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## Effect of carnauba wax nanoemulsion associated with *Syzygium aromaticum* and *Mentha piperita* essential oils as an alternative to extend lychee post-harvest shelf life

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The demand for tropical fruits worldwide has increased, but their short shelf life poses a challenge. Lychee, a highly perishable fruit used in various ways, lacks effective post-harvest preservation methods, particularly for export. To address this, plant-based edible coatings incorporating nanotechnology and essential oils offer a sustainable solution for maintaining lychee quality. This study aimed to assess the impact of carnauba wax nanoemulsion (CWN) coatings at different concentrations, with or without essential oils of *Syzygium aromaticum* (CEO) and *Mentha piperita* (MEO), on lychee preservation after harvesting. Two stages were conducted: first, determining the optimal CWN concentration (4.5%, 9%, and 18%) for storing lychee at 16 °C and 70% relative humidity for five days; second, selecting the 9% CWN concentration with 1% CEO and MEO for storing lychee for eight days under the same conditions. Physical and chemical analyses were performed during storage. GC-MS analysis showed that eugenol (89.73%) and isomenthol (49.46%) were the main components of clove essential oil and peppermint oil, respectively. The treatments with CWN (9%) and CWN (18%) significantly reduced weight loss by approximately 4% compared to the control while maintaining quality indicators such as  $L^*$  value, pH, and total soluble solids (TSS%). Lychee fruits coated with CWN (9%) combined with CEO and MEO showed a significant reduction in decay incidence and severity after 168 h of storage. Specifically, the CWN–MEO treatment exhibited a 20% incidence and severity compared to the control's 60% and 100%, respectively. Coatings with 9% CWN and 1% MEO have the potential to effectively preserve post-harvest lychee quality, minimize losses, reduce disease severity, extend shelf life, and enhance commercial opportunities.

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### Sustainability spotlight

Currently, approximately 40% of horticultural food production is lost due to post-harvest diseases. The limited shelf life of these foods presents a significant challenge. Moreover, the excessive use of chemical fungicides to combat these diseases results in problems such as environmental damage and potential harm to humans. By developing plant-based edible coatings that incorporate nanotechnology and essential oils, we are offering a sustainable solution to improving fruit quality and reduce waste in the supply chain. Our research focuses on reducing food waste and post-harvest losses, aligning with the ONU Sustainable Development Goal 12.3, which aims to halve global food waste per capita by 2030.

## 1 Introduction

Nowadays, consumers are looking for healthier and natural food, which demands a greater variety of fruits and vegetables.

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However, most of them tend to have a short shelf-life, especially tropical and subtropical fruits.<sup>1</sup>

Lychee (*Litchi chinensis* Sonn.) is a non-climacteric fruit<sup>2</sup> with high nutritional<sup>3</sup> and commercial value,<sup>4</sup> commonly cultivated in tropical and subtropical regions.<sup>5</sup> Lychees are only available for a few months per year and they have very short shelf-life between 2 and 3 days at room temperature.<sup>6</sup> Several problems after harvesting have been reported, such as darkening of the pericarp, desiccation and rot. Some post-harvesting technologies are applied to solve these problems, such as using refrigerated temperatures associated with acid leaching with sulfur treatment, although they are not very sustainable.<sup>6,7</sup> Therefore,

there are limited options available to extend the shelf life of lychee fruits as these fruits are susceptible to changes in the cold chain. Studies are needed on new fruit preservation alternatives to minimize food waste. Each fruit has its own distinct physiology, highlighting the need to individually evaluate new cost-effective technologies to increase their shelf life.

Edible coatings present an alternative to extend the shelf-life of fruits and vegetables. Most of them are not only biodegradable, but also made of natural compounds that form a layer on the surface that reduces gases and moisture exchange with the environment, thus reducing the respiration rate.<sup>8</sup> The respiration rate relates to fruit senescence, and its lower rates allow shelf-life to be increased.<sup>9</sup> Edible coatings, in addition to inhibiting respiration, act as a barrier against water loss, inhibiting oxidative reactions. Also, when antimicrobial compounds are added, they reduce the growth of pathogens on the food surface.<sup>10</sup>

Coating components must be Generally Recognized as Safe (GRAS) and approved by regulatory agencies such as the U.S. Food and Drug Administration – FDA and the European community. Edible coatings are composed of a matrix, and it is possible to incorporate essential oils to obtain their antimicrobial properties.<sup>8</sup>

Carnauba wax nanoemulsion (CWN) can be used as a matrix for edible coating. Its properties can be complemented by adding plant essential oils with antimicrobial properties.<sup>11</sup> The application of carnauba wax nanoemulsion (CWN) associated or not with other components, such as essential oils, has presented promising results for post-harvest conservation in strawberries,<sup>12</sup> papayas,<sup>13</sup> and tomatoes.<sup>14</sup> Those coatings involve the surface of the fruit and are a physical barrier that enables it to reduce the respiration rate and delay senescence.<sup>11</sup>

Essential oils usually present antibacterial and antifungal activities. Thus, they can be incorporated into edible coatings to improve their properties.<sup>15</sup> The essential oil of *Syzygium aromaticum* (clove) with main component eugenol, has been described as having antibacterial and antifungal activities against post-harvest pathogenic microorganisms in fruits<sup>16</sup> and a few foodborne pathogens.<sup>17</sup> *Mentha piperita* (peppermint) essential oil has menthol and menthone,<sup>18</sup> which are associated with the antifungal activities of these essential oils.<sup>19</sup>

Furthermore, essential oils can also effectively inhibit enzymatic browning and oxidative reactions in fruits, helping to maintain their appearance, texture, flavor and nutritional value, ensuring a longer shelf life.<sup>20</sup> Therefore, research and development of alternatives as suitable essential oil formulations associated with hydrophobic coatings can increase the overall effectiveness of fruit preservation.

The main goal of this research was to evaluate edible coating of carnauba wax nanoemulsion (CWN) in different concentrations associated or not with essential oils of *Syzygium aromaticum* (CEO) and *Mentha piperita* (MEO) on the post-harvest conservation of lychee. The study aims at post-harvest preservation of lychee, with visual quality as a physical aspect, delaying senescence and inhibiting the growth of fungal diseases.

## 2 Materials and methods

### 2.1. Materials

Carnauba wax type I (99% purity; CAS no. 8015-86-9) was provided by Pontes Indústria de Cera (Parnaíba, PI, Brazil). *Syzygium aromaticum* and *Mentha piperita* essential oils were acquired from Laszlo Aromaterapia (Belo Horizonte, MG, Brazil). Lychee fruits, cultivar Bengal, were carefully transported from a local producer to the post-harvest laboratory, Embrapa Instrumentation, São Carlos, SP. Then, they were separated by lack of standard defects, size and stage of maturity.

### 2.2. Essential oil composition

The composition of essential oils was determined by qualitative analysis using gas chromatography with Shimadzu equipment (GC-2010 Plus, Kyoto, Japan) coupled to a quadrupole mass spectrometer (GC-MS). A non-polar DB-5MS capillary column (30 m × 0.25 mm, i.d. × 0.25 µm) was used, and helium was the carrier gas at 1 mL min<sup>-1</sup> flow rate. Samples were diluted in dichloromethane (10% v/v) and injected (1 µL) in split mode (1 : 50). Injector temperature: 220 °C, oven temperature: 60 to 240 °C at 3 °C min<sup>-1</sup>; interface: 240 °C; ion source: +70 eV, m/z: 35–350. Co-injection of authentic standards confirmed sample components. A semi-quantitative analysis of the essential oils (% relative area) was also performed using a flame ionization detector (GC-FID). Qualitative and semi-quantitative analyses were performed in triplicate.

### 2.3. Edible coating preparation

The synthesis of the carnauba wax nanoemulsion (CWN) was based on the methodology adapted from Hagenmaier & Baker.<sup>21</sup> The zeta potential (−43.8 mV), the diameter size (44.1 ± 7.6 nm) and polydispersity indices (0.28) of CWN were measured using a Zetasizer Nano ZS (Malvern Instruments Inc., Westborough, MA, USA).<sup>22</sup>

The experiment was divided into two phases. First, the carnauba wax nanoemulsion (CWN) was applied to lychees randomly divided into four treatments, namely: control, CWN (4.5% solid phase in suspension), CWN (9% solid phase in suspension), and CWN (18% phase suspended solid). The fruits were immersed in the coating solution and stored for five days at 16 °C and 70% relative humidity. In the second phase, after evaluating which concentration of carnauba wax nanoemulsion best preserved the lychees, the fruits were subjected to treatments with essential oil: control, CWN (9% solid phase in suspension), CWN (9%) + CEO (1%), CWN (9%) + MEO (1%), and stored for eight days at 16 °C and 70% relative humidity.

### 2.4. Physicochemical parameters of lychees

**2.4.1 Non-destructive analysis: weight loss and colorimetric.** Lychee weight loss was determined according to Duarte *et al.*,<sup>23</sup> by weighing the fruits in the first phase of the research on day 0 (beginning of the experiment) and on days 2 and 4 of storage (0, 48 and 96 h) and in the second phase on days 0, 2 and 6 (0, 48 and 168 h). Peel color was measured with a Minolta®



CR-400 Chroma Meter colorimeter (Minolta Camera Co., Osaka, Japan) according to de Oliveira Filho *et al.*<sup>13</sup>

**2.4.2 Destructive analysis: pH, TSS, titratable acidity and texture.** The pH, total soluble solids (TSS), and titratable acidity (TA) were analyzed according to the methodology of Duarte & Picone.<sup>10</sup> The fruit pulp was composed of 10 fruits in triplicate. Firmness was assessed using a TA.XT plus Digital Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) with a 6 mm diameter probe, 120 mm min<sup>-1</sup> speed, 2 mm penetration distance and a 2 mm area of contact of 12 mm<sup>2</sup> in the peeled fruit. The results were expressed in newtons (N) and the average was calculated based on three penetrations in the distal region of each fruit. All analyses were performed in triplicate and data were calculated with mean and standard deviation.

## 2.5. Decay percentage and severity on lychees

The incidence was visually analyzed through the presence of fungi in lychees during storage. The percentage of rot was determined based on the number of lychees with visible fungus per treatment, with each treatment having five lychees. The severity of the diseases was analyzed using a 4-point scale (0 = no symptoms; 1 = 1% of the affected area; 2 = 2–5%; 3 = 6–100%) to assess the fungal activity of the coatings. Due to the fruit type, on score 3, 6% was considered to have high disease infestation.<sup>24</sup>

## 2.6. Statistical analysis

Parametric analysis of variance and multiple comparisons of Duncan's test or non-parametric ANOVA and multiple comparisons of Kruskal-Wallis were performed at the 5% significance level using the R software version 4.2.2 to assess the differences between treatments.

# 3 Results and discussion

## 3.1. Essential oil composition

As shown in Table 1, *Syzygium aromaticum* (clove) essential oil was mainly constituted by eugenol (89.73%) and  $\beta$ -Gurjunene (7.59%). This composition is similar to that obtained by Haro-González *et al.*<sup>25</sup> and Kačániová *et al.*<sup>26</sup> where eugenol was the dominant component of CEO. The antifungal activity of clove essential oil can be associated with the hydroxyl groups in eugenol that can interact with the cellular membrane and damage it.<sup>25</sup> Eugenol can also induce oxidative stress by generating reactive oxygen species (ROS) within fungal cells and thus cause damage to DNA, proteins, and lipids.<sup>27</sup> Eugenol also impairs fungal growth by interrupting energy production, consequently inhibiting ATP synthesis.<sup>28</sup>

*Mentha piperita* (peppermint) essential oil presented isomenthol (49.46%), menthone (18.97%) and menthol (15.25%) as major components, similar to what was described by Desam *et al.*<sup>19</sup> and Kamatou *et al.*<sup>29</sup> These components attribute anti-fungal activity to the oil, due to its hydrophobicity, which allows interaction with the cellular membrane and leads to its lyses.<sup>30</sup> Menthol inhibits the biosynthesis of ergosterol (an important component of the fungal membrane). Thus, it alters membrane fluidity by interrupting the normal functioning of membrane-

Table 1 Composition of essential oils

Compound	<i>Syzygium aromaticum</i> (% area)	<i>Mentha piperita</i> (% area)
$\alpha$ -Pinene	—	0.61
Sabinene	—	0.14
$\beta$ -Pinene	—	0.63
3-Octanol	—	0.16
<i>p</i> -Cymene	—	0.15
Limonene	—	1.76
1,8-Cineol	—	0.84
Linalool	—	0.10
Isopulegol	—	1.75
Menthone	—	18.97
Menthol	—	15.25
Isomenthol	—	49.46
Neoisomenthol	—	1.27
$\gamma$ -Terpineol	—	0.28
$\alpha$ -Terpineol	—	0.63
Pulegone	—	1.80
Piperitone	—	0.90
Menthyl acetate	—	0.19
Eugenol	89.73	—
Neoisomenthyl acetate	—	4.18
$\beta$ -Bourbonene	—	0.19
$\beta$ -Gurjunene	7.59	—
Caryophyllene	—	0.73
$\alpha$ -Humulene	2.10	—
$\gamma$ -Selinene	0.20	—
$\delta$ -Cadinene	0.25	—
Caryophyllene oxide	0.13	—
Total	100	99.97

bound proteins and enzymes, affecting essential cellular processes and cellular integrity.<sup>31</sup>

Essential oils also possess antioxidant activity crucial for fruit preservation as they eliminate and neutralize free radicals, preventing or delaying oxidative damage.<sup>20</sup> It helps maintain the appearance, texture, flavor and nutritional value of the fruits, ensuring that they remain attractive and safe for consumption for longer periods of time.<sup>32</sup>

The main antioxidant compounds in clove essential oil are eugenol and beta-caryophyllene, while menthol is the main antioxidant compound in peppermint essential oil.<sup>33,34</sup> These compounds are capable of donating electrons or hydrogen atoms to free radicals, neutralizing their reactivity and preventing them from causing oxidative damage to cells and tissues.<sup>35</sup> Therefore, essential oils in proper concentrations and formulations can safely and effectively preserve food.

Both essential oils presented variations in their percentage of composition compared to other authors, which can be explained due to different factors, such as the geographic origin of the plant, environmental conditions (climatic and seasonal conditions and sunlight), plant organ and age, genetics, nutrition of the plant, and extraction method.<sup>25</sup>

## 3.2. First phase: control, CWN 4.5%, CWN 9% and CWN 18%

**3.2.1 Weight loss.** Weight loss through time is a natural process that occurs with vapor diffusion, and it may be



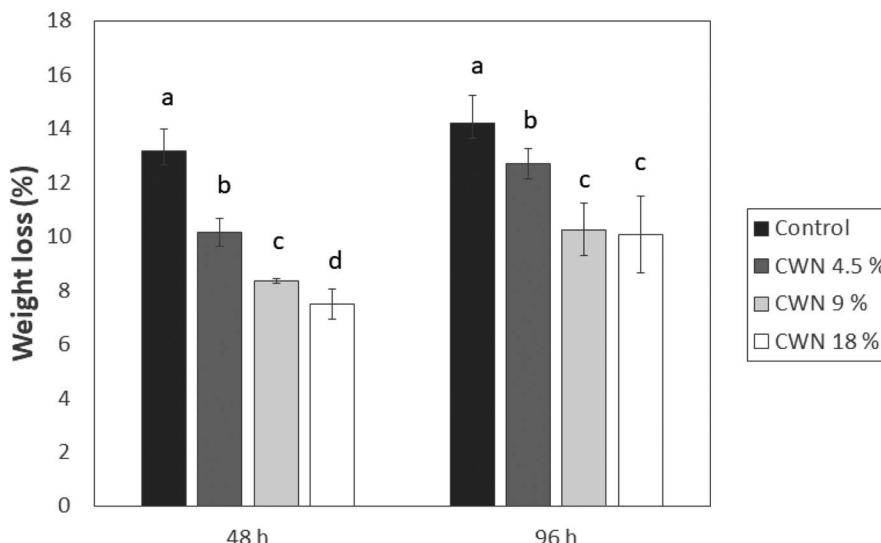


Fig. 1 Lychee weight loss during storage time at 16 °C and 70% RH. For each storage period, different letters indicate significant differences between treatments ( $p < 0.05$ ).

Table 2 pH and total soluble solids (TSS) of fruit over time<sup>a</sup>

Treatments	pH			TSS (%)		
	0	48	96	0	48	96
Storage time (h)	0	48	96	0	48	96
Control	4.09 ± 0.02 <sup>a</sup>	4.53 ± 0.01 <sup>a</sup>	4.94 ± 0.03 <sup>a</sup>	17.14 ± 0.05 <sup>a</sup>	18.95 ± 0.15 <sup>a</sup>	19.87 ± 0.19 <sup>a</sup>
CWN 4.5%	4.09 ± 0.02 <sup>a</sup>	4.40 ± 0.02 <sup>ab</sup>	4.67 ± 0.03 <sup>b</sup>	17.14 ± 0.05 <sup>a</sup>	17.55 ± 0.07 <sup>c</sup>	18.04 ± 0.02 <sup>c</sup>
CWN 9%	4.09 ± 0.02 <sup>a</sup>	4.28 ± 0.01 <sup>b</sup>	4.58 ± 0.04 <sup>c</sup>	17.14 ± 0.05 <sup>a</sup>	17.31 ± 0.21 <sup>c</sup>	18.00 ± 0.11 <sup>c</sup>
CWN 18%	4.09 ± 0.02 <sup>a</sup>	4.50 ± 0.13 <sup>a</sup>	4.72 ± 0.02 <sup>b</sup>	17.14 ± 0.05 <sup>a</sup>	17.87 ± 0.07 <sup>b</sup>	18.56 ± 0.13 <sup>b</sup>

<sup>a</sup> Means followed by different letters on the same column indicate significant differences between treatments ( $p$ -value < 0.05).

hampered to the fruit skin and can be reduced by applying CWN coating in lychee because the physical barrier formed reduces gas and moisture transfer, respiration rate and weight loss through time.<sup>23</sup>

The results presented in Fig. 1 show that lychee lost weight progressively over time during storage at 16 °C and that the control group presented higher weight loss than CWN 4.5%, CWN 9%, and CWN 18% treatment. Therefore, all CWN coatings efficiently prevented weight loss on lychee over time. After 48 h, we observed a reduction of approximately 4 and 5% of weight loss at CWN 9% and 18%, respectively, compared to the control. After 96 h of storage, the difference in weight loss between the control and the CWN 9% and 18% treatments remained at 4%, and between the control and the CWN 4.5% treatment there was only a 2% difference. Thus, CWN 9% and 18% were more effective. Carnauba wax nanoemulsions have already presented promising results on reducing weight loss in tomatoes, as shown by Miranda *et al.*<sup>14</sup> Weight loss is an important quality parameter especially for exporting fruits because their quality and value are related to that.<sup>36</sup>

**3.2.2 pH and total soluble solids (TSS).** The pH analyses (Table 2) demonstrated that all groups had an increase in pH during 96 h of storage. However, the control group showed

a higher increase and the CWN 9% treatment had the lowest change. Treatments CWN 4.5% and CWN 18% showed the same result after 96 h. Therefore, all treatments reduced changes in pH, but CWN 9% showed the lowest values. The increase in pH over time is related to the senescence process, in which organic acids are consumed during respiration to synthesize sugar. Therefore, pH becomes more basic.<sup>37</sup> So, it can be assumed that lychee treated with the CWN coating had its respiration rate reduced because it formed a barrier, delaying its senescence process. Therefore, coated lychee presented a lower increase in pH values compared to the control.

The results of total soluble solids (TSS) (Table 2) were that for all treatments with different concentrations of CWN, the TSS value increased over time with significant differences. These results were expected once sugar is synthesized during respiratory processes to be converted into CO<sub>2</sub> and water during fruit senescence.<sup>38</sup> All treatments with CWN showed little increase in TSS values compared to the control, which can be related to the reduction in the respiration rate on coated lychee and, consequently, a reduction in the fruit metabolic activity.<sup>9</sup>

After 48 h of storage, the treatment that best lowered the increase in TSS was CWN 9%. After 96 h of storage, the best results were by CWN 4.5% and CWN 9%, showing equal results.

Table 3 Color parameters  $L^*$ ,  $C^*$  and  $a^*$  of lychees stored at 16 °C and 70% RH<sup>a</sup>

Treatments	Time (h)			48			96		
	0			$L^*$	$C^*$	$a^*$	$L^*$	$C^*$	$a^*$
Control	27.70 ± 7.25 <sup>a</sup>	15.90 ± 4.16 <sup>ab</sup>	13.10 ± 3.27 <sup>a</sup>	20.20 ± 4.85 <sup>b</sup>	12.40 ± 5.67 <sup>b</sup>	8.50 ± 3.93 <sup>b</sup>	25.50 ± 2.99 <sup>a</sup>	15.00 ± 2.13 <sup>a</sup>	11.90 ± 1.88 <sup>a</sup>
CWN 4.5%	25.90 ± 6.14 <sup>a</sup>	14.50 ± 4.39 <sup>b</sup>	11.20 ± 3.92 <sup>a</sup>	27.10 ± 3.92 <sup>a</sup>	19.10 ± 3.25 <sup>a</sup>	13.60 ± 2.02 <sup>a</sup>	25.40 ± 3.54 <sup>a</sup>	14.30 ± 3.69 <sup>a</sup>	10.00 ± 1.99 <sup>b</sup>
CWN 9%	28.90 ± 8.53 <sup>a</sup>	18.20 ± 5.48 <sup>a</sup>	12.20 ± 3.49 <sup>a</sup>	21.20 ± 5.64 <sup>b</sup>	18.90 ± 4.92 <sup>a</sup>	5.90 ± 2.76 <sup>c</sup>	22.00 ± 7.22 <sup>a</sup>	16.00 ± 6.55 <sup>a</sup>	10.00 ± 3.64 <sup>b</sup>
CWN 18%	26.40 ± 9.73 <sup>a</sup>	17.40 ± 3.77 <sup>ab</sup>	12.60 ± 2.79 <sup>a</sup>	29.90 ± 3.22 <sup>a</sup>	19.40 ± 3.69 <sup>a</sup>	12.70 ± 3.58 <sup>a</sup>	19.50 ± 7.14 <sup>a</sup>	14.10 ± 3.51 <sup>a</sup>	8.10 ± 2.48 <sup>b</sup>

<sup>a</sup> Means followed by different letters on the same column indicate significant differences between treatments ( $p$ -value < 0.05).



Fig. 2 Uncoated or control treatment lychees (A) and CWN 9% coated lychees (B) after 96 h of storage at 16 °C.

**3.2.3 Color data.** Color data are presented in Table 3, and the skin browning is noticeable over time. This change happens naturally due to anthocyanin degradation and oxidative reactions of pigments by polyphenol oxidases.<sup>39</sup>

The  $L^*$  value represents the lightness,<sup>40</sup> and the results obtained showed that after 96 h of storage, the treatments control and CWN 9% had the  $L^*$  value decreased. The pericarp lightness of lychee coated with CWN 4.5% did not significantly change over the 96 h of storage. The  $L^*$  value of treatment CWN 18% increased and then decreased, during 96 h of storage. This type of  $L^*$  value behavior was previously described in lychee by Yang *et al.*<sup>24</sup> Generally, all samples had decreased  $L$  values indicating color darkness and fruit senescence for climacteric fruit.<sup>41</sup> On the other hand, for lychee, non-climacteric fruit, despite the decreased  $L$  values, there was no significant difference between treatments, and the absence of darkness (Fig. 2) can be corroborated with the maintenance of the fruits and with the lowest pH and TSS values during storage (Table 2) of CWN 9% and CWN 18%.

The  $C^*$  value indicates color saturation<sup>42</sup> and although some changes occurred over time for all the treatments, the color saturation can be considered significantly the same at 0 h and 96 h for all the treatments.

The  $a^*$  value represents the green-red axis,<sup>43</sup> and the results show variations over time for all treatments. However, the control treatment showed the highest value, which is significantly different from the CWN treatments, indicating less intensive red color for those treatments, with CWN 18% being the lowest one (Fig. 2).

After all the non-destructive and destructive analyses of the first phase were finished (control, CWN 4.5%, CWN 9% and CWN 18%), the second phase analysis was performed with CWN 9% since it was the treatment with the most promising results in tested parameters to maintain post-harvest quality. Therefore, the second phase analysis compared CWN 9% coating to CWN 9% coating related to 1% clove and 1% peppermint essential oil.

### 3.3. Second phase: control, CWN 9%, CWN-CEO, and CWN-MEO

**3.3.1 Weight loss.** The weight loss resulting from the second phase is presented in Fig. 3, and it shows that the control group had higher percentages of weight loss over time. Therefore, all treatments reduced weight loss by forming a physical barrier reducing transfers with the medium. After 48 h of storage, CWN 9% and CWN-CEO coating showed equal results, while CWN-MEO coating had a slightly better result on

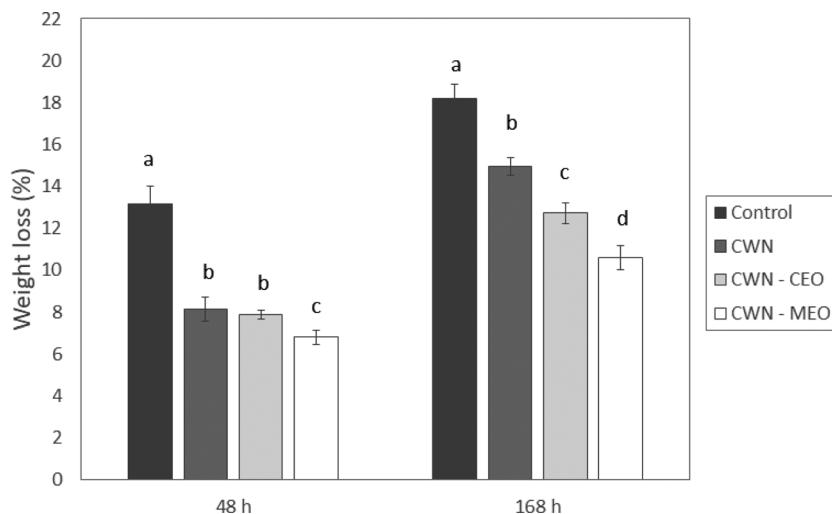


Fig. 3 Lychee weight loss during storage time at 16 °C and 70% RH. For each storage period, different letters indicate significant differences between treatments ( $p < 0.05$ ).

Table 4 pH, titratable acidity (TA) and total soluble solids (TSS) of lychees over time<sup>a</sup>

Treatments	pH			TA (%)			TSS (%)		
	0	48	168	0	48	168	0	48	168
Storage time (h)									
Control	3.87 ± 0.05 <sup>a</sup>	4.44 ± 0.05 <sup>a</sup>	4.83 ± 0.02 <sup>a</sup>	0.734 ± 0.006 <sup>a</sup>	0.724 ± 0.002 <sup>a</sup>	0.634 ± 0.002 <sup>c</sup>	16.74 ± 0.08 <sup>a</sup>	18.28 ± 0.08 <sup>a</sup>	19.86 ± 0.04 <sup>a</sup>
CWN 9%	3.87 ± 0.05 <sup>a</sup>	4.23 ± 0.02 <sup>b</sup>	4.60 ± 0.01 <sup>b</sup>	0.734 ± 0.006 <sup>a</sup>	0.706 ± 0.005 <sup>b</sup>	0.657 ± 0.003 <sup>b</sup>	16.74 ± 0.08 <sup>a</sup>	17.85 ± 0.03 <sup>b</sup>	18.94 ± 0.10 <sup>b</sup>
CWN-CEO	3.87 ± 0.05 <sup>a</sup>	4.13 ± 0.02 <sup>c</sup>	4.53 ± 0.02 <sup>c</sup>	0.734 ± 0.006 <sup>a</sup>	0.704 ± 0.002 <sup>b</sup>	0.653 ± 0.001 <sup>b</sup>	16.74 ± 0.08 <sup>a</sup>	17.55 ± 0.04 <sup>c</sup>	18.62 ± 0.01 <sup>c</sup>
CWN-MEO	3.87 ± 0.05 <sup>a</sup>	4.04 ± 0.02 <sup>d</sup>	4.46 ± 0.04 <sup>d</sup>	0.734 ± 0.006 <sup>a</sup>	0.701 ± 0.001 <sup>b</sup>	0.675 ± 0.004 <sup>a</sup>	16.74 ± 0.08 <sup>a</sup>	17.26 ± 0.01 <sup>d</sup>	18.16 ± 0.04 <sup>d</sup>

<sup>a</sup> Means followed by different letters on the same column indicate significant differences between treatments ( $p$ -value < 0.05).

reducing weight loss. After 168 h of storage, CWN-MEO, CWN-CEO, and CWN 9% were the most efficient ones to prevent weight loss, showing 10.58%, 12.72%, and 14.96% of weight loss, respectively, while the control group had 18.17% of weight loss. For the lowest weight value loss to the control (CWN-MEO), a reduction of 7.59% can be observed, which represents 41.77% of the difference in weight maintenance.

Many studies prove a lower weight loss in fruits with coatings with incorporated essential oils due to the hydrophobic contribution, thus providing protection against moisture loss.<sup>15</sup> Sapper *et al.*<sup>44</sup> discovered starch-gellan coatings with thyme essential oil, which prevented water loss from persimmons, and Sarengaowa *et al.*<sup>45</sup> found weight loss inhibition results in potatoes coated with chitosan and cinnamon essential oil.

**3.3.2 pH, titratable acidity (TA) and total soluble solids (TSS).** The pH of lychee (Table 4) increased over time independent of the treatment, but after 48 h of storage, the treatment CWN-MEO led to the lowest pH increase, and after 168 h, the treatments associated with essential oils (CWN-CEO and CWN-MEO) led to the lowest pH increase and presented equal results. As mentioned before, carnauba wax nanoemulsion was reported to reduce weight loss, and it can also be associated with essential oils as described by de Oliveira Filho *et al.*<sup>13</sup> and Miranda *et al.*<sup>46</sup> The lower pH increase can be associated with

a reduction in gas and moisture transfer and reduction in the reparation rate; besides this, the oil-associated coating might have been more efficient due to its more hydrophobic characteristics than CWN only.<sup>13,46</sup>

The titratable acidity (TA) presented a decreasing pattern for all the treatments over time, with the control group having the highest decrease. Therefore, CWN 9%, CWN-CEO, and CWN-MEO treatments were efficient in reducing changes in the TA value. After 168 h of storage, the CWN 9% and CWN-CEO treatments showed significantly equal results in reducing the decrease in TA, the CWN-MEO treatment being the most efficient. As previously mentioned, organic acids are consumed during the senescence process of the fruit, causing a decrease in the TA value. However, the coating reduces the respiration rate and organic acid consumption, such as the TA decrease over time.<sup>9,23</sup>

The total soluble solids (TSS) has increased over time for all treatments, but the control group had the highest increase in TSS, which evidences that CWN 9%, CWN-CEO, and CWN-MEO treatments reduced TSS changes over time due to delaying the senescence process. CWN-MEO showed a lower increase in TSS and presented the best results, followed by CWN-CEO and CWN 9% treatments. Similar results were found by de Oliveira *et al.*<sup>47</sup> with mangoes coated with chitosan and the essential oil

**Table 5** Firmness (*N*) of lychees during storage for 8 days at 16 °C and 70% RH<sup>a</sup>

Treatments	Storage time (h)		
	0	48	168
Control	0.221 ± 0.040 <sup>a</sup>	0.356 ± 0.042 <sup>a</sup>	0.383 ± 0.077 <sup>a</sup>
CWN	0.221 ± 0.040 <sup>a</sup>	0.248 ± 0.043 <sup>b</sup>	0.289 ± 0.049 <sup>b</sup>
CWN-CEO	0.221 ± 0.040 <sup>a</sup>	0.226 ± 0.021 <sup>b</sup>	0.264 ± 0.062 <sup>b</sup>
CWN-MEO	0.221 ± 0.040 <sup>a</sup>	0.223 ± 0.045 <sup>b</sup>	0.251 ± 0.055 <sup>b</sup>

<sup>a</sup> Means followed by different letters on the same column indicate significant differences between treatments (*p*-value < 0.05).

of *Mentha piperita* and by dos Passos Braga *et al.*<sup>48</sup> with papayas. In both studies, there were not only a few changes in TSS values but also the loss of weight, titratable acidity, and pH, thus indicating the effectiveness of the essential oil in delaying senescence.

**3.3.3 Firmness.** The firmness of lychee (Table 5) increased over time for all treatments. However, the control group showed an increase higher than other treatments. Thus, CWN 9%, CWN-CEO, and CWN-MEO reduced alterations in the lychee firmness and showed significantly equal results. The increase in the lychee firmness may be associated with the loss of fruit moisture during storage.<sup>49</sup> Edible coatings act as a semipermeable barrier restricting gas exchange and water vapor,<sup>50</sup> so the control group that lost more weight (Section 3.3.1) probably got stiffer because it dried out more. Generally, there is a reduction in fruit firmness over time due to the degradation of the cell walls by increased activity of endogenous enzymes. However, refrigeration (lychees were stored at 16 °C) inhibits the rates of enzymatic and non-enzymatic reactions. Thus, it slows the softening of fruit texture.<sup>51</sup> Mahajan & Goswami<sup>49</sup> also observed an increase in the firmness of lychees after 22 days of storage.

**3.3.4 Color data.** Lychee color changed over time due to the browning of the pericarp, which occurred naturally due to oxidative reactions of fruit pigments and degradation of anthocyanins, which produced a brown pigment.<sup>52</sup> Table 6 shows that the pericarp lightness, represented by the *L*\* value, decreased after 168 h of storage for the control, CWN-CEO and CWN-MEO, with a higher decrease occurring in the control. On the other hand, CWN 9% treatment maintained the *L*\* value over time, significantly.

The *C*\* value (saturation) significantly decreased after 168 h of storage for all the treatments. The treatments CWN showed the lowest values for this parameter and for the *a*\* value (green-red), indicating less intensive red color, significantly different from the control. CWN-CEO and CWN-MEO presented final storage results that were significantly equal. The browning rate of lychees was associated with the dehydration rate or weight loss during storage.<sup>53</sup> Coated fruits lost less weight than uncoated fruits, the red color was less intense in all coated fruits, and similar results were found in the first step (Fig. 2).

#### 3.4. Decay percentage and severity on lychees

As shown in Fig. 4A, disease incidence in the control group was higher than that in lychee revested with CWN, CWN-CEO, and

**Table 6** Color parameters *L*\*, *C*\* and *a* of lychees stored at 16 °C and 70% RH<sup>a</sup>

Treatments	0			48			168		
	<i>L</i> *	<i>C</i> *	<i>a</i>	<i>L</i> *	<i>C</i> *	<i>a</i>	<i>L</i> *	<i>C</i> *	<i>a</i>
Control	48.20 ± 10.87 <sup>a</sup>	40.59 ± 5.49 <sup>a</sup>	31.89 ± 5.63 <sup>a</sup>	33.27 ± 4.09 <sup>ab</sup>	28.98 ± 5.57 <sup>ab</sup>	19.98 ± 3.20 <sup>b</sup>	30.95 ± 6.08 <sup>a</sup>	27.32 ± 4.85 <sup>a</sup>	17.71 ± 1.78 <sup>a</sup>
CWN	35.88 ± 11.27 <sup>b</sup>	35.82 ± 9.08 <sup>ab</sup>	26.71 ± 11.44 <sup>a</sup>	34.87 ± 5.42 <sup>a</sup>	29.43 ± 5.66 <sup>ab</sup>	22.21 ± 3.54 <sup>a</sup>	31.14 ± 6.71 <sup>a</sup>	20.97 ± 3.38 <sup>b</sup>	12.92 ± 2.02 <sup>b</sup>
CWN-CEO	48.62 ± 5.12 <sup>a</sup>	39.37 ± 4.40 <sup>ab</sup>	32.19 ± 4.08 <sup>a</sup>	31.80 ± 4.39 <sup>b</sup>	27.40 ± 5.51 <sup>b</sup>	21.44 ± 4.31 <sup>ab</sup>	27.95 ± 7.39 <sup>a</sup>	20.51 ± 3.12 <sup>b</sup>	13.07 ± 1.77 <sup>b</sup>
CWN-MEO	43.66 ± 6.84 <sup>ab</sup>	34.98 ± 5.35 <sup>b</sup>	35.31 ± 3.59 <sup>a</sup>	31.56 ± 4.94 <sup>a</sup>	22.87 ± 2.57 <sup>a</sup>	20.82 ± 6.77 <sup>a</sup>	21.49 ± 4.08 <sup>b</sup>	13.27 ± 2.18 <sup>b</sup>	

<sup>a</sup> Means followed by different letters on the same column indicate significant differences between treatments (*p*-value < 0.05).



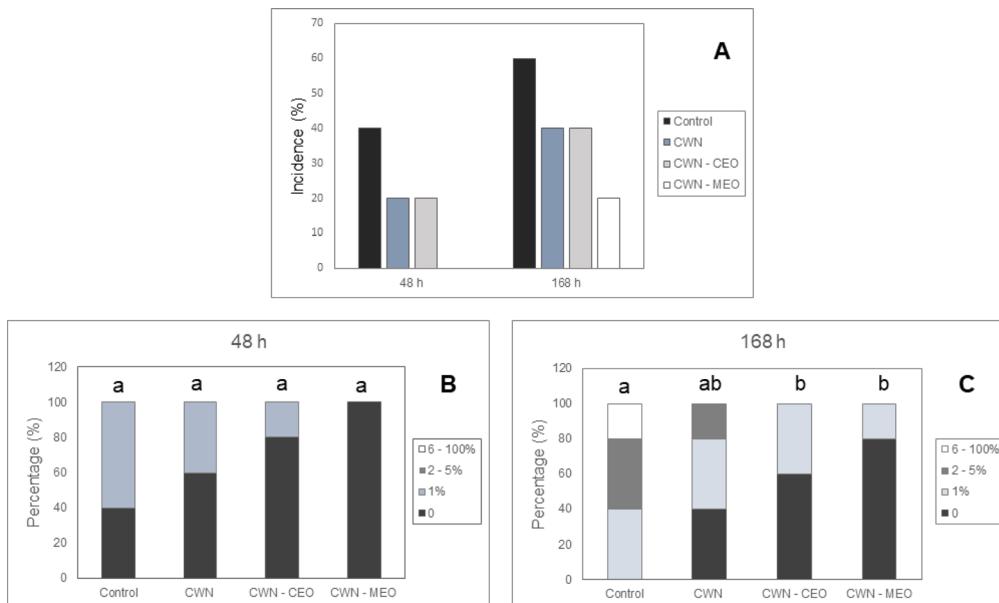


Fig. 4 Fruit disease incidence (A) and severity of lychee post-harvest deterioration after 48 h (B) and 168 h (C) of storage at 16 °C. Different letters indicate significant differences between treatments ( $p < 0.05$ ) for each storage period.

CWN-MEO. After 48 h of storage, disease incidence was 40% for the control, 20% for CWN and CWN-CEO, and 0% for CWN-MEO. After 168 h of storage, the control group presented 60% of the incidence, CWN and CWN-CEO had 40% of the incidence and CWN-MEO presented 20% of the incidence. The results obtained showed that all treatments reduced the postharvest disease incidence, the CWN-MEO coating led to lower incidence than the others, and CWN and CWN-CEO coatings presented significantly equal results.

Lychee coated with CWN, CWN-CEO and CWN-MEO showed lower disease severity compared to control group after 48 h and 168 h of storage (Fig. 4B and C). After 48 h of storage (Fig. 4B), 60% of group control presented 1% of the affected area; 40% of CWN coated lychee had 1% of the area affected; 20% of CWN-CEO coated lychee had 1% of the area affected and none of CWN-MEO coated lychee had the area affected. After 168 h of storage (Fig. 4C), all lychee in the control group had at least 1% of the area affected; CWN coated lychee present 40% of the group with 0% area affected; CWN-CEO coated lychee present 60% of the group with 0% area affected; CWN-MEO coated lychee present 80% of the group with 0% area affected. Therefore, CWN-MEO coating was the most efficient in preventing the increase in disease severity.

It is possible that the superior efficacy of CWN-MEO coatings arises from the synergy between the antimicrobial and antioxidant properties of peppermint essential oil and the barrier-forming abilities of carnauba wax. The bioactive compounds in the oil fortify the wax's defense against microbes and oxidative stress on the lychee surface, resulting in enhanced preservation.

de Oliveira Filho *et al.*<sup>13</sup> report post-harvest decay inhibition in papayas with a coating of carnauba nanoemulsion with clove (*Z. aromaticum*) and mint essential oil (*Mentha spicata*), the last

association being more effective. Both oils belong to the same *Mentha* genus, although *Mentha spicata* and *Mentha piperita* have differences in their major compounds, which may result in variations in their antifungal properties.<sup>54</sup> Similar antifungal efficacy results of coatings with *Mentha piperita* were obtained in mangoes<sup>55</sup> and grapes.<sup>56</sup>

Essential oils are bioactive compounds of plant origin that have antimicrobial and antioxidant activities due to their phenolic compounds. Recent work has also explored other bioactive compounds of plant origin for food preservation using mango seed grains<sup>57</sup> and date seed extracts<sup>58-60</sup> due to the presence of polyphenols in these compounds.

Fruits coated with CWN (9%) associated with essential oils CEO and MEO could preserve post-harvest physical-chemical parameters even at temperatures higher than recommended because, in addition to maintaining the physical-chemical parameters such as pH, titratable acidity and total soluble solids, they prolong the shelf life of these fruits as they inhibit the growth of antifungal diseases due to the antimicrobial properties of essential oils. The coating of carnauba wax with essential oils can be an alternative for the conservation of lychees that, when stored at low temperatures, can suffer browning on the skin and pulp and changes in their texture.<sup>61</sup>

## 4 Conclusion

The results of this study indicate the effectiveness of carnauba wax nanoemulsion (CWN) coatings, particularly at 9% concentration, in preserving lychee post-harvest quality. At a storage temperature of 16 °C, application of CWN (9%) significantly mitigated the weight loss by 4% compared to untreated fruits, while simultaneously maintaining crucial physicochemical parameters such as pH and soluble solids content during the

five-day storage period. Furthermore, the incorporation of *Syzygium aromaticum* (CEO) and *Mentha piperita* (MEO) essential oils into CWN demonstrated control over the incidence and severity of diseases in stored lychee fruits. The CWN (9%)-MEO (1%) combination was more effective in reducing the incidence and severity of post-harvest diseases. These findings highlight the potential of using edible coatings based on carnauba wax nanoemulsions (CWNs) at a concentration of 9% with essential oils, particularly *Mentha piperita* (MEO), in significantly extending the post-harvest shelf life of lychee. This sustainable and safe alternative not only reduces losses, but also ensures greater commercial viability, offering promising prospects for the preservation and commercialization of lychee on a wider scale.

## Ethical statement

This article does not contain any studies with human or animal subjects.

## Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Author contributions

Conny W. T. Fukuyama: writing – original draft, formal analysis, investigation. Larissa G. R. Duarte: conceptualization, data curation, investigation, methodology, project administration, visualization, writing – review & editing. Isadora C. Pedrino: investigation, formal analysis. Milene C. Mitsuyuki: formal analysis, software. Stanislau Bogusz Junior: methodology, project administration. Marcos D. Ferreira: conceptualization, funding acquisition, methodology, project administration, resources, supervision, writing – review & editing.

## Conflicts of interest

The authors declare no competing interests.

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