levels were defined as follows: 1 = no symptoms; 2 = 1–5%; 3 = 6–10%; 4 = 11–20%; 5 > 20% (Figure S2). The browning index = the number of lichi fruit with browning levels/total number of litchi fruit. When the browning index exceeded 5, the fruit was considered poor quality without commercial value. The total soluble solids (TSSs) of the fruit juice were extracted by hand and determined by a digital refractometer (PAL–1, Atago Co., Ltd., Tokyo, Japan). A total of 25 litchi fruit were randomly sampled from different precooling treatments to measure quality traits.

2.8. Statistical Analysis

Data were analyzed using CoStat 6 software (CoHort Software, Pacific Grove, CA, USA) and subjected to analysis of variance. Mean values within treatments were compared using the least significant difference (LSD) test at a 5% significance level ($p < 0.05$).

3. Results

3.1. Mycelial Growth of M. lusitanicus at Different Temperatures

The optimal temperature for the growth of *M. lusitanicus* was 25 °C, where radial mycelial growth exceeded 9 cm over 6 days (Table 1). Growth persisted up to 35 °C, though at a slower rate. Below 10 °C, mycelial growth slowed significantly and was completely inhibited after 1 day at 10 °C. Notably, at 5 °C, mycelial growth ceased after 2 days, with complete suppression at 1 °C for 7 days (Table 1). Therefore, the critical temperature for inhibiting the growth of *M. lusitanicus* was between 5 and 1 °C. These two temperatures (1 and 5 °C) were chosen for precooling and storage, respectively, because the former can remove the heat and quickly prevent pathogen growth, and the latter would be used for transportation and storage of litchi fruit in retail stores to avoid chilling injury.

Table 1. Effect of different temperatures on the mycelial growth of *Mucor lusitanicus* for 7 days.

Temp (°C)	Colony Diameter (cm)						
	1 Day	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
1	0.00 ± 0.00 ^{f}	0.00 ± 0.00 ^{g}	0.00 ± 0.00 ^{h}	0.00 ± 0.00 ^{h}	0.00 ± 0.00 ^{h}	0.00 ± 0.00 ^{f}	0.00 ± 0.00 ^{f}
5	0.00 ± 0.00 ^{f}	0.00 ± 0.00 ^{g}	0.25 ± 0.00 ^{g}	0.50 ± 0.00 ^{g}	0.83 ± 0.02 ^{g}	1.13 ± 0.03 ^{e}	1.72 ± 0.02 ^{e}
10	0.00 ± 0.00 ^{f}	0.28 ± 0.02 ^{f}	0.97 ± 0.02 ^{f}	1.33 ± 0.02 ^{f}	1.87 ± 0.02 ^{f}	2.33 ± 0.03 ^{d}	2.70 ± 0.03 ^{d}
15	0.65 ± 0.00 ^{e}	1.27 ± 0.03 ^{e}	1.97 ± 0.02 ^{e}	2.55 ± 0.03 ^{e}	3.18 ± 0.04 ^{e}	3.85 ± 0.00 ^{c}	4.57 ± 0.03 ^{c}
20	1.18 ± 0.06 ^{c}	2.15 ± 0.07 ^{c}	3.03 ± 0.04 ^{c}	3.95 ± 0.00 ^{d}	5.20 ± 0.03 ^{d}	6.63 ± 0.02 ^{b}	8.05 ± 0.00 ^{a}
25	1.95 ± 0.03 ^{a}	3.17 ± 0.07 ^{a}	5.23 ± 0.04 ^{a}	6.85 ± 0.00 ^{a}	8.58 ± 0.02 ^{a}	– ^{z}	–
30	1.68 ± 0.03 ^{b}	3.02 ± 0.03 ^{b}	4.98 ± 0.03 ^{b}	6.47 ± 0.03 ^{b}	8.22 ± 0.02 ^{b}	8.60 ± 0.00 ^{a}	–
35	0.87 ± 0.02 ^{d}	1.62 ± 0.02 ^{d}	2.88 ± 0.02 ^{d}	4.07 ± 0.02 ^{c}	5.35 ± 0.03 ^{c}	6.65 ± 0.03 ^{b}	7.85 ± 0.00 ^{b}

Means (n = 3) within columns followed by the same letter are not significantly different by the least significant difference (LSD) test at $p < 0.05$. ^{z} Greater than 9 cm.

3.2. Effect of HClO on Mycelial Growth and Spore Germination

HClO concentrations between 40 and 80 mg L^{-1} effectively inhibited mycelial growth for 7 days, while lower concentrations (5–20 mg L^{-1}) suppressed growth for only 2 days (Table 2). Sporangiospore germination was partially inhibited at 20 mg L^{-1} HClO and completely suppressed at concentrations of 40–80 mg L^{-1} (Table 3). Thus, HClO at 40 mg L^{-1} was identified as the critical concentration needed to entirely suppress both mycelial growth and spore germination of *M. lusitanicus*.

Table 2. Effect of 0–80 mg L^{-1} hypochlorous acid (HClO) on the mycelial growth of *Mucor lusitanicus* at 25 °C for 7 days.

HClO (mg L^{-1})	Colony Diameter (cm)						
	1 Day	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
0	1.90 ± 0.03 ^{a}	3.12 ± 0.02 ^{a}	5.13 ± 0.02 ^{a}	6.83 ± 0.02 ^{a}	8.60 ± 0.00 ^{a}	- ^{z}	-
5	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	2.32 ± 0.02 ^{b}	3.53 ± 0.04 ^{b}	4.48 ± 0.02 ^{b}	5.62 ± 0.07 ^{a}	6.65 ± 0.08 ^{a}
10	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	2.00 ± 0.03 ^{c}	2.97 ± 0.03 ^{c}	4.07 ± 0.04 ^{c}	5.20 ± 0.05 ^{b}	6.30 ± 0.08 ^{b}
20	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	1.73 ± 0.02 ^{d}	2.75 ± 0.03 ^{d}	3.77 ± 0.02 ^{d}	4.65 ± 0.08 ^{c}	5.67 ± 0.07 ^{c}
40	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{e}	0.00 ± 0.00 ^{e}	0.00 ± 0.00 ^{e}	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}
80	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{e}	0.00 ± 0.00 ^{e}	0.00 ± 0.00 ^{e}	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}

Means (n = 3) within columns followed by the same letter are not significantly different by the least significant difference (LSD) test at $p < 0.05$. ^{z} Greater than 9 cm.

Table 3. Germination and inhibition rates of sporangiospore at 25 °C under various concentrations of hypochlorous acid (HClO).

HClO (mg L^{-1})	Germination Rate (%)	Inhibition Rate (%)
0	91.33 ± 1.69 ^{a}	0.00
5	68.67 ± 1.61 ^{b}	24.81
10	42.33 ± 0.95 ^{c}	53.29
20	14.67 ± 0.99 ^{d}	83.94
40	0.00 ± 0.00 ^{e}	100.00
80	0.00 ± 0.00 ^{e}	100.00

Means (n = 3) within columns followed by the same letter are not significantly different by the least significant difference (LSD) test at $p < 0.05$.

3.3. Suppression of M. lusitanicus by Low Temperature and HClO In Vivo

In vivo assays on wound-inoculated litchi fruit showed significant differences in mycelial growth between treated and control groups (Table 4). In control fruit, pathogenic symptoms were evident after 2 days, whereas no symptoms were observed in fruit treated

with either 1 °C cold water or 40 mg L^{-1} HClO. However, disease symptoms at 25 °C appeared in fruit treated with either 1 °C water or HClO alone after 4 days or 7 days, respectively (Table 4). The combined treatment of 1 °C water and 40 mg L^{-1} HClO effectively prevented the growth of *M. lusitanicus* in inoculated litchis for up to 7 days.

Table 4. Effects of different treatments on lesion diameters in inoculated 'Yu Her Pao' litchis stored at 25 °C for 7 days.

Treatment	Colony Diameter (cm)						
	1 Day	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
Control	0.00 ± 0.00 [a]	0.26 ± 0.03 [a]	0.66 ± 0.00 [a]	1.32 ± 0.02 [a]	1.85 ± 0.00 [a]	2.31 ± 0.02 [a]	2.65 ± 0.03 [a]
Tap water	0.00 ± 0.00 [a]	0.00 ± 0.00 [b]	0.29 ± 0.01 [b]	0.58 ± 0.02 [b]	0.82 ± 0.02 [b]	1.23 ± 0.03 [b]	1.50 ± 0.03 [b]
1 °C water	0.00 ± 0.00 [a]	0.00 ± 0.00 [b]	0.00 ± 0.00 [c]	0.25 ± 0.00 [c]	0.40 ± 0.03 [c]	0.73 ± 0.03 [c]	0.75 ± 0.03 [c]
HClO [z]	0.00 ± 0.00 [a]	0.00 ± 0.00 [b]	0.00 ± 0.00 [c]	0.00 ± 0.00 [d]	0.00 ± 0.00 [d]	0.00 ± 0.00 [d]	0.30 ± 0.04 [d]
1 °C water + HClO [z]	0.00 ± 0.00 [a]	0.00 ± 0.00 [b]	0.00 ± 0.00 [c]	0.00 ± 0.00 [d]	0.00 ± 0.00 [d]	0.00 ± 0.00 [d]	0.00 ± 0.00 [e]

[z] 40 mg L^{-1} hypochlorous acid (HClO). Means (n = 3) within columns followed by the same letter are not significantly different by the least significant difference (LSD) test at $p < 0.05$.

3.4. Respiration Rate and Ethylene Production at Different Temperatures

The respiration rate and ethylene production of 'Yu Her Pao' litchis increased with temperature (Table 5). The highest respiration rate occurred at 40 °C, nearly 30 times higher than the rates observed between 1 °C and 10 °C, where respiration rates showed no significant differences. Ethylene production exhibited a similar trend, with concentrations remaining undetectable between 1 °C and 15 °C. At 20 °C, ethylene production began to increase and reached 2.34 μL C_2H_4 Kg^{-1} H^{-1} at 40 °C (Table 5). Based on the stable and low respiration and ethylene production rates observed below 10 °C, this is the recommended temperature for the end of precooling.

Table 5. Respiration rates and ethylene production of 'Yu Her Pao' litchis at different temperatures.

Temp (°C)	Respiration Rate (mL CO_2 Kg^{-1} H^{-1})	Ethylene Production (μL C_2H_4 Kg^{-1} H^{-1})
1	0.65 ± 0.08 [h]	0.00 ± 0.00 [d]
5	1.13 ± 0.20 [h]	0.00 ± 0.00 [d]
10	1.51 ± 0.14 [h]	0.00 ± 0.00 [d]
15	4.56 ± 0.25 [g]	0.00 ± 0.00 [d]
20	11.42 ± 0.47 [f]	0.82 ± 0.08 [b,c]
25	16.25 ± 0.94 [e]	0.52 ± 0.16 [c,d]
30	18.49 ± 1.22 [d]	1.24 ± 0.32 [b]
35	24.04 ± 0.84 [c]	1.39 ± 0.27 [b]
40	42.48 ± 0.74 [a]	2.34 ± 0.21 [a]

Means (n = 3) within columns followed by the same letter are not significantly different by the least significant difference (LSD) test at $p < 0.05$.

3.5. Effects of Delayed Precooling on Disease Incidence

There were no significant differences in disease symptoms in any treated litchi fruit after 2-week storage at 5 °C (Figure 1). After 21 days at 5 °C, there were no significant differences in disease incidence between fruit subjected to immediate hydrocooling and those with an 8 h delay, but a 12 h delay resulted in significantly higher disease rates. After rewarming at 26 °C for 1 day, the disease incidence in fruit with a 12 h hydrocooling delay was almost double that of litchi fruit without precooling delay (Figure 1).

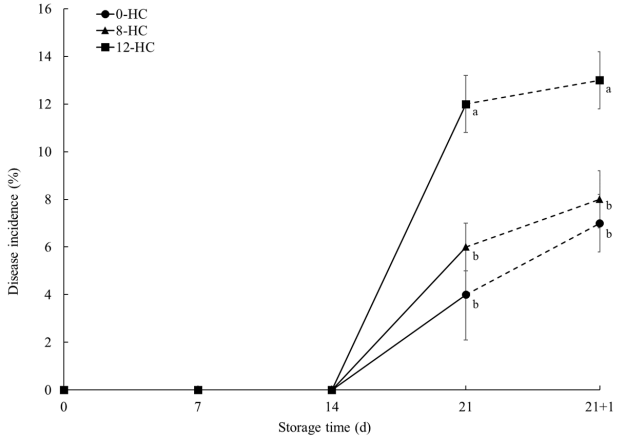


Figure 1. Effect of precooling delay on disease incidence in 'Yu Her Pao' litchis stored at 5 °C for 21 days, followed by 26 °C for 1 day (dashed line). Means (n = 5) with the same letter within each storage time are not significantly different by the least significant difference (LSD) test at $p < 0.05$. Error bars represent the standard error of the mean. Treatments comprised hydrocooling with 40 mg L^{-1} hypochlorous acid delayed by 0, 8, and 12 h (denoted as 0-HC, 8-HC, and 12-HC, respectively).

3.6. Effects of Delayed Hydrocooling on Browning and Quality Traits

The browning index increased with both storage duration and delayed precooling. The highest browning index was observed in fruit with a 12 h hydrocooling delay for 14 and 21 days of storage at 5 °C, and this remained the highest even after 1 day at 26 °C (Figure 2). Although immediately hydrocooled litchi fruit showed the lowest browning index across all treatments during 14 days of storage at 5 °C, similar values were observed in fruit with an 8 h hydrocooling delay after 21 days at 5 °C, followed by 1 day at 26 °C (Figures 2 and 3). For optimal quality preservation over extended storage, hydrocooling within 8 h of harvest is recommended, as it leads to lower browning indices during long-term storage. Wight loss increased with storage time, but no significant differences were detected among treatments during 3 weeks of low-temperature storage (Table 6). However, the litchi fruit precooled without delay showed relatively lower weight loss than that with a 12 h precooling delay after 21 days at 5 °C and 1 day at 26 °C (Table 6). Although total soluble solids (TSS) decreased slightly in all groups as storage time increased, they remained above 19% in all treatments, even after 1 day of shelf life at 26 °C (Table 7).

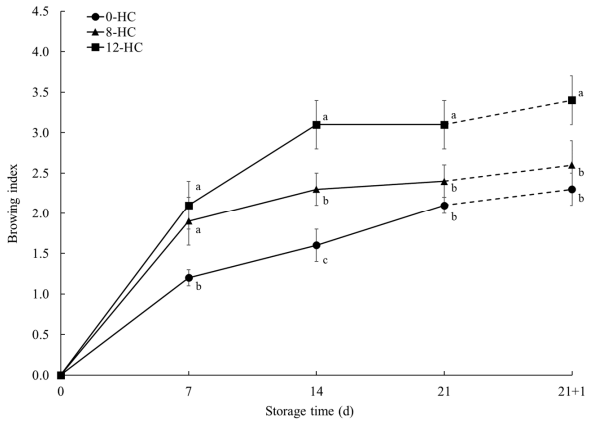


Figure 2. Effect of precooling delay on the browning index in 'Yu Her Pao' litchis stored at 5 °C for 21 days followed 26 °C for 1 day (dashed line). Means (n = 5) with the same letter within each storage time are not significantly different by the least significant difference (LSD) test at $p < 0.05$. Error bars represent the standard error of the mean. Treatments comprised hydrocooling with 40 mg L^{-1} hypochlorous acid delayed by 0, 8, or 12 h (denoted as 0-HC, 8-HC, or 12-HC, respectively).

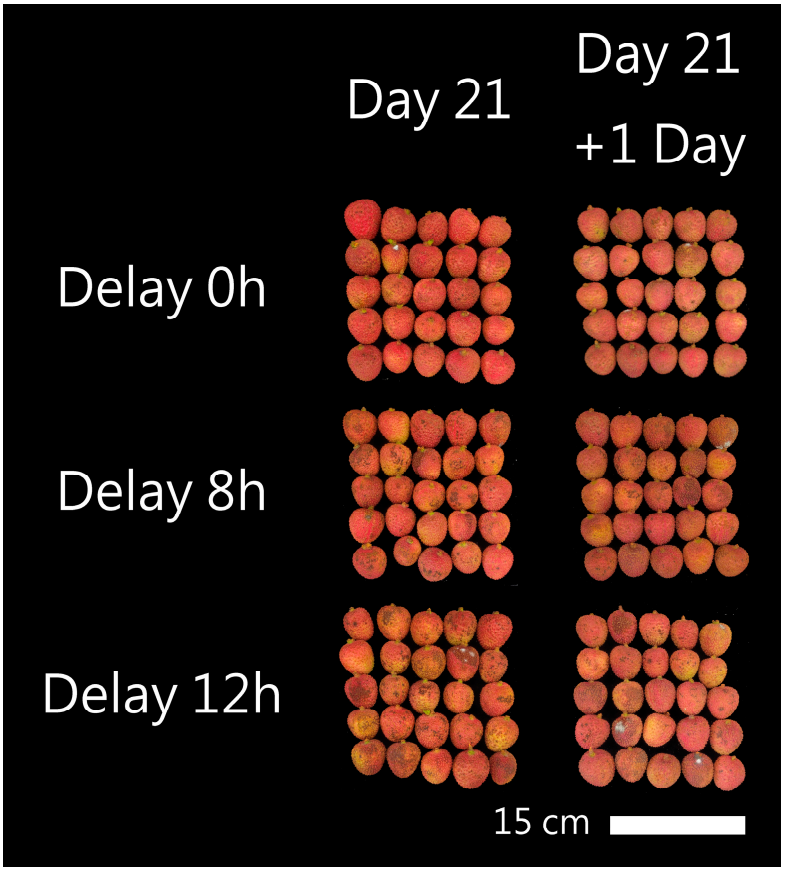


Figure 3. Effects of hydrocooling with 40 mg L^{-1} HClO delay on the appearance of 'Yu Her Pao' litchis stored at 5 °C for 21 days followed by 26 °C for 1 day.

Table 6. Weight loss (%) of 'Yu Her Pao' litchis with delayed hydrocooling with 40 mg L^{-1} HClO after storage for 21 days at 5 °C and rewarming 1 day at 26 °C.

Delay Time (h)	Storage Time (Days)			
	7	14	21	21 + 1
0	0.42 ± 0.02 [a]	0.81 ± 0.02 [a]	1.23 ± 0.02 [a]	1.43 ± 0.02 [b]
8	0.50 ± 0.11 [a]	0.93 ± 0.11 [a]	1.38 ± 0.11 [a]	1.60 ± 0.11 [a,b]
12	0.49 ± 0.01 [a]	1.08 ± 0.14 [a]	1.53 ± 0.20 [a]	1.82 ± 0.17 [a]

Means (n = 5) within columns followed by the same letter are not significantly different by the least significant difference (LSD) test at $p < 0.05$.

Table 7. Total soluble solids (%) of 'Yu Her Pao' litchis with delayed precooling after storage for 21 days at 5 °C and rewarming 1 day at 26 °C.

Delay Time (h)	Storage Time (Days)				
	0	7	14	21	21 + 1
0	20.24 ± 0.19 [a]	19.89 ± 0.14 [a]	19.89 ± 0.09 [a]	19.26 ± 0.16 [a]	19.21 ± 0.15 [a]
8	20.13 ± 0.12 [a]	19.88 ± 0.09 [a]	19.53 ± 0.12 [b]	19.25 ± 0.16 [a]	19.22 ± 0.13 [a]
12	19.89 ± 0.19 [a]	19.83 ± 0.14 [a]	19.63 ± 0.10 [a,b]	19.03 ± 0.18 [a]	19.08 ± 0.13 [a]

Means (n = 5) within columns followed by the same letter are not significantly different by the least significant difference (LSD) test at $p < 0.05$.

4. Discussion

This disease was first observed in litchi fruit from farmers in Taiwan that had been stored at cold temperatures (from 5 to 10 °C) for a period in 2023. The decayed litchi fruit was collected, and the pathogen was identified as *M. lusitanicus* from 2023 to 2024.

Although *Mucor* spp. were isolated from 17 litchi packinghouses, pathogenicity was not confirmed [18]. Pathogenicity tests showed that *M. lusitanicus* was able to cause decay on wound-inoculated litchi fruit (Table 4). After storage, the mucor rot has become the dominant postharvest disease in mandarin fruit in California, where no official chemical treatments are currently recommended for cultivation [19]. Similarly, it is highly likely that commonly used fungicides for litchi production target major diseases in fields, such as litchi sour rot, anthracnose, downy blight, and fruit rot, which may render these chemicals ineffective against decay caused by *Mucor* spp. Further research is needed to investigate the infection pathways of litchi fruits from farms to retail stores in Taiwan and to determine whether *Mucor* spp. are the dominant pathogens during long-term cold storage. The optimal growth temperature for *M. lusitanicus* was identified as 25 °C, and complete growth suppression occurred at 1 °C, with partial inhibition at 5 °C (Table 1). This finding could be applied to control the disease and evaluate the effect of delayed precooling on the litchi fruit. The seven of nine *Mucor* spp. isolates could not grow at 0 °C, but all isolates exhibited slower growth at 5 °C than at 25 °C [19]. However, a temperature of 5 °C was chosen for litchis to avoid chilling injury during long-term storage. In vitro experiments, the growth of *M. lusitanicus* was completely suppressed at 40 mg L^{-1} HClO (Table 2). In vivo, a combination of 1 °C water and 40 mg L^{-1} HClO fully inhibited the pathogen growth in inoculated litchis, whereas either treatment alone only partially suppressed it (Table 4). This result suggested that integrating hydrocooling with HClO into postharvest handling could extend storage ability and decrease microbial populations, particularly *M. lusitanicus*. Hypochlorous acid (HClO) is widely utilized in agriculture, medicine, and the food industry as a safe and effective disinfectant [23,24]. For example, HClO treatment has been shown to reduce microbial contamination and extend the shelf life of tomatoes compared to untreated controls [27].

During summer, harvested litchis are exposed to high temperatures (around 30 °C), which accelerates pathogen growth and increases CO_2 and C_2H_4 production (Table 5). These conditions shorten the storage ability of the fruit due to disease incidence, browning, and fast metabolism. Therefore, precooling is crucial to reduce respiration rates, delay senescence, and improve the appearance of the fruit during postharvest handling. Since the mycelial growth of *M. lusitanicus* thrives optimally around 25 °C (Table 1), it is critical to implement effective strategies to inhibit its expansion under postharvest conditions. Precooling below 10 °C was optimal, as it maintained low respiration and ethylene production rates (Table 5). Both hydrocooling and forced-air cooling have been applied to maintain the color and quality of litchis [4,10,28]. While hydrocooling effectively removes field heat rapidly, it carries a risk of postharvest decay [29]. Some packinghouses are hesitant to use hydrocooling due to concerns about decay and microbial contamination. Previous studies have shown that hydrocooled litchis exhibit higher decay incidence during storage at 5 °C [4]. However, adding HClO to the hydrocooling system significantly reduced decay incidence in our study, consistent with findings in tomato storage [27].

In addition to disease prevention, hydrocooling within 8 h of harvest significantly improved the appearance of the fruit by reducing browning during 21 days' storage and 1 day of shelf life at 26 °C (Figures 2 and 3). Browning in litchis resulted in a loss of market value, and several studies have indicated that desiccation leads to membrane degradation, allowing phenolic compounds and anthocyanins to leak from vacuoles and be catalyzed by PPO [7,8]. Precooling litchis prevents the rapid decline of anthocyanins to maintain fruit quality [10]. Hydrocooling maintains high humidity, which helps retain water content in the pericarp, quickly removes heat, and keeps cell membranes intact, which in turn reduces PPO activity in the cytosol [4,10].

Although combining hydrocooling with HClO extends storage ability, implementing this approach can be challenging when orchards are distant from packinghouses. Therefore, this study also investigated the impact of delayed precooling on disease incidence and fruit quality. Our results indicated that earlier hydrocooling with HClO reduced disease symptoms and browning. After 21-day storage at 5 °C, fruit subjected to an 8 h delay in hydrocooling exhibited similar disease incidence rates as those hydrocooled immediately. In contrast, a 12 h delay in precooling resulted in higher disease incidence than those with a 0 or 8 h delay in precooling (Figures 1–3). These findings suggest that hydrocooling should be performed within 8 h after harvest to minimize disease risk in litchis. This recommendation aligns with studies on Irwin mangoes, where precooling within 3–6 h mitigated anthracnose, while longer delays led to disease symptoms during storage and over-ripening during shelf life [30]. In our study, hydrocooling with 40 mg L^{-1} HClO within 8 h prevented disease and preserved fruit color during 21 days of storage and subsequent shelf life.

5. Conclusions

To prevent *M. lusitanicus*, critical HClO concentrations and optimal temperatures were identified and integrated into the hydrocooling stage of the postharvest process. Taking into account the operational capacity and workflow of packinghouses, determining the maximum allowable delay for precooling while preserving product value may present challenges in practical applications. Applying hydrocooling within 8 h after harvest offers significant economic benefits by extending the storage life. Hydrocooling with HClO, appropriate packaging, and cold chain management could effectively prolong storage by reducing respiration rates and maintaining a favorable appearance, including delaying browning and disease symptoms. These postharvest treatments maintain the internal and external quality of litchis, making them suitable for local markets and export to countries requiring sulfur-free agricultural products. Since precooling is already a standard practice in some packinghouses, integrating hydrocooling with HClO sanitation can be incorporated into existing postharvest handling systems.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae11010083/s1. Figure S1: Symptoms of mucor rot caused by *Mucor lusitanicus* on litchi fruit from packinghouses. Figure S2: The browning levels were defined as follows: 1 = no symptoms; 2 = 1–5%; 3 = 6–10%; 4 = 11–20%; 5 > 20%. Figure S3: Effects of hydrocooling with 40 mg L^{-1} HClO delay on the appearance of 'Yu Her Pao' litchis stored at 5 °C for 21 days followed by 26 °C for 1 day.

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