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Novel Approach for Improving Papaya Fruit Storage with Carnauba Wax Nanoemulsion in Combination with *Syzigium aromaticum* and *Mentha spicata* Essential Oils

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Abstract: Application of hydrophobic coatings, such as carnauba wax nanoemulsions, combined with natural antimicrobials, has been demonstrated to be an effective solution in extending the shelf life of fruits. The present study evaluated the effectiveness of carnauba wax nanoemulsion (CWN) coatings containing free or encapsulated with β-cyclodextrin (β-CD) essential oils of *Syzigium aromaticum* (CEO) and *Mentha spicata* (MEO) for the post-harvest conservation of papaya fruit. The chemical composition of the essential oils (EOs) was analyzed using GC-MS. Subsequently, coatings incorporating free and encapsulated EOs were prepared and applied to papaya fruit. Fruit was evaluated for post-harvest quality parameters during 15 days of storage. Clove essential oil presented as main compounds eugenol (89.73%), spearmint and carvone (68.88%), and limonene (20.34%). The observed reduction in weight loss in coated fruit can be attributed to the formation of a physical barrier provided by the coating. Compared to the control group, which experienced the highest weight loss of 24.85%, fruit coated with CWN and CWN-MEO:β-CD exhibited significantly lower weight loss percentages of only 5.78% and 7.5%, respectively. Compared to the control group, which exhibited a release of ethylene at a rate of 1.3 μg kg^{-1} h^{-1}, fruit coated with CWN, CWN-MEO:β-CD, and CWN-MEO coatings demonstrated a lower ethylene release rate at 0.7 μg kg^{-1} h^{-1}. Although the physical-chemical properties of papayas, including pH, Brix, titratable acidity, color, and texture, remained largely unchanged during storage with the coatings, analysis of incidence and severity of papaya post-harvest deterioration revealed that coatings containing essential oils effectively acted as antifungals in the fruit. Microscopy images showed that CWN and CWN-MEO:β-CD coatings are more uniform compared to the others. The edible coatings, especially CWN and CWN-MEO: β-CD, can act as antimicrobial coatings on papaya fruit, increasing their conservation during post-harvest storage.

Keywords: natural antifungal compounds; post-harvest; preservative; hydrophobic coatings

Citation: Oliveira Filho, J.G.d.; Duarte, L.G.R.; Silva, Y.B.B.; Milan, E.P.; Santos, H.V.; Moura, T.C.; Bandini, V.P.; Vitolano, L.E.S.; Nobre, J.J.C.; Moreira, C.T.; et al. Novel Approach for Improving Papaya Fruit Storage with Carnauba Wax Nanoemulsion in Combination with *Syzigium aromaticum* and *Mentha spicata* Essential Oils. *Coatings* **2023**, *13*, 847. https://doi.org/10.3390/coatings13050847

Academic Editors: María B. Pérez-Gago and Lluís Palou

Received: 25 March 2023
Revised: 24 April 2023
Accepted: 25 April 2023
Published: 28 April 2023

1. Introduction

Currently there is a higher demand for healthy foods without synthetic preservatives by consumers [1]. Furthermore, foods rich in vitamins such as fruit and vegetables are

highly perishable and susceptible to microbial deterioration [2–4]. Natural antimicrobial coatings have proven to be an excellent alternative to increase food shelf life [5,6].

Edible coatings based on lipids such as waxes and oils prevent the diffusion of water vapor and decrease the respiration rate because of their hydrophobic character [7,8]. The high rates of water loss and respiration in fruit lead to significant decreases in firmness, crispness, and weight resulting from biochemical changes that accelerate the process of deterioration [9,10].

Carnauba wax is extracted from the Brazilian palm tree *Copernicia prunifera* and is recognized as generally recognized as safe (GRAS) by the FDA, and its use is authorized by Anvisa, FAO-Food and Agriculture Organization of the United Nations, and the European Union [7,11,12].

Carnauba wax hydrophobicity is due to the high content of fatty alcohols and long-chain alkanes present in its structure [13], preventing water loss and also being described with antifungal action [14]. Nanoemulsions, with particle diameters ranging from 10 to 100 nm, possess higher clarity and translucency compared to conventional emulsions as their average size is smaller than the visible light wavelength ($r << \lambda$) [15,16]. Moreover, decreasing the particle diameter offers a promising strategy for generating more thermodynamically stable emulsions [17]. Previous studies have demonstrated that carnauba wax nanoemulsion has great potential as a fruit coating material, capable of prolonging their shelf life and imparting shine [7,12,18].

In order to impart antimicrobial properties to coatings, natural antimicrobial agents such as essential oils have been incorporated [12]. Essential oils have also been considered GRAS substances by the FDA since 2008 [19]. In addition to being hydrophobic, essential oils have antimicrobial activity [20,21] which may vary depending on the composition of the oil and species of microorganisms [22].

Syzigium aromaticum EO's main compound is eugenol, showing antimicrobial, antiviral and antioxidant activity [23,24]. On the other hand, for *Mentha spicata*, carvone is the main one, which is also an antioxidant, and due to this bioactivity, it increased shelf life of fresh meats [25]. The antimicrobial activity of carvone was proven in the study by [26] with the inhibition of the growth of the fungus *Colletotrichum gloeosporioides* in papayas and with antifungal action against *Botrytis cinerea* in plums [27].

Natural antimicrobials such as essential oils can be added directly to foods, acting as biopreservatives [28–30]. However, the effectiveness of these bioactive compounds can be impaired by their high volatility and they are prone to degradation caused by exposure to light, high temperatures, and the presence of oxygen [22,31]. In addition, essential oils have low solubility in water and a very intense aroma that can interfere with the sensory attributes of the food to which they are applied [32,33]. Encapsulation of essential oils offers potential solutions to overcome several challenges, including enhanced stability and protection, better control over compound release, reduced intensity of flavors and odors, prolonged shelf life, and improved bioavailability and palatability of the encapsulated materials [34].

Several studies have investigated the antibacterial properties of green mint nanoemulsions [35], while others have explored the antifungal potential of essential oil nanoemulsions containing thymol and were incorporated into quinoa and chitosan films [36]. However, there is a scarcity of research focused on nanoemulsions containing encapsulated clove and mint oils despite their notable antifungal activity and potential to provide a safer and more natural alternative to conventional antifungal agents.

The primary objective of this study was to assess the effectiveness of edible coatings composed of nanoemulsions containing carnauba wax, *S. aromaticum*, and *M. spicata* essential oils in preserving papayas. A post-harvest quality evaluation was conducted to determine the impact of free and encapsulated essential oils in the coatings on the retardation of fruit ripening and deterioration.

2. Materials and Methods

2.1. Materials

A sample of Carnauba wax type I with 99% purity and CAS No.: 8015-86-9 was obtained from Pontes Indústria de Cera in Fortaleza, CE, Brazil. MEO and CEO were acquired from Laszlo Aromaterapia in Belo Horizonte, MG, Brazil. Papaya fruit of the cultivar THB from the solo group were transported from a commercial farm in Bahia State to the postharvest laboratory at Embrapa Instrumentação in São Carlos, SP, where they were sanitized using a specialized fruit detergent and chlorine dioxide. Only papayas that lacked standard defects, met size requirements, and were at stage 1 of maturation (with less than 15% of their skin surface covered in yellow) were selected for the study [37].

2.2. Essential Oil Composition

A qualitative analysis of essential oils (EOs) was conducted via gas chromatography using a Shimadzu (GC-2010 Plus, Kyoto, Japan) coupled with a quadrupole mass spectrometer (GC-MS). A non-polar DB-5MS capillary column (30 m $\times$ 0.25 mm, i.d. $\times$ 0.25 µm) was used for gas chromatography analyses with helium as the carrier gas at a flow rate of 1 mL/min. Essential oil samples were diluted in dichloromethane (10% v/v) and injected (1 µL) in a split mode (1:50). The chromatographic conditions were as follows: injector temperature: 220 °C, oven temperature: 60 to 240 °C at 3 °C/min; interface: 240 °C; ion source: +70 eV, m/z: 35–350. The linear temperature programmed retention index (RI) was calculated using an alkane solution (C_7–C_{30}). Identification of analytes was conducted by comparing the RI and mass spectra obtained from the sample with mass spectra and RI of the literature, with at least 85% similarity for the mass spectra and maximum variation in RI of $\pm$ 10. The identification of analytes was confirmed by co-injection of authentic standards whenever available. Semi-quantitative analysis of essential oils (% relative area) was performed using the flame ionization detector (GC-FID) in the same gas chromatography system. All qualitative and semi-quantitative analyses were performed in triplicate.

2.3. Encapsulation of Essential Oils with β-Cyclodextrin

MEO:β-CD and CEO:β-CD microcapsules were prepared by the co-precipitation method as reported [38]. The MEO:β-CD and CEO:β-CD ratios of 10:90 and 20:80 (% w/w), respectively, were selected as these ratios provides the maximum inclusion of MEO or CEO in β-CD according to previous tests. Obtained MEO:β-CD and CEO:β-CD microcapsules were stored in a desiccator at 25 °C until use.

2.4. Edible Coating Preparation

A carnauba wax nanoemulsion (CWN) was prepared using an oil phase and water phase via a high-pressure process with ammonia in a morpholine-free method adapted for this study [39] in a high-pressure process. The diameter size of the CWN obtained was 44.1 $\pm$ 7.6 nm with a narrow polydispersion index of 0.28 and a zeta potential of $-$43.8 mV as measured by the Zetasizer Nano ZS (Malvern Instruments Inc., Westborough, MA, USA) [40]. The incorporation of MEO and CEO free and microencapsulated, as antimicrobial agents, was done by mixing the 1.0% concentration with CWN in a high-speed mixer (UltraTurrax T25, IKA Werke GmbH & Co, Staufen, Alemanha) for 5 min at 5.000 rpm.

The coatings were applied to the fruit, which were randomly divided into 6 treatment groups as follows: CWN (9% solid phase in suspension), CWN (9%) with MEO (1%), CWN (9%) with CEO (1%), CWN (9%) with MEO:β-CD (1%), CWN (9%) with CEO:β-CD (1%), and non-treated fruit as a control. The coatings were applied manually by pouring 1 mL of the coating solution onto latex-gloved hands and then manually spreading it on sanitized papayas. For non-destructive analyses, five papayas were used per treatment, and for destructive analyses, ten papayas were used. The fruit was stored for 15 days at 16 °C and a relative humidity of 70%. The quality attributes of the papayas were evaluated at the beginning of the experiment (0 days) and after 5, 10, and 15 days of storage.

2.5. Physicochemical Parameters of Papayas

The fruit weight loss was determined using the [41] standard method by measuring the fruit weight on day 0 (start of the experiment) and on days 5, 10, and 15 of storage. The percentage difference between the initial and final weight on each day was used to calculate the weight loss.

The soluble solids (SS) content was measured with an Atago RX-5000cx digital refractometer (Tokyo, Japan) and expressed as Brix following the [41] standard method. The pH of the samples was assessed using a PHS-3B digital pH meter following the same standard method. The titratable acidity was determined using 0.1 N NaOH and phenolphthalein as an indicator, and the results were expressed as g of citric acid per 100 g of fruit.

The color measurements were performed on the external surface of the fruit (on the peel) with a Konica Minolta CR-400 colorimeter (Konica Minolta, Osaka, Japan) equipped with a C illuminant using the CIELAB scale. Hue angle (h°), chroma (C^*), and total color difference (ΔE) were calculated with Equations (1)–(3), respectively.

$$h^{\circ} = tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (1)$$

$$C^* = \left((a^*)^2 + (b^*)^2\right)^{1/2} \quad (2)$$

$$\Delta E^* = \sqrt{(L_t{}^* - L_{t0}{}^*)^2 + (a_t{}^* - a_{t0}{}^*)^2 + (b_t{}^* - b_{t0}{}^*)^2} \quad (3)$$

where subscripts t and 0 correspond to parameters evaluated at time t and at the beginning of the study, respectively.

The firmness of the fruit was assessed using a digital TA.XTplus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with a 6 mm diameter probe, 15 mm/s velocity, 5 mm penetration distance, and 12 mm^2 contact area with the peel removed. The results were reported in Newtons (N) and the mean value was calculated based on three penetrations in the distal region of each fruit. All analyses were performed in triplicate and the data were presented as mean ± standard deviation.

2.6. Respiration Rate and Ethylene of Papayas

The respiration rate was determined following the method described by [42], using a respirometer (model 6600, Illinois Instrument, Inc., Johnsburg, IL, USA). Two papayas were placed in 2000 mL glass containers with a silicone septum in the lids, which were hermetically sealed. The concentrations of O_2 and CO_2 were measured at each time point by suctioning air samples from the containers using a paramagnetic sensor and an infrared sensor, respectively. Ethylene production was determined according to the method described by [18]. Two papayas of the same treatment were packed in pairs in hermetic glass jars with screw caps and held for 2 h. At the end of this period, 1 mL of the headspace was collected through a rubber septum located on the cap. This volume was injected with Varian Gas Chromatograph model CP 3800, with TCD/FID detectors, in order to detect the peaks corresponding to ethylene. Results were expressed in $\mu g \cdot kg^{-1} \cdot h^{-1}$. All analyses were carried out in triplicate, and the data were calculated as means ± standard deviations.

2.7. Scanning Electron Microscopy

Images of papaya peels with or without coating were determined according to [43] by emission gun scanning electron microscopy (SEM-SEM JEOL JSM-6701F, Tokyo, Japan). Surface and fracture micrographs of the fruit peel were obtained. Both were first dried and then coated with gold. The accelerating voltage used for microscopy was 10 kV.

2.8. Decay Percentage and Severity on Papayas

The presence or absence of mold growth in papayas during storage was evaluated visually, and any visible spoilage was considered as decay. The percentage of decay was

determined based on the number of decayed papayas per treatment, with each treatment having ten papayas. The severity of the disease in the fruit was assessed using a six-point scale (0 = no symptoms; 1 = 1%–20% affected area; 2 = 21%–40%; 3 = 41%–60%; 4 = 61%–80%; and 5 = 81%–100%) and was used to evaluate the antifungal activity of the treatments [12].

2.9. Statistical Analysis

Data were described using means and standard deviations and comparison of means was performed by parametric analysis of variance and Duncan's multiple comparisons test or non-parametric ANOVA and Kruskal–Wallis multiple comparisons test, depending on the homogeneity condition of variance, verified by the Bartlett test, or the level of measurement of the response variable. The significance level was set at 5% and the software used for the analyses was R version 4.2.2.

3. Results and Discussion

3.1. Essential Oil Composition

The major compounds of clove (*Syzigium aromaticum*) and spearmint (*Mentha spicata*) essential oils obtained from chromatograms are shown in Table 1. Clove essential oil presented as main compounds eugenol (89.73%), spearmint and carvone (68.88%), and limonene (20.34%). These results are close to those found by [44], who obtained eugenol values (70.58%), and [45], who found 96.33% eugenol for clove oil. Reference [46] obtained 62.9% carvone and 8.5% limonene for spearmint oil. The variation in chemical composition in the comparison of the mentioned works may be due to factors such as geographic origin, environmental conditions, age and part of the plant, seasonal and climatic conditions, genetic factors, and even plant nutrition [47,48].

Table 1. Composition of essential oils.

Compound	*Syzigium aromaticum* (% Area)	*Mentha spicata* (% Area)
α-Pinene	-	0.69
Sabinene	-	0.32
β-Pinene	-	0.76
Myrcene	-	0.95
3-Octanol	-	0.25
p-Cymene	-	0.23
Limonene	-	20.34
1,8-Cineol	-	1.10
γ-Terpinene	-	0.13
Menthone	-	0.50
cis-Sabinene hydrate	-	0.17
Menthol	-	0.15
Isomenthol	-	1.06
(E)-dihydrocarvone	-	1.40
cis-Dihydrocarvone	-	0.15
trans-Carveol	-	0.28
Carvone	-	68.88
Piperitone	-	0.18
Menthyl acetate	-	0.39
Dihydrocarvyl acetate	-	0.13
Eugenol	89.73	-
cis-Carvyl acetate	-	0.11
β-bourbenene	-	0.77
β-Gurjenene	7.59	-
Caryophyllene	-	1.03
α-Humulene	2.10	-
γ-Selinene	0.20	-
δ-Cadinene	0.25	-
Caryophyllene oxide	0.13	-
Total	100	99.97

Eugenol, present in clove essential oil, is a phytochemical that confers antimicrobial and antioxidant properties in addition to the characteristic flavor and odor of this oil [49–51].

The antifungal efficacy of carvone present in spearmint essential oil has been proven in other studies [52,53].

3.2. Physicochemical Parameters of Papayas

Based on the results presented in Figure 1, the control group exhibited the greatest weight loss (24.85%) after 15 days of storage, which was significantly different from all other treatments. In contrast, papayas treated with only carnauba wax nanoemulsion demonstrated the lowest weight loss (5.78%), followed by CWN-MEO:β-CD, which exhibited 7.5% weight loss. Comparison of the treatments containing essential oils revealed that papayas treated with carnauba nanoemulsion containing MEO, either free or encapsulated, exhibited lower weight loss, particularly during the first 10 days of storage, compared to those treated with CEO.

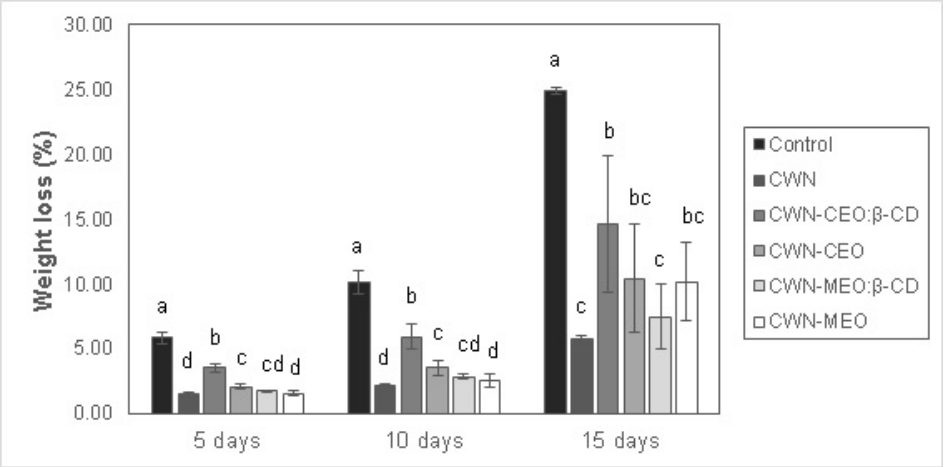

Figure 1. Papaya weight loss during storage time at 16 °C and 70% RH. For each storage period, different letters indicate significant differences between treatments ($p < 0.05$).

Fruit weight loss occurs through transpiration due to the respiration that takes place in the stomata of the epidermis [54,55]. The main component of the coating, carnauba wax, is highly hydrophobic; therefore, the coating acted as a barrier to gas exchange and thus reduced the transpiration rate of the fruit [7,56]. Similar results were obtained in cucumbers [57], apples [58], and papayas [12].

Ideal coatings should allow controlled gas exchange, avoiding the formation of anaerobic conditions and the accumulation of undesirable compounds, such as acetaldehydes and other off-flavors [59]. Since the weight loss of all treatments involving carnauba wax nanoemulsion with or without essential oils differed significantly from that of the control, the findings suggest that the coatings created a physical barrier that reduced the extent of fruit weight loss.

The results of pH, titratable acidity, and soluble solids analysis of papayas are presented in Table 2. The pH values of all treatments increased over time and were not significantly different among them (Table 2). A delay in the fruit ripening process occurs when there is a decrease in the use of some organic acids that are converted into sugars [12,60].

The increase in TSS values is directly related to the ripening of the fruit; as time passes, starch hydrolysis occurs and consequently the synthesis of sucrose and hexose in plant tissues [28,61]. The increase in soluble solids content is also attributed to a reduction in the water content of the fruit, resulting in a higher concentration of soluble solids [62,63].

Over time, the TA values of fruit tend to decrease as organic acids such as citric acid are used up during respiration [64,65]. Even though there were no significant differences in the pH, TA, and TSS values, the weight loss results indicate that the coating process inhibited the fruit's respiratory system.

Table 3 shows the values of the color parameters L^*, C^*, h°, and ΔE^*. The luminosity values (L^*) decreased over time for all treatments, indicating fruit ripening [66], with the exception of the group with encapsulated spearmint essential oils (CWN-MEO:β-CD), which showed higher values compared to the first day. The chroma (C^*) values of all groups also decreased, especially after the 5th day of storage, due to oxidative phenomena and the synthesis of papaya pigments such as carotene, lycopene, and anthocyanins during storage [67]. At the end of 15 days, the h° values of all treatments also decreased; as papaya matures, its color changes from greenish to yellowish due to chlorophyll degradation and carotenoid biosynthesis [68]. However, the CWN-MEO:β-CD treatment showed a statistical difference compared to the other treatments in relation to h° values. These changes in color parameters led to an increase in the total color difference (ΔE) of the fruits during storage, highlighting differences in h° values compared to day 0 (Table 3). By the end of 15 days, the fruits showed significantly equal ΔE values, indicating visible similarity among treatments.

During storage, fruit firmness decreased over time for all groups (as shown in Table 4). On the last day of storage (15th day), CWN-CEO showed the lowest reduction in firmness, while CWN-MEO showed the highest reduction (Table 4). The lowest firmness values observed for CWN-MEO may be the result of the interaction of the components of this oil with the cellular tissue of the fruit, causing structural changes that lead to softening and an increased release of enzymes or substrates that favor this process [69,70]. Some essential oils, depending on the concentration, can penetrate the cell tissue of the fruit and cause structural changes, decreasing firmness [6]. A similar behavior was reported by [71] for fresh-cut melons with alginate-based coatings that contained geraniol.

Table 2. Titratable acidity (TA), soluble solid content (TSS), and pH of fruit over time.

Treatments	pH			TSS (%)			TA (%)		
Storage Time (Days)	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**
Control	5.35 ± 0.14 ^{b}	5.68 ± 0.10 ^{b}	6.05 ± 0.19 ^{ab}	10.02 ± 1.44 ^{a}	8.06 ± 1.45 ^{b}	8.97 ± 2.31 ^{a}	0.066 ± 0.013 ^{bc}	0.097 ± 0.090 ^{a}	0.055 ± 0.014 ^{a}
CWN	5.64 ± 0.05 ^{a}	6.00 ± 0.13 ^{a}	6.28 ± 0.23 ^{a}	8.59 ± 1.51 ^{a}	8.87 ± 1.19 ^{ab}	7.75 ± 1.32 ^{a}	0.065 ± 0.009 ^{c}	0.052 ± 0.007 ^{a}	0.059 ± 0.010 ^{a}
CWN-CEO:β-CD	5.48 ± 0.11 ^{ab}	5.69 ± 0.14 ^{b}	6.21 ± 0.14 ^{ab}	8.76 ± 0.76 ^{a}	9.43 ± 1.40 ^{ab}	9.27 ± 1.99 ^{a}	0.070 ± 0.010 ^{bc}	0.057 ± 0.005 ^{a}	0.051 ± 0.009 ^{a}
CWN-CEO	5.49 ± 0.08 ^{ab}	5.61 ± 0.21 ^{b}	6.09 ± 0.12 ^{ab}	8.80 ± 0.87 ^{a}	9.02 ± 0.67 ^{ab}	7.43 ± 1.20 ^{a}	0.085 ± 0.012 ^{a}	0.056 ± 0.005 ^{a}	0.050 ± 0.008 ^{a}
CWN-MEO:β-CD	5.45 ± 0.10 ^{ab}	5.54 ± 0.14 ^{b}	6.11 ± 0.10 ^{ab}	9.02 ± 0.70 ^{a}	8.67 ± 0.81 ^{b}	7.90 ± 1.11 ^{a}	0.081 ± 0.009 ^{ab}	0.051 ± 0.006 ^{a}	0.059 ± 0.006 ^{a}
CWN-MEO	5.46 ± 0.27 ^{ab}	5.72 ± 0.21 ^{b}	6.00 ± 0.24 ^{b}	9.84 ± 1.24 ^{a}	10.37 ± 1.17 ^{a}	8.25 ± 1.90 ^{a}	0.068 ± 0.004 ^{bc}	0.048 ± 0.011 ^{a}	0.056 ± 0.020 ^{a}

Means followed by different letters on the same column indicate significant differences between treatments ($p < 0.05$).

Table 3. Color parameters L*, C*, h°, and ΔE of papayas stored for 15 days at 16 °C and 70% RH.

Treatments	Time (Days)														
	0			**5**				**10**				**15**			
	L*	*C**	(*h*°)	***L****	*C**	(*h*°)	**Δ*E****	***L****	*C**	(*h*°)	**Δ*E****	***L****	*C**	(*h*°)	**Δ*E****
Control	54.85 ± 3.91 ^{a}	39.00 ± 2.13 ^{a}	102.92 ± 2.55 ^{a}	61.48 ± 5.77 ^{a}	49.53 ± 6.98 ^{a}	101.74 ± 9.47 ^{a}	13.73 ± 5.74 ^{a}	53.76 ± 6.93 ^{a}	27.00 ± 4.31 ^{a}	88.83 ± 11.20 ^{a}	16.60 ± 1.54 ^{a}	47.52 ± 10.88 ^{b}	22.55 ± 7.14 ^{b}	74.59 ± 12.82 ^{a}	24.31 ± 6.82 ^{a}
CWN	52.00 ± 3.39 ^{b}	37.43 ± 1.89 ^{a}	103.70 ± 2.34 ^{a}	49.37 ± 4.75 ^{c}	23.73 ± 3.05 ^{b}	102.44 ± 7.42 ^{a}	14.55 ± 3.05 ^{a}	52.53 ± 6.55 ^{a}	25.92 ± 3.06 ^{a}	96.70 ± 10.69 ^{a}	14.32 ± 2.41 ^{a}	50.84 ± 8.58 ^{b}	24.85 ± 4.75 ^{b}	85.66 ± 15.19 ^{a}	18.45 ± 3.33 ^{a}
CWN-CEO:β-CD	53.10 ± 3.40 ^{ab}	37.63 ± 2.04 ^{a}	104.08 ± 2.33 ^{a}	53.28 ± 7.65 ^{bc}	31.79 ± 11.88 ^{b}	103.79 ± 9.23 ^{a}	13.14 ± 6.30 ^{a}	54.43 ± 6.51 ^{a}	27.80 ± 3.47 ^{a}	92.87 ± 10.63 ^{a}	14.15 ± 2.78 ^{a}	49.83 ± 10.22 ^{b}	24.58 ± 4.24 ^{b}	80.35 ± 14.02 ^{a}	20.19 ± 3.78 ^{a}
CWN-CEO	54.86 ± 4.94 ^{a}	38.45 ± 3.75 ^{a}	102.89 ± 2.97 ^{a}	61.49 ± 4.76 ^{a}	48.17 ± 5.11 ^{a}	103.86 ± 5.61 ^{a}	12.32 ± 5.16 ^{a}	56.55 ± 6.08 ^{a}	28.43 ± 3.84 ^{a}	87.93 ± 8.81 ^{a}	15.15 ± 2.54 ^{a}	52.44 ± 10.35 ^{ab}	26.21 ± 6.18 ^{b}	74.64 ± 10.14 ^{a}	21.59 ± 3.42 ^{a}
CWN-MEO:β-CD	53.63 ± 4.10 ^{ab}	37.11 ± 2.60 ^{a}	103.82 ± 2.66 ^{a}	53.96 ± 5.89 ^{bc}	26.25 ± 3.31 ^{b}	97.05 ± 30.40 ^{ab}	12.17 ± 1.27 ^{a}	58.54 ± 5.65 ^{a}	30.05 ± 3.48 ^{a}	86.57 ± 27.61 ^{a}	14.17 ± 3.51 ^{a}	58.01 ± 7.39 ^{a}	31.75 ± 4.56 ^{a}	71.07 ± 29.77 ^{a}	21.28 ± 3.73 ^{a}
CWN-MEO	55.16 ± 4.21 ^{a}	39.71 ± 4.25 ^{a}	101.21 ± 4.05 ^{b}	54.38 ± 5.80 ^{b}	26.69 ± 3.84 ^{b}	94.43 ± 9.61 ^{b}	14.48 ± 2.01 ^{a}	57.84 ± 5.97 ^{a}	29.38 ± 4.21 ^{a}	87.37 ± 10.81 ^{a}	15.03 ± 2.49 ^{a}	50.84 ± 8.58 ^{b}	24.85 ± 4.75 ^{b}	85.66 ± 15.19 ^{a}	20.63 ± 5.07 ^{a}

Means followed by different letters on the same column indicate significant differences between treatments ($p < 0.05$).

Table 4. Firmness (N) of papaya during storage for 15 days at 16 °C and 70% RH.

Treatments	Storage Time (Days)		
	5	**10**	**15**
Control	5.46 ± 2.22 ^{a}	5.52 ± 3.07 ^{a}	4.02 ± 3.07 ^{ab}
CWN	4.32 ± 3.02 ^{a}	3.68 ± 1.33 ^{a}	4.68 ± 2.20 ^{ab}
CWN-CEO:β-CD	5.53 ± 3.10 ^{a}	4.34 ± 0.92 ^{a}	3.87 ± 2.30 ^{ab}
CWN-CEO	6.59 ± 3.79 ^{a}	3.83 ± 1.04 ^{a}	6.60 ± 2.41 ^{a}
CWN-MEO:β-CD	5.65 ± 3.35 ^{a}	7.12 ± 2.10 ^{a}	4.71 ± 1.70 ^{ab}
CWN-MEO	5.15 ± 2.98 ^{a}	3.88 ± 0.32 ^{a}	2.82 ± 0.58 ^{b}

Means followed by different letters on the same column indicate significant differences between treatments ($p < 0.05$).

The effectiveness of the carnauba wax nanoemulsion coating without and with essential oils in ethylene release, CO_2 production rate, and O_2 consumption rate of papayas can be seen in Figure 2. Applying coatings to papayas during storage resulted in a reduction in the ethylene production of the fruit, ultimately leading to delayed maturation [72]. No significant difference in ethylene levels during storage was observed among the different coatings. Ethylene is a hormone related to fruit ripening, and high levels indicate fast ripening [73]. Similar behavior was observed by [74] for plums coated with hydroxypropylmethylcellulose and two different essential oils (oregano essential oil (OEO) and bergamot essential oil (BEO)), fruit coated with H-OEO showed no significant difference in the production of ethylene compared to fruit coated with H-BEO, and both oils were effective in reducing and delaying ethylene production.

The levels of CO_2 and O_2 were also measured during storage and are shown in Figure 2. The control group showed a significant increase in CO_2 production (Figure 2B) and O_2 consumption (Figure 2C), indicating high metabolism and accelerated ripening, which ultimately led to a shorter shelf life. Regarding the coating treatments, it is worth noting that fruit coated with CWN exhibited lower CO_2 production after 5, 10, and 15 days of storage, as well as lower O_2 consumption.

The balance between those two gases enhances post-harvest life. High levels of CO_2 in the fruit restrict the Krebs cycle and low levels of O_2 inhibit the activities of respiratory enzymes [75]. Association with OEs did not show a significant decrease in CO_2 or in O_2 at 5 and 10 days of storage. It can be seen that the CWN-CEO coating presented O_2 concentrations close to that of the control (Figure 2C) as coatings can present different degrees of permeability due to the formation of irregular structures and thicknesses during film consolidation [41]. However, at 15 days of storage, the treatments wit OE encapsulated demonstrated a reduction on O_2 consumption when compared to control, a possible indication of reduction in metabolism.

3.3. Scanning Electron Microscopy

For a better understanding of the deposition of the coatings on the fruit, microscopic analyses were carried out as shown in Figure 3 (micrographs of the surface of the peels and micrographs of the fractures of the peels). Microscopic analysis showed that the CWN and CWN-MEO:β-CD coatings were more uniformly applied over the fruit surface compared to the other treatments, which exhibited more cleavage or cracking. This result is consistent with the findings for the CWN coating, which showed the lowest weight loss (Figure 1) and the highest inhibition of ethylene biosynthesis and gas exchange (CO_2 and O_2) (Figure 2) in papayas, followed by the CWN-MEO:β-CD coating.

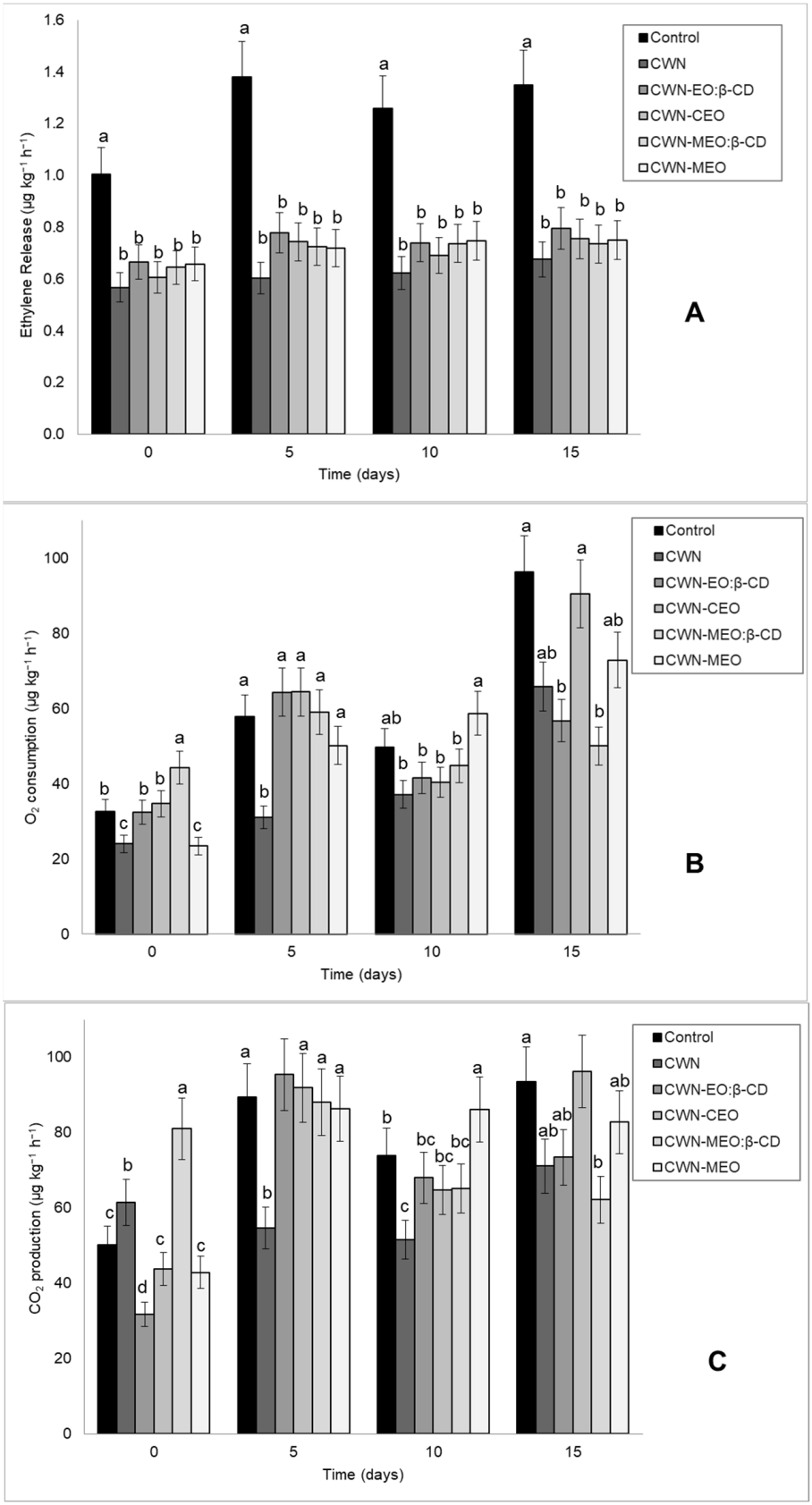

Figure 2. (**A**) Ethylene release, (**B**) CO_2 production rate, and (**C**) O_2 consumption rate of papayas during storage for 15 days at 16 °C and 70% RH. For each storage period, different letters indicate significant differences between treatments ($p < 0.05$).

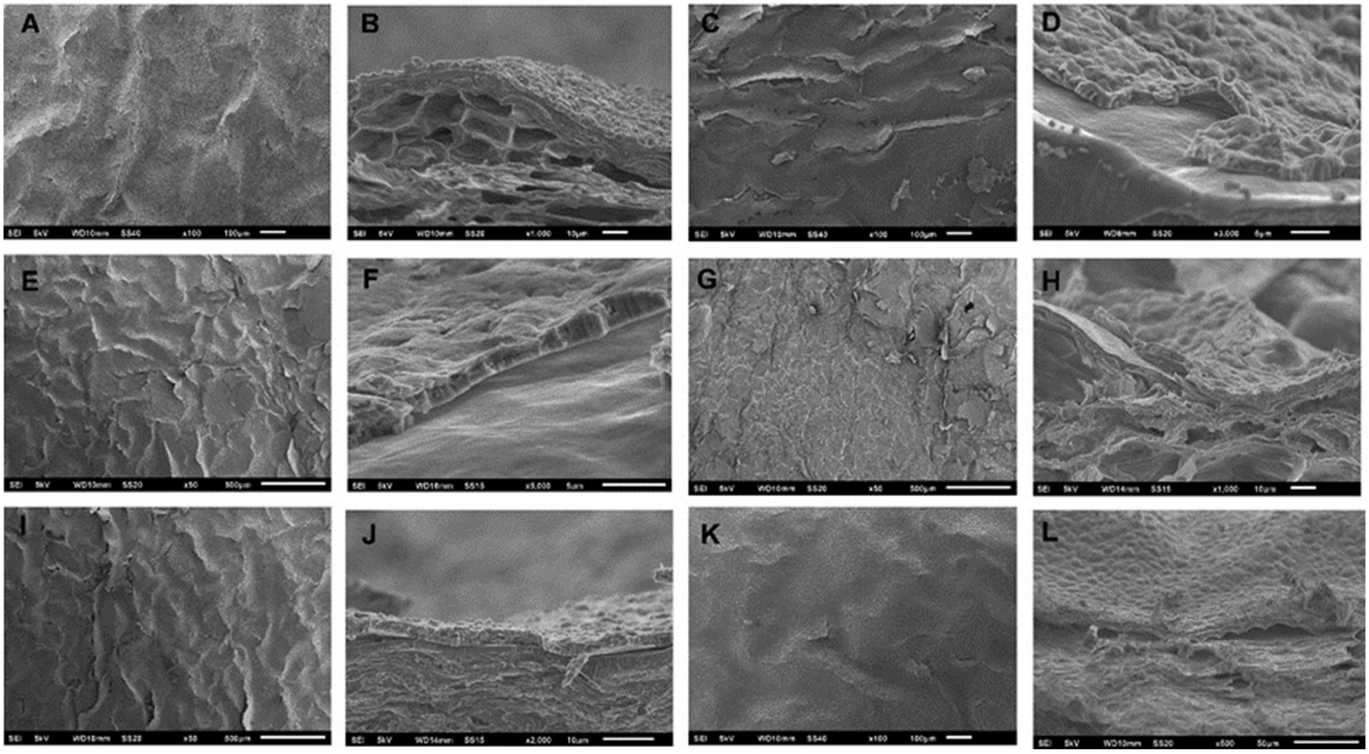

Figure 3. Micrographs of the surface (magnification 50× and scale bar 500 μm) and fractures (magnification 2000× and scale bar 10 μm) of the peels of papaya Control (**A**,**B**), CWN (**C**,**D**) CWN-CEO:β-CD (**E**,**F**) CWN-CEO (**G**,**H**) CWN-MEO:β-CD (**I**,**J**) and CWN-MEO (**K**,**L**).

The greater the chemical homogeneity of the nanometric coatings, the greater the uniformity and adhesion to the fruit [76]. CWN presents a regular structure due to the stability of the nanoemulsion (with the diameter size parameters of 44, 1 nm, PDI 0.28, and zeta potential of −43.8 mV) [38]. The uniformity of CWN-MEO:β-CD is due to encapsulation avoiding aggregation and flocculation of EO droplets ensuring a better distribution of EO in coatings [77]. However, the chemical composition of the EO determines its polarity and viscosity; thus, the type of EO can affect the average droplet size of the nanoemulsion [78], so CWN-CEO:β-CD may not have shown as much uniformity compared to CWN-MEO:β-CD (Figure 3).

3.4. Decay Percentage and Severity on Papayas

Coatings reduced postharvest disease incidence (Figure 4A) and severity (Figure 4B–D) in papayas when compared to control fruit. The CWN-MEO:β-CD coating showed the lowest incidence of disease compared to the other treatments at the end of 15 days. Encapsulated essential oils show greater stability in vivo tests due to the slow release of active compounds from the EO, reducing fruit rot in the long term. This behavior was also described by [79], who developed polylactic acid (PLA) nanocapsules with lemongrass EO and evaluated in vivo against the postharvest activity of *C. gloeosporioides* in apples.

Coatings with CWN-CEO and CWN-MEO essential oils had the lowest rot severities with 100% and 90% scores of 1%–20% affected area, respectively, at the end of the storage period. *S. aromaticum* and *M. spicata* essential oils added to CWN acted as antifungals. The antifungal mechanism of essential oils is through depolarization of the mitochondrial membrane and consequently greater cell permeability and imbalance in ion transport and thus cell death by apoptosis [80]. The antifungal action of the oils delayed fruit rot.

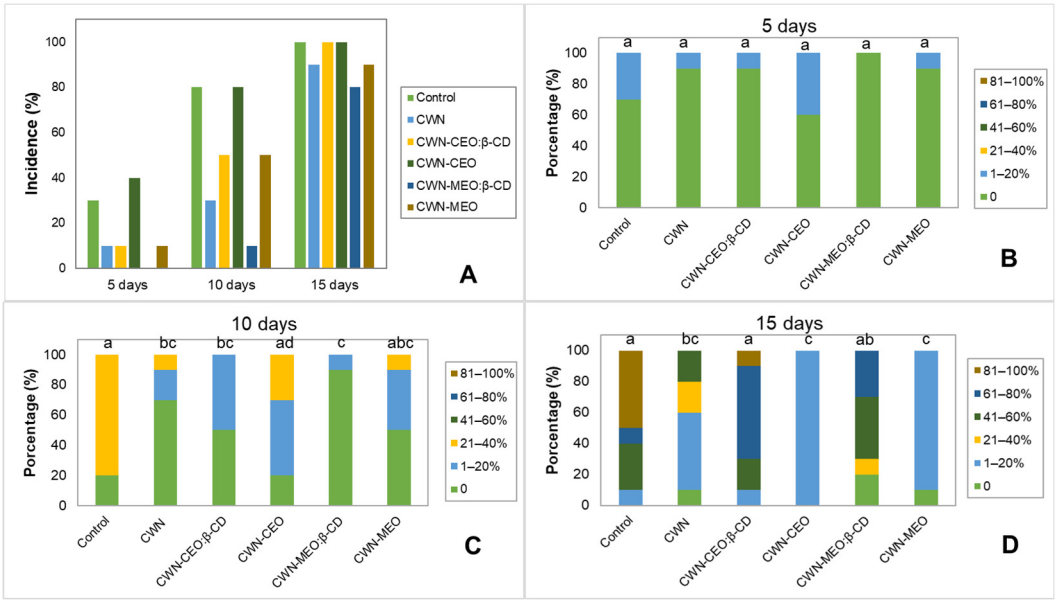

Figure 4. Fruit incidence (**A**) and severity of papaya post-harvest deterioration after 5 days (**B**), 10 days (**C**), and 15 days (**D**) of storage at 16 °C. For each storage period, different letters indicate significant differences between treatments ($p < 0.05$).

4. Conclusions

CWN coatings with or without essential oils reduced weight loss and delayed fruit rot due to physical barrier on gas exchange and presence of antifungal compounds. The microscopy images indicated that the CWN and CWN-MEO:β-CD coatings exhibited more uniformity and improved stability resulting from encapsulation in spearmint oil. The coating based on CWN-MEO was less effective in reducing fruit firmness loss due to negative interactions between MEO components and fruit tissue. The CWN-CEO-based coating was also inefficient in reducing the respiration rate of the fruit. Additionally, this coating did not show good uniformity when applied to papaya fruit, as observed in SEM images. This lack of uniformity negatively impacted gas exchange reduction, resulting in low coating efficiency. Coatings with carnauba nanoemulsion and essential oils inhibited the growth of fungi evaluated by the incidence and severity in the fruit. Therefore, CWN coatings with essential oils delayed fruit rot and thus can be a good alternative for natural antifungals and fruit preservation.

Author Contributions: Conceptualization, J.G.d.O.F. and M.D.F.; methodology, J.G.d.O.F., S.B.J. and M.D.F.; software, M.C.M.; formal analysis, L.G.R.D., Y.B.B.S. and M.C.M.; investigation, Y.B.B.S., E.P.M., H.V.S., T.C.M., V.P.B., L.E.S.V., J.J.C.N. and C.T.M.; resources, S.B.J. and M.D.F.; data curation, J.G.d.O.F.; writing—original draft preparation, J.G.d.O.F. and L.G.R.D.; writing—review and editing, M.D.F.; visualization, J.G.d.O.F. and L.G.R.D.; supervision, S.B.J. and M.D.F.; project administration, M.D.F. and J.G.d.O.F.; funding acquisition, M.D.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Embrapa (process 20.19.03.024.00.00), FAPESP (process 2022/10686-6), CAPES (Grant No. 001), and the Brazilian National Council for Scientific and Technological Development-CNPq (process 381280/2023-3).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article.

Acknowledgments: The authors thank Empresa Brasileira de Pesquisa Agropecuária–Embrapa, Rede Agronano, and CNPq/MCTI Sisnano (process 442575/2019-0) from Brazil. Ferreira thanks CNPq for his Research Productivity fellowship (process 307141/2022-5).

Conflicts of Interest: The authors declare no conflict of interest.

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