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Improve the Effectiveness of Inhibiting Pathogenic Fungus and Maintaining the Quality of Rambutan (*Nephelium lappaceum* L.) Post-Harvest by Indigenous Lactic Bacteria

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Abstract: The change in the quality of rambutan (*Nephelium lappaceum* L.) fruit after harvest is mainly dehydration. Rambutan fruit peel is often dark (brown) and fruit rot due to fungal disease. This study investigated the effects of *Lactobacillus* bacteria strains [*Lactobacillus plantarum* CC6 (CC6), *Lactobacillus fermentum* DC2 (DC2), *Lactobacillus fermentum* DGMC2 (DGMC2)] with/without supplement combinations [chitosan 0.03% (Chito), alginate 0.03% (SA) and carboxymethyl cellulose 0.5% (CMC)] on the quality of Java rambutan fruit during storage at 13 °C, and included a sample without treatment with bacteria and chemical considered as the control. The results showed that among 16 treatments on rambutan fruits immediately after harvesting, the four treatments that showed the best results were CC6-Chito, DC2-Chito, CC6-CMC and DC2–SA, and DC2-Chito was more effective than the others. However, all four of these treatments supported the preservation of rambutan fruit, reducing both weight loss and browning of the skin and stem, and kept the infection rate at the lowest level. The brightness and peel color values were highest for these treatments.

Keywords: rambutan; coating treatment; indigenous lactic bacteria; post-harvest qualities

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1. Introduction

Rambutan (*Nephelium lappaceum* L.) is a non-climacteric and one of the most important tropical commercial fruits in Southeast Asia, Australia, South America and Africa. In Vietnam, rambutan is widely grown in the southern provinces, with a total area of 26,000 hectares and an annual output reaching 340,000 tons, concentrated mainly in provinces such as Dong Nai, Ben Tre, Vinh Long, and Tien Giang [1]. Rambutans are harvested two times per year—from June to July and from November to January. Rambutans are ready to harvest when they have enough color (yellow or red) and are of suitable eating quality [2]. Growers often harvest rambutans based on their personal assessment of fruit color, or by counting the number of days since full bloom or fruit set. In addition, it is a fruit rich in sugars (glucose and sucrose), proteins, vitamins, minerals and antioxidants, and is highly appreciated for its fresh taste, pleasant aroma and exotic appearance [3,4]. Thanks to its attractive appearance, beautiful color, delicious taste and rich nutritional value [5], rambutan is becoming increasingly popular.

However, rambutan peels are susceptible to browning, rotting and damage during transportation, and can only be distributed 3 days after harvest. The rambutan storage time is very short, only approximately 2 to 3 days at room temperature, which makes it difficult to adapt for market consumption [6,7]. The fruit skin dehydrates, turns brown and wilts [8], accompanied by a rapid loss of fruit quality at ambient temperature. Post-harvest treatments can control browning, reduce aging, maintain color, quality and control

post-harvest diseases on rambutan, which includes salicylic acid combination and warm water treatment [9], hot water treatment [10], $CaCl_2$ dipping [11], ultraviolet radiation [12] and sodium nitroprusside soaking [13]. Consumers of nutritious food are becoming more conscious of the detrimental effects that rigorous conservation methods have on the biological and nutritional qualities of processed foods. Customers therefore demand products that are guaranteed as well as the complete preservation of sensory qualities, biological and nutritional contents. Food technologists and consumers can benefit greatly from a conservation strategy that tackles these issues [14,15]. Biopolymers are a promising resource for a sustainable planet in the future since they are abundant, renewable, and biocompatible materials. The inclusion of probiotics in biopolymeric edible films and coatings is difficult because these products need to protect microorganisms by offering microbiological stability while preserving probiotic viability; they also need to have an adequate barrier and mechanical properties; be non-toxic, safe, and compatible with food; and be inexpensive [16]. In addition, biological control is a environmentally friendly technique, which could have potential in maintaining fruit quality and limiting fungal diseases [14]. Methods of combining chitosan with various biological control agents (*Candida satoiana* or *Cryptococcus laurentii*) that have antagonistic effects on post-harvest pathogens have also been implemented [17–19]. The combination of *Candida saitoana* with 0.2% glycolchitosan was more effective in controlling gray and green mold on apples and green mold caused by *Penicillium digitatum* on oranges and lemons than using yeast or glycolchitosan alone [20]. Martínez-Castellanos et al. [5] also reported that using *Lactobacillus plantarum* alone or in combination with 2% chitosan preserved the quality characteristics of rambutan fruit. The aim of this study was to select appropriate treatment methods (single or combined) from the carriers (low-molecular-weight chitosan, sodium alginate and carboxymethyl cellulose) and biological agents (native lactic bacteria strains) to preserve rambutan fruit quality and extend the post-harvest storage time of the Java rambutan variety in Vietnam.

2. Materials and Methods

2.1. Materials

Rambutan (*Nephelium lappaceum* L. Java) fruits were harvested from local orchards (Cho Lach district, Ben Tre province, Vietnam) when they reached commercial maturity (red-orange fruit with green spintern tips) and we followed the method described by Ummarat and Seraypheap [15] in order to minimize water loss (weight loss) from the fruit after harvest.

The bacterial strains *L. plantarum* CC6, *L. fermentum* DC2 and *L. fermentum* DGMC2 were isolated, characterized and identified by using 16S rRNA gene sequence analysis, which was preserved at the Department of Microbiology—Postharvest Department—Southern Crop Research Institute [21]. Bacterial strains were inoculated in Man, Rogosa and Sharpe (MRS) broth and incubated at 30 °C for 24 h [21]. Subsequently, cells were centrifuged (959× *g*) at 5 °C for 15 min and the pellet was washed with 0.02 M phosphate buffer (pH 7.0) [21]. Then, the cell suspension was adjusted to 10^8 CFU/mL and stored at 10 °C for no longer than 24 h. It was then used for the coating formulation.

Fungal strains that cause disease on rambutan fruits after harvest, including Lasiodiplodia pseudotheobromae, Fusarium verticillioides, Phomopsis mali, Lasmenia sp., Gliocephalotrichum cylindrosporum, Pestalotiopsis virgatula and Pestalotiopsis clavispora, have been isolated and preserved at the Department of Microbiology—Postharvest Department—Southern Crop Research Institute [22].

Low-molecular-weight chitosan (187 kDa) has 15% acetylation [chitosan (20 g/L), Sigma-Aldrich, USA] dissolved in 0.1 M acetic acid)]. Sodium alginate and carboxymethyl cellulose (CMC), from Himedia (India), were used in this study.

Polyethylene bags (24 × 24 cm, 40 µm thick) and cardboard boxes (30 × 22.5 × 12.5 cm) were used to contain fruit after treatment and during storage.

2.2. Treatment with Single and Combined Bacteria

Experiments Design

The rambutan fruits were harvested by cutting the pedicel from the tree using sharp scissors and leaving the pedicel approximately 2 mm in size. Following a satisfactory harvest, the rambutan fruits were promptly conveyed to the laboratory in a temperature-regulated transport vehicle set at 25 °C. In order to conduct the experiment, rambutan fruits were chosen based on their uniform size and color, and were ensured to be free from any defects, damage, or bruising. Subsequently, the fruits were cleansed using distilled water and then dried for a duration of 5 min prior to being coated. Fifteen treatments along with a control sample were designed according to Table 1. Each treatment (using 20 fruits per treatment) was repeated three times. In each treatment, rambutan fruits were dipped into the prepared solution for 5 min. When enough time elapsed, the fruit was removed and dried at ambient temperature (25 ± 3 °C) for 1 h. Then, the fruits were put into perforated polyethylene (PE) bags (each PE bag containing 20 fruits). The bags containing the fruit were put into cardboard boxes, stored at a temperature of 13 ± 3 °C and RH 80–90% to observe the quality of the fruits during storage [15].

Table 1. Arrangements of experimental treatments.

No.	Experiments	Explain the Experiments
1	Chito	Chitosan 0.03%
2	SA	Sodium alginate 0.03%
3	CMC	Carboxymethyl cellulose 0.5%
4	CC6	*L. plantarum* CC6 (10^8 cfu/mL)
5	DC2	*L. fermentum* DC2 (10^8 cfu/mL)
6	DGMC2	*L. fermentum* DGMC2 (10^8 cfu/mL)
7	CC6-Chito	Chitosan 0.03% with *L. plantarum* CC6 (10^8 cfu/mL)
8	DC2-Chito	Chitosan 0.03% with *L. fermentum* DC2 (10^8 cfu/mL)
9	DGMC2-Chito	Chitosan 0.03% with *L. fermentum* DGMC2 (10^8 cfu/mL)
10	CC6-CMC	Carboxymethyl cellulose 0.5% with *L. fermentum* CC6 (10^8 cfu/mL)
11	DC2-CMC	Carboxymethyl cellulose 0.5% with *L. fermentum* DC2 (10^8 cfu/mL)
12	DGMC2-CMC	Carboxymethyl cellulose 0.5% with *L. fermentum* DGMC2 (10^8 cfu/mL)
13	CC6-SA	Alginate 0.03% with *L. fermentum* CC6 (10^8 cfu/mL)
14	DC2-SA	Alginate 0.03% with *L. fermentum* DC2 (10^8 cfu/mL)
15	DGMC2-SA	Alginate 0.03% with *L. fermentum* DGMC2 (10^8 cfu/mL)
16	Control	No bacterial treatment or chemical additives were used

2.3. Quality Measurements

To measure the quality of fruit, the evaluation was performed with 3 repetitions, and each treatment contained 15 fruits after storage periods of 5, 10 and 15 days. The color of the fruit peel of each treatment was measured using a CR-400 Chroma Meter (Minolta, Osaka, Japan) inside the box with controlled LED light conditions, expressed through L* values representing brightness, a* values representing redness (+) or greenness (−), and b* value represents yellowness (+) or Blueness (−). A white reference standard was used to calibrate the instrument. The lower the brightness value, the more severe the browning. The soft thorns of the fruit were removed before measuring. Three measurements were taken per sample at equally spaced points on the equatorial axis.

The browning index was determined using the method described by Zhang et al. [23] with some modifications. The brown color of rambutan fruit peel was assessed visually by assessing the extent of brown area on each fruit surface using the following scale: Grade 0 = no browning; Type 1 = 1–25% browning; Type 2 = browning 26–50%; Type 3 = browning 51–75%; Type 4 = 76–100% browning. The browning index is calculated as ∑(browning scale × number of corresponding fruits within each treatment)/(4 × total number of fruit) [23].

Disease incidence was calculated as the ratio of the number of fruits with disease spots to the total number of fruits (n = 20) [24].

Weight loss was measured using digital scales. Fruits were weighed at the beginning of the storage and at two-day intervals of analysis and expressed as the percentage of weight loss. The weight loss of fruit (% WL) was calculated with the following equation:

$$WL\ (\%) = [(W_i - W_t)/W_i] \times 100\%$$

where W_t is the weight (g) at each time and W_i is the initial weight (g) of each sample.

The juices were extracted to given measured volumes. The total soluble solids (TSS) content and the titratable acidity (TA) from rambutan juice were determined by a pocket Brix-acidity meter (ATAGO®) reported as % Brix and % TA, respectively. Vitamin C content (mg/100 g) was determined by the titration method [25].

2.4. Statistical Analysis

The experiment consisted of a completely randomized design with a factorial arrangement of variables and each treatment comprised three replicates for evaluating fruit quality. The means were analyzed using a one-way ANOVA of SAS version 9.0 (SAS Institute, Cary, NC, USA). Significant differences between the means were compared using the least significant difference (LSD) at $p \leq 0.05$.

3. Results and Discussion

3.1. Change in Fruit Peel Color

The changes in fruit peel color during storage stages are presented in Tables 2–4.

Table 2. Change in brightness (L*) of rambutan peel during storage at 13 °C.

Treatments	Storage Time (Days)			
	0	5	10	15
Chito	45.17 ^{ab}	46.21 ^{a}	36.61 ^{cde}	27.87 ^{cdef}
SA	45.22 ^{ab}	43.97 ^{ab}	36.13 ^{def}	26.09 ^{f}
CMC	45.57 ^{a}	44.04 ^{ab}	35.48 ^{def}	26.86 ^{ef}
CC6	44.88 ^{abc}	43.01 ^{abc}	37.31 ^{cd}	29.07 ^{bcde}
DC2	44.55 ^{abcd}	43.09 ^{ab}	36.72 ^{cde}	27.64 ^{def}
DGMC2	45.63 ^{a}	43.33 ^{abcd}	36.33 ^{cdef}	26.92 ^{f}
CC6-Chito	44.92 ^{abcd}	43.23 ^{abc}	38.85 ^{bc}	31.37 ^{abc}
DC2-Chito	44.47 ^{abcd}	42.11 ^{bc}	41.69 ^{a}	32.58 ^{ab}
DGMC2-Chito	44.58 ^{abcd}	42.72 ^{abc}	41.30 ^{ab}	31.04 ^{abcd}
CC6-CMC	43.30 ^{d}	41.47 ^{bc}	40.10 ^{ab}	32.92 ^{a}
DC2-CMC	44.41 ^{abcd}	43.48 ^{abc}	34.07 ^{f}	31.37 ^{abc}
DGMC2-CMC	47.74 ^{abc}	43.46 ^{ab}	33.97 ^{f}	31.60 ^{ab}
CC6-SA	45.51 ^{cd}	42.19 ^{bc}	35.31 ^{def}	30.87 ^{abcd}
DC2-SA	42.20 ^{abcd}	42.13 ^{bc}	33.70 ^{ef}	27.97 ^{cdef}
DGMC2-SA	43.92 ^{bcd}	43.16 ^{abc}	33.93 ^{ef}	27.48 ^{ef}
Control	44.36 ^{abcd}	39.45 ^{c}	36.84 ^{cde}	27.02 ^{ef}
CV (%)	1.94	3.84	2.6	3.59

Mean values with different superscripts in the same column differ significantly at $p \leq 0.05$. CV is the coefficient of variation. These treatments are described in Table 1.

The results showed that the various effects of the treatments on fruit brightness showed statistically significant differences compared to the control sample, with significant differences shown in the DC2-Chito, DGMC2-Chito and CC6-CMC at storage times of 5, 10 and 15 days (Table 2). Initial L* values of all index treatments ranged from 42.2 to 47.74. On the 5th day of storage, the brightness of the fruit peel did not changed much compared to the original (Table 2). On the 10th day of storage, the fruit peel brightness generally decreased in all treatments; however, treatments CC6-CMC, DC2-Chito, and DGMC2-Chito showed a smaller decrease in peel color—L* values of 40.10, 41.69, and 41.3, respectively (Table 2). These values were significantly higher than that of the control sample (L* = 36.84).

The highest value of L* was found for the DC2-Chito sample (Table 2). On the 15th day of storage, the brightness of the fruit peel continued to decrease, approximately 8 to 25% of the fruit's brightness compared to the original (Table 2). However, in the treatments CC6-Chito, DC2-Chito, CC6-CMC, DC2-CMC and DGN2-CMC, rambutan fruit brightness decreased less but there was still a statistically significant difference compared to the control sample (Table 2). The lactic acid bacteria combined with chitosan or CMC could have the highest potential to slow down the reduction in lightness of Java rambutan after 15 days of storage, which also similar with the result of Martínez-Castellanos et al. [5].

Table 3. Change in a* values of rambutan peel during storage at 13 °C.

Treatments	Storage Time (Days)			
	0	5	10	15
Chito	22.15 ^{b}	18.67 ^{abc}	19.21 ^{ab}	16.98 ^{ab}
SA	22.74 ^{ab}	20.24 ^{ab}	18.59 ^{abc}	17.58 ^{a}
CMC	22.66 ^{ab}	18.57 ^{abc}	17.86 ^{abc}	16.55 ^{ab}
CC6	22.44 ^{ab}	18.52 ^{abc}	17.16 ^{abc}	15.15 ^{abc}
DC2	22.03 ^{ab}	18.52 ^{abc}	18.13 ^{abc}	16.81 ^{ab}
DGMC2	26.38 ^{a}	18.15 ^{abc}	18.20 ^{abc}	17.60 ^{a}
CC6-Chito	23.87 ^{ab}	18.58 ^{abc}	19.82 ^{a}	15.51 ^{abc}
DC2-Chito	23.65 ^{ab}	17.06 ^{abc}	19.15 ^{ab}	15.53 ^{bc}
DGMC2-Chito	24.45 ^{ab}	17.27 ^{bc}	19.54 ^{a}	15.42 ^{abc}
CC6-CMC	23.58 ^{ab}	18.39 ^{abc}	16.44 ^{c}	13.73 ^{c}
DC2-CMC	24.19 ^{ab}	16.22 ^{c}	17.10 ^{bc}	13.38 ^{bc}
DGMC2-CMC	24.66 ^{ab}	16.53 ^{c}	17.49 ^{abc}	14.61 ^{bc}
CC6-SA	23.64 ^{ab}	18.22 ^{abc}	17.68 ^{abc}	15.94 ^{abc}
DC2-SA	23.41 ^{ab}	18.73 ^{abc}	19.31 ^{ab}	16.57 ^{ab}
DGMC2-SA	24.32 ^{ab}	18.05 ^{abc}	18.99 ^{abc}	16.49 ^{ab}
Control	23.37 ^{ab}	21.38 ^{a}	18.41 ^{abc}	17.58 ^{a}
CV (%)	7.65	3.63	3.01	2.65

Mean values with different superscripts in the same column differ significantly at $p \leq 0.05$. CV is the coefficient of variation. These treatments are described in Table 1.

Table 4. Change in b* values of rambutan peel during storage at 13 °C.

Treatments	Storage Time (Day)			
	0	5	10	15
Chito	28.67 ^{ab}	32.161 ^{b}	28.73 ^{a}	23.50 ^{abcde}
SA	31.11 ^{a}	31.27 ^{ab}	28.81 ^{a}	23.11 ^{bcde}
CMC	27.33 ^{b}	31.27 ^{ab}	28.20 ^{abc}	24.07 ^{abcd}
CC6	29.47 ^{a}	31.48 ^{ab}	28.45 ^{ab}	24.05 ^{abcd}
DC2	28.86 ^{ab}	32.06 ^{ab}	28.06 ^{abcde}	23.87 ^{abcd}
DGMC2	29.45 ^{a}	31.25 ^{ab}	28.39 ^{ab}	24.65 ^{ab}
CC6-Chito	28.61 ^{ab}	31.66 ^{ab}	27.81 ^{abcde}	24.44 ^{abc}
DC2-Chito	31.18 ^{a}	31.06 ^{ab}	27.68 ^{abcde}	21.90 ^{cde}
DGMC2-Chito	31.51 ^{a}	31.81 ^{ab}	27.51 ^{abcde}	20.98 ^{e}
CC6-CMC	30.65 ^{a}	30.72 ^{ab}	27.82 ^{abcde}	21.60 ^{de}
DC2-CMC	27.65 ^{ab}	31.67 ^{ab}	26.86 ^{bcde}	21.00 ^{e}
DGMC2-CMC	28.77 ^{ab}	31.14 ^{ab}	27.08 ^{abcde}	21.83 ^{de}
CC6-SA	33.46 ^{a}	30.84 ^{ab}	26.24 ^{e}	22.38 ^{bcde}
DC2-SA	31.25 ^{a}	31.79 ^{ab}	26.50 ^{cde}	22.84 ^{bcde}
DGMC2-SA	31.18 ^{a}	32.41 ^{a}	26.30 ^{de}	23.50 ^{abcde}
Control	28.50 ^{ab}	30.21 ^{b}	28.14 ^{abcd}	25.93 ^{a}
CV (%)	7.24	7.90	4.25	6.70

Mean values with different superscripts in the same column differ significantly at $p \leq 0.05$. CV is the coefficient of variation. These treatments are described in Table 1.

While the L* value indicates the darkness of the skin, the a* and b* values indicate the red and yellow color of the skin of the fruit [26]. Tables 3 and 4 showed that the values of a* and b* of rambutan changed significantly during storage. Before storage, the redness value (a*) was in the range of 22.15–26.38. After storage, on day 5, 10, and 15, the redness value (a*) reduced to with the range of 16.22–21.38, 16.44–19.54, and 13.73–17.58, respectively (Table 3). Similarly, the yellowness (b*) also slightly reduced during storage (Table 4). It was observed that the rambutan appeared dark pink, less reddish and yellowish, and also reduced from the acceptable score during over time. This is due to natural water evaporation—the fruit shrinks, causing the color of the fruit to change [27]. The peel gradually changed to brown, which could be further discussed in the next section. Therefore, it could be concluded that the treatments have a significant effect on the change in the values of L*, a*, and b*.

3.2. Degree of Browning

Enzymatic browning is a natural phenomenon that widely occurs in many fruits and vegetables. Fruit darken rapidly when exposed to air due to the formation of brown melanin from the oxidation of phenolic compounds. Polyphenol oxidase (PPO) is found in most fruits and vegetables and is responsible for enzymatic browning. In addition to PPO, the presence of peroxidase, a similar oxidative enzyme, can cause enzymatic browning of fruits and vegetables [28]. Enzymatic oxidation of phenolic compounds combined with the breakdown of anthocyanins causes browning. The acquired values of the browning index and the slight variations in the values of L* and chroma (C*) are compatible with the experimental data presented here, which suggest that phenolic oxidation leads to the generation of brown polymers, which is the primary cause of browning [29]. The degree of browning on rambutan fruit peel also shows the activity of these enzymes. Along with the change in peel color, the degree of browning of the peel in the treatments also showed a gradual increase with storage time on the 5th, 10th and 15th days of storage (Table 5).

Table 5. Degree of browning of rambutan fruit peel.

Treatments	Storage Time (Day)		
	5	10	15
Chito	0.6 ^{ef}	2.0 ^{gh}	2.5 ^{ijh}
SA	0.8 ^{cd}	2.5 ^{efd}	2.4 ^{ij}
CMC	0.7 ^{de}	2.7 ^{cde}	3.2 ^{cdef}
CC6	0.6 ^{ef}	2.8 ^{cd}	3.0 ^{defg}
DC2	0.9 ^{bc}	2.3 ^{efg}	3.2 ^{cdef}
DGMC2	0.8 ^{cd}	2.1 ^{fgh}	3.3 ^{cde}
CC6-Chito	0.5 ^{fg}	1.7 ^{h}	2.5 ^{ijh}
DC2-Chito	0.4 ^{g}	1.8 ^{h}	2.3 ^{j}
DGMC2-Chito	1.0 ^{b}	2.9 ^{cd}	2.8 ^{ghij}
CC6-CMC	0.5 ^{g}	2.6 ^{cde}	2.6 ^{ghij}
DC2-CMC	0.9 ^{bc}	2.3 ^{efg}	3.2 ^{cdef}
DGMC2-CMC	0.8 ^{cd}	2.9 ^{cd}	3.4 ^{bcd}
CC6-SA	0.9 ^{bc}	2.1 ^{fgh}	2.9 ^{efgh}
DC2-SA	0.8 ^{cd}	3.0 ^{c}	3.8 ^{b}
DGMC2-SA	1.0 ^{b}	3.5 ^{b}	3.6 ^{bc}
Control	1.3 ^{a}	4.8 ^{a}	5.0 ^{a}
CV%	12.35	10.76	7.98

Mean values with different superscripts in the same column differ significantly at $p \leq 0.05$. CV is the coefficient of variation. These treatments are described in Table 1.

Also shown in Table 5, on the 5th day of storage, the browning index ranges from 0.4 to 1.3, the highest value in the control treatment (1.3). At the 10th day of storage, the browning index was approximately 1.7 to 4.8, mostly increased in all treatments. The lowest values were observed for the treatments CC6-Chito and DC2-Chito (index 1.7 and

1.8, respectively) compared to the control sample with a browning index of 4.8, which could be seen in Table 5.

Table 5 also showed that on the 15th day of storage, the browning index increased the most in the control treatment (browning index was 5) and was lowest in the treatments Chito (2.5), SA (2.4), CC6-Chito (2.5), DC2-Chito (2.3), and CC6-CMC (2.6). The results clearly showed that the browning index was proportional to the L* value measured on the fruit peel. This can be explained by the activity of bacteria in the presence of appropriate supplements (chitosan 0.03%, CMC 0.5% and alginate 0.03%) that could produce antifungal compounds. However, different carriers as well as types of bacteria have an effect. Clearly, the lowest browning index after 15 days of storage was found for the DC2-Chito sample. Moreover, while the inhibitory effects varied depending on the specific bacterium and chitosan molecular weight, chitosan had stronger antibacterial activities than chitosan oligomers and significantly reduced the development of most examined bacteria in the study of No et al. [30]. Martínez-Castellanos et al. [29] also reported that *L. plantarum* showed the potential to maintain the color of lychee and a high content of polyphenol was found in the rind when the fruit was treated. Thus, it can be inferred that the presence of antioxidants and the formation of a protective layer contribute to the retention of moisture in the fruit peel and enhance the antifungal properties of bacteria.

3.3. Post-Harvest Fungal Infections of Rambutan

The disease rate on fruits (stored in cold storage at 13 °C) was observed on the 5th, 10th and 15th days of storage (Figure 1 and Table A1 in Appendix A). The treated rambutans fruits (according to the treatments) were packaged in PE bags and placed in a cardboard box to minimize mechanical impact (similar to the export conditions of Vietnamese rambutan). The results in Figure 1 and Table A1 in Appendix A showed that on the 5th day of storage, the control showed fungal attacks on fruit peels (1%). By the 10th day of storage, the treatments Chito, SA, CMC, DGMC2, DGMC2-CMC, DGMC2-SA and the control samples were infected with fungus, and the highest infection rate in the control sample (16%). After 15 days of storage, four treatments showed the best inhibitory effect on fungal pathogens, namely CC6-Chito, DC2-Chito, CC6-CMC and DC2-SA (Figure 2).

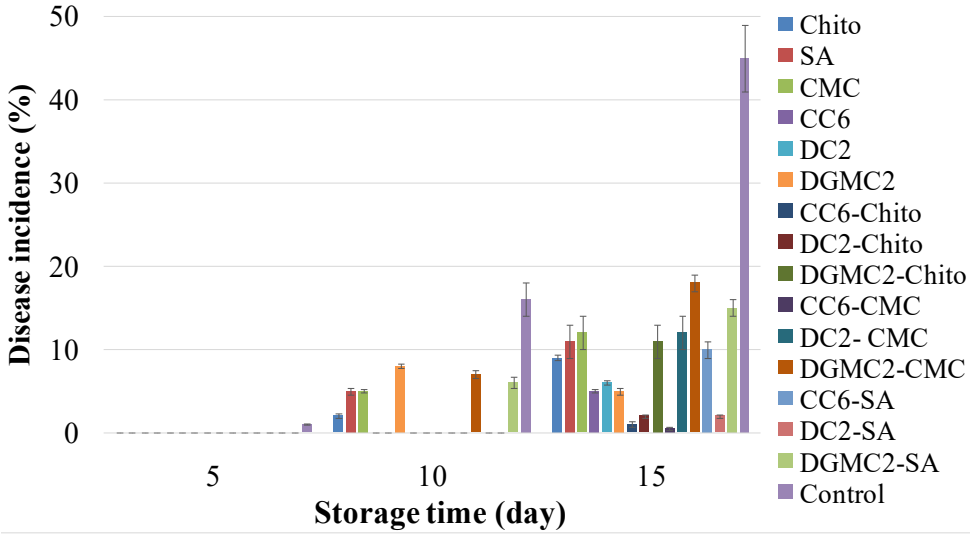

Figure 1. Post-harvest fungal infection incidence on rambutan at 5, 10 and 15 days of storage at 13 °C. The column shows the mean disease index of each treatment and the error bar shows the standard deviation. More data are shown in Table A1 (Appendix A). These treatments are described in Table 1.

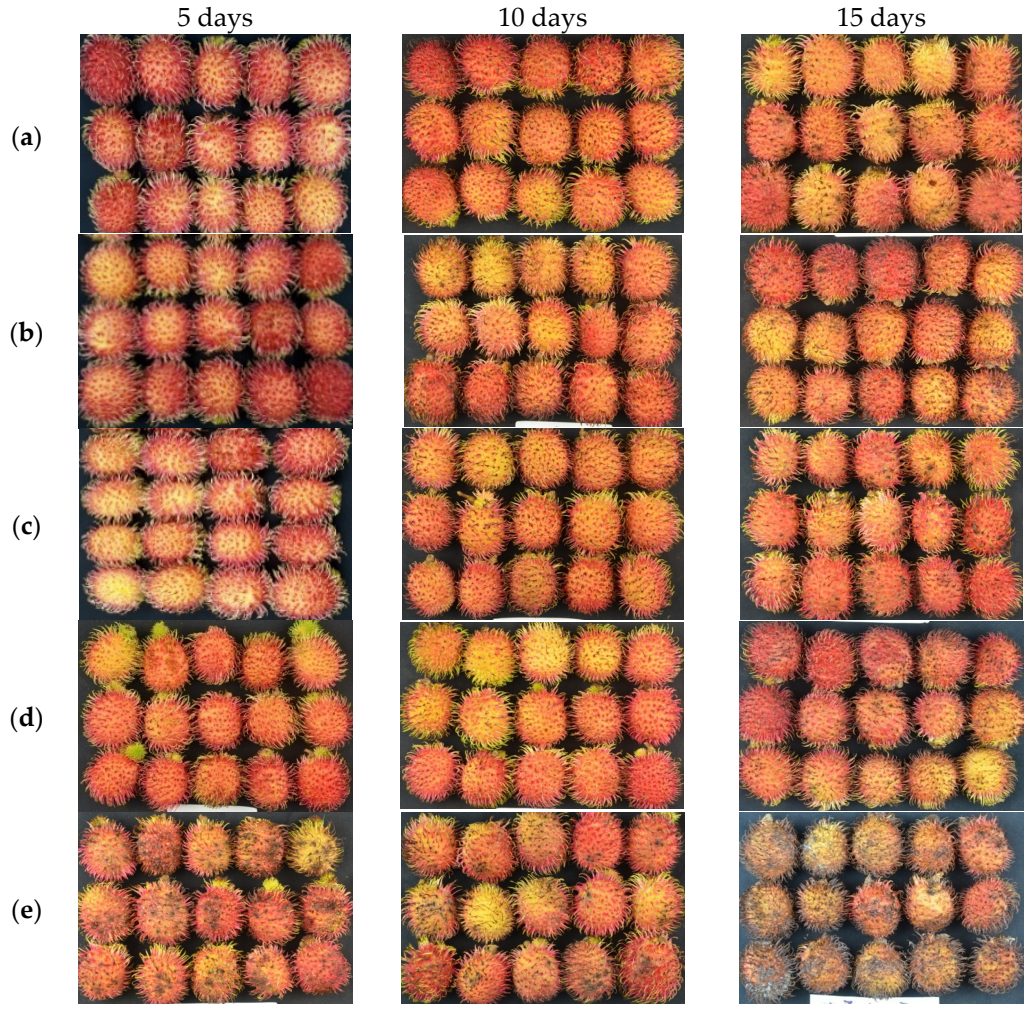

Figure 2. Post-harvest disease of rambutan fruit at 5, 10 and 15 days of storage. (**a**) CC6-Chito; (**b**) DC2-Chito; (**c**) CC6-CMC; (**d**) DC2-SA; and (**e**) Control sample. These four treatments are also presented in Table 1.

Fruit samples in these four treatments (CC6-Chito, DC2-Chito, CC6-CMC and DC2-SA) had a significant low fungal infection rate, which is less than 5% on the 15th day of storage. Fruits in other treatments showed a fungal infection rate of greater than or equal to 5% and the highest was found for the control sample (45%). When treating rambutans with a mixture of *L. plantarum* CC6 culture, 0.03% chitosan and 0.5% CMC, the pH of the rambutan juice was 3.28 and the TSS of the juice was 17.9° Brix, efficiently reducing weight loss, maintaining the fruit peel color, and lowering disease in post-harvest rambutans [31].

3.4. Weight Loss

Analytical results showed that fruit weight gradually decreased over storage time (Table 6). Moreover, it could also be seen from Table 6 that the treated samples lost less weight than the control sample during the storage period. After 5 days, the weight loss was recorded from 0.27 to 1.93% (Table 6). Fruit weight decreased the most in the control treatment and the least in the Chito treatment. On the 10th day of storage, the level of weight loss increased (from 1.1 to 3.37%), and the highest loss was seen in the control treatment (Table 6). Table 6 also showed that fruit treatment had less weight loss, and the lowest weight loss was seen in treatments SA, CMC, and Chito (less than 1.2%). On the 15th day of storage, fruit weight loss increased, ranging from 1.63 to 3.97%, with the highest still being in the control treatment. At this time, the lowest mass loss was found in the CC6-Chito, DC2-Chito and DGMC2-Chito treatments (Table 6). Thus, bacterial

treatment combined with chitosan was highly effective in limiting water loss of fruit peel and reducing weight loss.

Table 6. The weight loss (%) of rambutan fruits after different treatments and stored at 13 °C.

Treatments	Storage Time (Days)		
	5	10	15
Chito	0.27 ^{g}	1.15 ^{gf}	2.27 ^{de}
SA	0.35 ^{gf}	1.18 ^{gf}	2.28 ^{de}
CMC	0.37 ^{efg}	1.1 ^{g}	2.23 ^{def}
CC6	0.68 ^{bcd}	1.38 ^{efg}	2.0 ^{fgh}
DC2	0.64 ^{cd}	1.31 ^{efg}	2.23 ^{def}
DGMC2	0.71 ^{bcd}	1.73 ^{cd}	2.17 ^{efg}
CC6-Chito	0.77 ^{bc}	1.47 ^{def}	1.75 ^{hij}
DC2-Chito	0.53 ^{def}	1.46 ^{def}	1.63 ^{j}
DGMC2-Chito	0.58 ^{cde}	1.47 ^{def}	1.72 ^{ij}
CC6-CMC	0.58 ^{cde}	1.46 ^{def}	2.31 ^{cde}
DC2-CMC	0.87 ^{b}	1.85 ^{bc}	2.20 ^{def}
DGMC2-CMC	0.62 ^{cd}	2.13 ^{b}	2.43 ^{bcd}
CC6-SA	0.56 ^{cdef}	1.38 ^{efg}	2.57 ^{bc}
DC2-SA	0.61 ^{cd}	1.57 ^{cde}	1.93 ^{ghi}
DGMC2-SA	0.68 ^{bcd}	1.6 ^{cde}	2.6 ^{b}
Control	1.93 ^{a}	3.37 ^{a}	3.97 ^{a}
CV%	20.09	11.72	6.94

Mean values with different superscripts in the same column differ significantly at $p \leq 0.05$. CV is the coefficient of variation. These treatments are described in Table 1.

Researchers also reported that the microbial density of the biofilms and the nature of the exopolymeric substances influence the biofilms' resistance to the mass transfer of water, gases, nutrients, and antimicrobials from the external environment, as well as the diffusion properties of the biofilms. In this case, biofilm may improve a micro-environment's preservation, keeping fruit from losing too much weight [29,32].

This result is also quite consistent with the study of Martínez-Castellanos et al. [5].

3.5. Total Soluble Solid and Titratable Acidity

The changes in the TSS and TA content of rambutan under different treatments and storage times are presented in Table 7. The TSS and TA content of rambutan samples after harvest (before storage) were 19.16 ± 0.15% and 4.2 ± 0.26%, respectively. Depending on growing region and variety, the pulp of rambutan has a high TSS ranging from 17 to 21% and TA from 0.7 to 5.5% at harvestable maturity [33].

In general, TSS values in treatments show significant differences ($p < 0.05$) over the same storage time. On the 5th day of storage, TSS varied slightly compared to TSS in the starting material, ranging from 18.20 to 19.90%, with little variation between treatments (Table 7). Also shown in Table 7, on the 10th day of storage, TSS values tended to increase slightly in some treatments compared to 5 days of storage (Chito, SA, CMC, DC2-Chito, CC6-SA and DC2-SA), the TSS value was highest (20.07%) in the Chito sample, and TSS decreased slightly in the remaining treatments. On the 15th day of storage, TSS ranged from 17.33 to 19.17%. In most treatments, TSS values tend to decrease, except for samples DC2 and DGMC2, where TSS values tend to increase slightly compared to 10 days of storage. This result is similar to the study of Martínez-Castellanos et al. [5].

A variation in TA content was also observed. On the 5th day of storage, TA values in most treatments increased significantly and differed from the initial TA value, except for sample DGMC2-SA (4.95 ± 0.79%), which did not show a significant difference (Table 7). Table 7 showed that on days 10 and 15, TA values changed slightly but did not show a clear difference. TA values on day 10 and day 15 ranged from 5.12 to 6.03% and 4.93 to 6.13%,

respectively. The highest TA value was found in CMC treatment, and the analyzed values were 6.03% and 6.13% at 10 and 15 days of storage, respectively (Table 7).

Table 7. The TSS and TA of rambutan fruits after different treatments and stored at 13 °C.

Treatments	TSS (%)			TA (%)		
	5 Days	10 Days	15 Days	5 Days	10 Days	15 Days
Chito	18.80 ^{cde}	20.07 ^{a}	18.93 ^{ab}	5.65 ^{abc}	6.00 ^{ab}	5.17 ^{bc}
SA	19.50 ^{abc}	19.80 ^{ab}	18.87 ^{ab}	5.43 ^{abc}	5.77 ^{bc}	5.47 ^{b}
CMC	19.10 ^{abcde}	19.60 ^{ab}	18.47 ^{bcd}	6.48 ^{abc}	6.03 ^{a}	6.13 ^{a}
CC6	19.57 ^{abc}	18.87 ^{bc}	18.47 ^{bcd}	5.53 ^{abc}	5.67 ^{cd}	4.93 ^{c}
DC2	19.90 ^{a}	18.20 ^{c}	18.60 ^{ab}	5.95 ^{abc}	5.12 ^{f}	5.13 ^{bc}
DGMC2	19.50 ^{abc}	18.87 ^{bc}	19.17 ^{a}	5.53 ^{abc}	5.30 ^{ef}	5.20 ^{bc}
CC6-Chito	19.73 ^{ab}	19.07 ^{abc}	18.63 ^{abc}	6.58 ^{a}	5.50 ^{de}	5.33 ^{bc}
DC2-Chito	19.43 ^{abcd}	19.47 ^{ab}	18.00 ^{cde}	5.97 ^{abc}	5.12 ^{f}	5.20 ^{bc}
DGMC2-Chito	19.10 ^{abcde}	19.33 ^{ab}	18.90 ^{ab}	5.90 ^{abc}	5.43 ^{de}	5.37 ^{bc}
CC6-CMC	18.83 ^{abcd}	18.17 ^{bc}	17.50 ^{ef}	5.91 ^{abc}	5.42 ^{de}	5.34 ^{bc}
DC2-CMC	18.20 ^{e}	18.20 ^{c}	17.33 ^{f}	5.37 ^{abc}	5.15 ^{f}	5.47 ^{b}
DGMC2-CMC	19.40 ^{abcd}	18.87 ^{bc}	18.77 ^{ab}	5.87 ^{abc}	5.47 ^{de}	5.52 ^{b}
CC6-SA	18.57 ^{de}	18.67 ^{bc}	17.87 ^{def}	5.70 ^{abc}	5.50 ^{de}	5.37 ^{bc}
DC2-SA	18.83 ^{bcde}	19.57 ^{ab}	18.90 ^{ab}	5.88 ^{abc}	5.67 ^{abc}	5.40 ^{b}
DGMC2-SA	19.47 ^{abcd}	19.40 ^{ab}	18.83 ^{ab}	4.95 ^{abc}	5.65 ^{cd}	5.13 ^{bc}
Control	19.73 ^{ab}	19.40 ^{ab}	18.50 ^{ab}	5.50 ^{c}	5.37 ^{ef}	5.23 ^{bc}
CV%	2.38	3.95	2.12	12.05	2.76	5.13

Mean values with different superscripts in the same column differ significantly at $p \leq 0.05$. CV is the coefficient of variation. These treatments are described in Table 1.

3.6. Vitamin C

Results of the vitamin C content analysis according to treatments and storage times are presented in Table 8. On the 5th day of storage, the vitamin C content of the treatments showed a significant difference, changing from 58.12 to 85.12 mg/100 g (Table 8). The highest vitamin C content was in the control sample (85.12 mg/100 g) as seen in Table 8. Also shown in Table 8, on the 10th day of storage, the vitamin C content tended to decrease, fluctuating between 45.20 and 62.52 mg/100 g and decreasing sharply in the control sample; however, this content tended to increase in the treatment sample DC2-CMC. On the 15th day of storage, the vitamin C content increased slightly in most treatments. This change shows that the vitamin C content of rambutan is well maintained when there is a combination of carriers and bacterial strains. Similar to the study of Xu et al. [34], soaking lychees in preservative *L. plantarum* (10^8–10^9 cfu/mL DGNKZX003 and 2% citric acid, 1% chitosan and 1% sodium lactate) significantly reduced the loss of nutrients, including vitamin C, titratable acids, and total sugar. It was explained that the soaked aqueous mixture, which included live *L. plantarum*, citric acid, chitosan, and sodium lactate, served as a composite preservative. Each of these ingredients works in concert to preserve lychee. Sodium lactate has a bacteriostatic effect, chitosan primarily forms a membrane on the lychee surface, and *L. plantarum* DN003 can inhibit peroxidase, polyphenol oxidase, *Penorophythora litchi*, and *Colletotrichum gloeosporioides*. The producing citric acid also aids in maintaining the color of the lychee pericarp.

Table 8. The vitamin C (mg/100 g) of rambutan fruits after different treatments and stored at 13 °C.

Treatments	Storage Time (Days)		
	5	**10**	**15**
Chito	72.21 ^{cd}	46.37 ^{e}	66.93 ^{ab}
SA	77.49 ^{abc}	45.20 ^{e}	59.88 ^{defg}
CMC	68.69 ^{d}	49.31 ^{de}	61.05 ^{cdef}
CC6	83.36 ^{ab}	60.76 ^{abc}	63.40 ^{bcde}
DC2	72.21 ^{cd}	61.05 ^{abc}	58.12 ^{g}
DGMC2	69.68 ^{cd}	60.61 ^{abc}	55.57 ^{g}
CC6-Chito	69.28 ^{cd}	62.23 ^{abc}	58.12 ^{efg}
DC2-Chito	67.22 ^{d}	65.48 ^{ab}	68.86 ^{a}
DGMC2-Chito	74.56 ^{cd}	65.88 ^{a}	66.19 ^{abc}
CC6-CMC	75.14 ^{cd}	58.41 ^{abc}	64.58 ^{abcd}
DC2-CMC	58.12 ^{e}	62.23 ^{abc}	66.93 ^{ab}
DGMC2-CMC	72.80 ^{cd}	62.43 ^{abc}	66.57 ^{abc}
CC6-SA	58.12 ^{e}	55.77 ^{cd}	65.16 ^{abcd}
DC2-SA	73.38 ^{cd}	56.95 ^{cd}	63.99 ^{bcd}
DGMC2-SA	69.27 ^{cd}	57.40 ^{bcd}	63.81 ^{bcd}
Control	85.12 ^{a}	62.52 ^{abc}	63.99 ^{bc}
CV%	7.8	7.82	5.23

Mean values with different superscripts in the same column differ significantly at $p \leq 0.05$. CV is the coefficient of variation. These treatments are described in Table 1.

4. Conclusions

Coating formulation could affect on the capacity to preserve the quality of Java rambutan. The combination of a carrier and a bacterial strain showed greater effectiveness. In particular, *L. fermentum* DC2 bacterial strains combined with chitosan 0.03% treatment maintained post-harvest rambutan quality, in which Java rambutan fruits showed low levels of browning, the lowest weight loss, the highest level of vitamin C and minimal post-harvest fungal infection rates up to 15 days of storage at 13 °C. The peel color is bright and fruit quality is maintained during the storage period. The obtained results can be applied in controlling post-harvest diseases of rambutan fruit, maintaining rambutan fruit quality for export activities as opposed to large quantities required by importing countries.

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Appendix A

Table A1. Data of post-harvest fungal infection incidence (%) on rambutan at 5, 10 and 15 days of storage at 13 °C.

Treatment	Storage Day (Days)		
	5	**10**	**15**
Chito	nd	2^{f}	9^{f}
SA	nd	5^{e}	11^{de}
CMC	nd	5^{e}	12^{d}
CC6	nd	0	5^{g}
DC2	nd	0	6^{g}
DGMC2	nd	8^{b}	5^{g}
CC6-Chito	nd	0	1^{hi}
DC2-Chito	nd	0	2^{h}
DGMC2-Chito	nd	0	11^{de}
CC6-CMC	nd	0	0.5^{i}
DC2-CMC	nd	0	12^{d}
DGMC2-CMC	nd	7^{c}	18^{b}
CC6-SA	nd	0	10^{ef}
DC2-SA	nd	0	2^{hi}
DGMC2-SA	nd	6^{d}	15^{c}
Control	1 ± 0.1	16^{a}	45^{a}
CV%		6.03	9.71

nd: fungal infection was not detected. Mean values with different superscripts in the same column differ significantly at $p \leq 0.05$. CV is the coefficient of variation. These treatments are described in Table 1.

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