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# Impact of Chitosan Coatings Enriched with Clove Essential Oil on Quality of Minimally Processed Strawberries

**Sandriane Pizato**<sup>1\*</sup>

<https://orcid.org/0000-0002-4184-7457>

**Sergio Sebastian Vega-Herrera**<sup>2</sup>

<https://orcid.org/0000-0003-1930-1439>

**Raquel Costa Chevalier**<sup>3</sup>

<https://orcid.org/0000-0003-2738-0050>

**Rosalinda Arevalo Pinedo**<sup>4</sup>

<https://orcid.org/0000-0001-7413-3322>

**William Renzo Cortez-Vega**<sup>4</sup>

<https://orcid.org/0000-0001-7772-1998>

<sup>1</sup>Universidade Federal do Amazonas, Faculdade de Ciências Agrárias, Departamento de Engenharia Agrícola e de Solos, Manaus, Amazonas, Brasil; <sup>2</sup>Universidade Federal da Grande Dourados, Faculdade de Ciências Biológicas e Ambientais, Dourados, Mato Grosso do Sul, Brasil; <sup>3</sup>Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos, Departamento de Engenharia de Alimentos, Campinas, São Paulo, Brasil; <sup>4</sup>Universidade Federal da Grande Dourados, Faculdade de Engenharia, Departamento de Engenharia de Alimentos, Dourados, Mato Grosso do Sul, Brasil.

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\*Correspondence: sandrianepizato@yahoo.com.br; Tel.: +55-67-981003564 (S.P.).

## HIGHLIGHTS

- Chitosan and clove essential oil to improve the characteristics of strawberries.
- Different treatments were applied in minimally processed strawberries.
- The applied coatings were efficient to increase the preservation time of strawberries.
- T3 treatment presented better results in retarding the growth of molds and yeasts.

**Abstract:** The work aimed to evaluate the effects of applying chitosan edible coating with the addition of different concentrations of clove essential oil in minimally processed strawberries. The strawberries were selected and sanitized, had their leaves removed, and were submerged in the coatings. Three treatments were obtained: T1 - control (uncoated strawberries); T2 - 2% chitosan + 1% clove essential oil; T3 - 2% chitosan + 1.5% clove essential oil. After receiving the coatings, the strawberries were placed in PET containers and stored at 5±1°C for 12 days. Physical, chemical and microbiological analysis were performed. T2 and T3 samples were more efficient in reducing mass loss (13.78% and 13.51%) when compared the control sample

(24.19%). They were also effective in maintaining texture and color for longer. The treatment T3 was more effective to increasing the shelf life and slowing growth, especially of molds and yeasts. The use of different concentrations of clove oil prolonged the quality of strawberries during refrigerated storage.

**Keywords:** chitosan; physicochemical; shelf life; microbiological analysis; clove oil.

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## INTRODUCTION

Strawberry (*Fragaria ananassa*) is a non-climacteric fruit containing a great variety of bioactive compounds including phenolic constituents, anthocyanins, vitamins and minerals [1]. The changes in fruit composition are mainly due to the ripening process which may take place after harvesting and/or even when the fruit is attached to the mother plant. The continuous active metabolism and high physiological activities after harvesting the strawberries leads to reduction in senescence and ripening periods [2,3]. In addition, the mechanical injuries, excessive texture softening, spoilage caused by microbial growth, moisture loss, and physiological disorders also contributes to heavy damage to strawberries during growth, harvesting and storage. Due to these factors, the shelf life of strawberries is very short (1–2 days) at normal storage temperature [4].

Edible coatings are being used in minimally processed food as a strategy to reduce the effects the minimally process causes in vegetable tissues [5]. Besides, edible coatings can contribute to the increase the life of minimally processed fruits, reducing its moisture and solute migration, respiration and oxidation reactions and can also be applied in the liquid form of the film-forming dispersion, or as thin sheets (films) used to wrap food products. Both are well considered for food preservation purposes due to their ability to improve food quality [6].

In order to control the decay and improve the shelf life of strawberries, different postharvest treatments have been studied, such calcium chloride treatment combined with nano-chitosan [7], chitosan, gelatin, starch, and sorbitol with or without monoterpenes (geraniol and thymol) [8], alginate-based with natural antimicrobials [9], polysaccharide-based enriched with essential oils [10], sodium alginate and sodium alginate in combination with ascorbic acid [11].

Chitosan is a natural non-toxic polymer, biodegradable and economic available, which are reasons for its use in a variety of applications in food industry, counting on the quality control as a substitute for non-biodegradable polymers [6]. In previous reports, chitosan coating functioned delaying loss of fruit quality, including inhibiting the growth of microbes, water loss, loss of nutrition value and postharvest softening in strawberry fruit during ripening [12,13]. Chitosan, as an edible coating, has advantages such as improving color, firmness, maintaining fruit freshness, decreasing the respiratory rate, oxidative stress and the growth of pathogenic microorganisms [14].

The clove essential oil is obtained from the shoots and leaves of the *Syzygium aromaticum* plant, presenting several functions due to the high content of eugenol, a phenolic compound that has anti-inflammatory, healing and analgesic effects [15]. In addition to eugenol clove essential oil it contains carvacrol, both acting as antioxidants and antimicrobials [16].

Essential oils are aromatic and volatile liquids extracted from plant components e.g. roots, flowers, stems, leaves, seeds, fruits and the whole plant, and are one of the compounds with these characteristics [17]. The essential clove oil, e.g., has eugenol in its composition substance responsible for analgesic, anti-inflammatory and antioxidant activity [18]. The use of clove essential oil aims to reduce the microbial growth of minimally processed fruits [19].

Thus, the objective of the present work was to evaluate the effect of an edible coating based on chitosan with the addition of essential clove oil to increase the shelf life of minimally processed strawberries.

## MATERIAL AND METHODS

### Preparation of strawberries

Fresh strawberries (*Fragaria ananassa*), chitosan (degree of deacetylation around 89%, and molecular mass between 150 and 170 kDa) and clove oil were obtained from the local market in Dourados-MS, Brazil were used. The fruits were selected and classified according to their skin color, without physiological defects, injuries and detectable infections from the presence of microorganisms. The strawberries were transported to the bioengineering laboratory of the Federal University of Grande Dourados in plastic boxes, free of solar rays and at a temperature of approximately 10-12°C. They were then stored at 5±1°C until processing.

The leaves and stalk of the strawberry were removed with the help of a stainless steel knife, then they were sanitized in an organic chlorine solution at 2 g.L<sup>-1</sup>, for 5 minutes, the water was drained for 2 to 3 minutes on sieves.

The chitosan solution was prepared by dissolving it in distilled water containing 1% (v/v) acetic acid and agitated for 30 minutes until complete dissolution at a temperature of 25°C, using a propeller agitator (Fisaton 313D), then the clove essential oil (CEO) was added and the agitation continued for another 5 minutes. The strawberries were totally submerged in the different coverings for three minutes and then drained with the help of sieves. Three treatments were obtained: T1 - control (uncoated strawberries); T2 - 2% chitosan + 1% clove essential oil; T3 - 2% chitosan + 1.5% clove essential oil.

After applying the treatments, the strawberries were stored in a quantity of nearly 50 grams per package in PET (polyethylene terephthalate), with lid (SANPACK) and with external measurements of 15x10x4cm, and these packages were stored under refrigeration at 5±1°C for a period of 12 days.

### Physical, chemical and microbiological analysis

The physical, chemical and microbiological analyses was performed in triplicate, on the day of processing, being considered as day 0 and after 1, 3, 5, 7, 9 and 12 days of storage.

For the weight loss the strawberries were stored at a cooling temperature of 5±1°C and UR of 60±2% and weighed in an analytical balance on days 0, 1, 3, 5, 7, 9 and 12 of storage. The weight loss was obtained through the difference in the initial and final mass of the strawberry at each analysis, using the following equation:

$$\text{Weight loss} = \frac{\text{initial mass} - \text{final mass}}{\text{initial mass}} \times 100$$

The results were expressed as a percentage of weight loss.

The total soluble solids content was determined using an Abbé bench refractometer, and the results were expressed in °Brix [20].

The color measurements were made using a previously calibrated Konica Minolta colorimeter (Model CR-400/CR-410), using the CIE L\*a\*b\* (Comission Internationale de L'Eclairage) system. The results were expressed as L\* (which represents the percentage of brightness, 0 = dark and 100 = light), a\* (where -a\* represents direction to green and +a\* direction to red) and b\* (where -b\* represents direction to blue and +b\* direction to yellow) [21].

The determination of the texture of the strawberries was obtained by cutting and shearing the samples in a uniaxial way, with the aid of the texturometer (TA-TX Plus). The tests were performed with the aid of a 12 mm diameter cylindrical probe. The samples were centered on the slit of the blade, which was sheared at a speed of 2 mm s<sup>-1</sup> uniaxially. The distance traveled was pre-fixed in 35mm and the complete shearing of the sample occurred. The shearing force was expressed in Newton (N) [22].

The microbiological tests performed were for molds and yeasts, psychrotrophic, *Salmonella* ssp, and *Escherichia coli* (ATCC 25922), following the methods described in APHA [23].

The results obtained were statistically evaluated through the Analysis of Variance (ANOVA) and Tukey's test at a 5% significance level, with the help of the STATISTICA® 7.0 program.

## RESULTS AND DISCUSSION

Table 1 shows the values of weight loss (%) found for minimally processed strawberries evaluated over a period of 12 days.

For all treatments, there was a progressive weight loss during all time of experiment. The coating treatments did not show any significant difference (p<0.05) at end of the experiment. The lowest weight loss was recorded for strawberries with 2% chitosan + 1.5% clove essential oil (13.51%) followed by the strawberries with 2% chitosan + 1% clove essential oil (13.78%). These results are aligned with reported by Martínez-González [24], which tested nanostructured chitosan-propolis coatings on strawberries obtaining values between 9.7 and 13.2% of weight loss after 8 days of storage.

**Table 1.** Weight loss (%) of strawberries coated with chitosan and different concentration of clove essential oil at 5±1°C, for 12 days.

Days	Treatments		
	T1	T2	T3
0	0 <sup>gA</sup>	0 <sup>gA</sup>	0 <sup>gA</sup>
1	0.47 ± 0.16 <sup>fA</sup>	0.53 ± 0.24 <sup>fA</sup>	0.49 ± 0.11 <sup>fA</sup>
3	2.61 ± 0.12 <sup>eA</sup>	1.94 ± 0.33 <sup>eB</sup>	2.05 ± 0.14 <sup>eB</sup>
5	4.94 ± 0.22 <sup>dA</sup>	3.31 ± 0.24 <sup>dB</sup>	3.17 ± 0.08 <sup>dB</sup>
7	10.54 ± 0.48 <sup>cA</sup>	7.41 ± 0.43 <sup>cB</sup>	7.26 ± 0.19 <sup>cB</sup>
9	19.11 ± 0.09 <sup>bA</sup>	11.76 ± 0.07 <sup>bB</sup>	11.59 ± 0.13 <sup>bB</sup>
12	24.19 ± 0.27 <sup>aA</sup>	13.78 ± 0.65 <sup>aB</sup>	13.51 ± 0.31 <sup>aB</sup>

Means of 3 repetitions ± standard deviation, followed by the same lowercase letter in the column and uppercase in the line do not differ from each other by the Tukey test ( $p < 0.05$ ). (T1) control (uncoated strawberries); (T2) 2% chitosan + 1% clove essential oil; (T3) 2% chitosan + 1.5% clove essential oil.

Many studies show a reduction weight loss of strawberries coated with chitosan-based polymers such as chitosan-whey protein isolate conjugate coated (16.15%) after 8 days [3], nano-chitosan 0.2% with CaCl<sub>2</sub> 3% [7].

The weight loss in strawberries is associated to thin skin and rate of transpiration making prone to rapid water loss, further causing deterioration and shriveling [25]. Chitosan coating act as a semipermeable barrier creating a suitable atmosphere for strawberries delaying a water loss. Coatings with essential oil reduce the water loss in many fruits and vegetables. Similar results were reported by Perdones [26] where strawberries coated with chitosan 1% and lemon essential oil 3% showed a weight loss of 30% after 11 days. The incorporation of essential oil enhanced the moisture barrier of the composite coating, resulting from its hydrophobic nature which increased the distance travelled through the film by water molecules [27,28].

Table 2 shows the total soluble solids (<sup>o</sup>Brix) of strawberries coated with chitosan and different concentration of clove essential oil at 5±1°C, for 12 days.

**Table 2.** Total soluble solids content (<sup>o</sup>Brix) values of strawberries coated with chitosan and different concentrations of clove oil at 5±1°C, for 12 days.

Days	Treatments		
	T1	T2	T3
0	7.26±0.09 <sup>dA</sup>	7.24±0.12 <sup>dA</sup>	7.26±0.11 <sup>dA</sup>
1	7.28±0.12 <sup>dA</sup>	7.25±0.08 <sup>dA</sup>	7.27±0.05 <sup>dA</sup>
3	7.57±0.19 <sup>dA</sup>	7.35±0.16 <sup>dA</sup>	7.30±0.11 <sup>dA</sup>
5	8.20±0.05 <sup>cA</sup>	7.92±0.09 <sup>cB</sup>	7.87±0.08 <sup>cB</sup>
7	8.83±0.21 <sup>bA</sup>	8.22±0.11 <sup>bB</sup>	8.02±0.07 <sup>cB</sup>
9	9.08±0.04 <sup>bA</sup>	8.86±0.23 <sup>aAB</sup>	8.56±0.13 <sup>bB</sup>
12	9.30±0.11 <sup>aA</sup>	9.06±0.06 <sup>aB</sup>	8.94±0.03 <sup>aC</sup>

Means of 3 repetitions ± standard deviation, followed by the same lowercase letter in the column and uppercase in the line do not differ from each other by the Tukey test ( $p < 0.05$ ). (T1) control (uncoated strawberries); (T2) 2% chitosan + 1% clove essential oil; (T3) 2% chitosan + 1.5% clove essential oil.

Sugar and organic acids compose much of the total soluble solids in strawberries [25]. In respect to the total soluble solids (TSS), there were no significant statistical differences between the treatments after three days of storage at 5±1°C (Table 3).

During 12 days of storage, the TSS of strawberry fruit increased in 2.04, 1.82 and 1.68, for the treatments T1, T2 and T3 respectively, which seems to be due to break down of organic matters to sugars and their involvement in the respiration cycle [29,30]. Once, strawberry's initial metabolic process converts carbohydrates to sugars and others soluble compounds, which increases total soluble solids [31]. However, it is possible verify that the coated strawberries had the lowest TSS values.

The color attributes of the fruit tissue are generally expressed in terms of L\* (Luminosity), a\* (green–red chromaticity), and b\* (yellow–blue chromaticity) and are shown in the Table 3 for strawberries minimally processed.

**Table 3.** Color obtained of strawberries coated with chitosan and different concentrations of clove oil at 5±1°C, for 12 days.

Parameter analyzed	Days	Treatments		
		T1	T2	T3
L*	0	33.84 ± 0.13 <sup>aA</sup>	34.07 ± 0.09 <sup>aA</sup>	34.04 ± 0.11 <sup>aA</sup>
	1	33.80 ± 0.07 <sup>aB</sup>	34.05 ± 0.03 <sup>aA</sup>	34.01 ± 0.09 <sup>aA</sup>
	3	32.30 ± 0.24 <sup>bB</sup>	33.68 ± 0.25 <sup>bA</sup>	33.91 ± 0.17 <sup>aA</sup>
	5	30.94 ± 0.11 <sup>cA</sup>	33.11 ± 0.03 <sup>cA</sup>	33.16 ± 0.09 <sup>bA</sup>
	7	30.22 ± 0.34 <sup>dB</sup>	32.73 ± 0.19 <sup>dA</sup>	32.78 ± 0.14 <sup>cA</sup>
	9	29.89 ± 0.27 <sup>dB</sup>	32.14 ± 0.29 <sup>eA</sup>	32.44 ± 0.04 <sup>dA</sup>
	12	29.63 ± 0.21 <sup>dB</sup>	31.06 ± 0.05 <sup>fA</sup>	31.54 ± 0.09 <sup>eA</sup>
Chroma a*	0	41.83 ± 0.12 <sup>aA</sup>	41.89 ± 0.09 <sup>aA</sup>	41.87 ± 0.14 <sup>aA</sup>
	1	41.80 ± 0.06 <sup>aA</sup>	41.85 ± 0.03 <sup>aA</sup>	41.85 ± 0.09 <sup>aA</sup>
	3	41.51 ± 0.13 <sup>bA</sup>	41.69 ± 0.11 <sup>aA</sup>	41.81 ± 0.11 <sup>aA</sup>
	5	41.02 ± 0.09 <sup>cB</sup>	41.31 ± 0.34 <sup>aAB</sup>	41.80 ± 0.19 <sup>aA</sup>
	7	40.73 ± 0.16 <sup>dC</sup>	40.94 ± 0.02 <sup>bB</sup>	41.71 ± 0.08 <sup>aA</sup>
	9	40.14 ± 0.28 <sup>eB</sup>	40.89 ± 0.07 <sup>bA</sup>	41.23 ± 0.42 <sup>abA</sup>
	12	39.87 ± 0.21 <sup>eB</sup>	40.68 ± 0.17 <sup>bA</sup>	40.77 ± 0.09 <sup>bA</sup>
Chroma b*	0	30.88 ± 0.26 <sup>aA</sup>	30.89 ± 0.14 <sup>aA</sup>	30.91 ± 0.08 <sup>aA</sup>
	1	30.80 ± 0.21 <sup>aA</sup>	30.80 ± 0.12 <sup>aA</sup>	30.89 ± 0.17 <sup>aA</sup>
	3	29.18 ± 0.12 <sup>bC</sup>	30.29 ± 0.24 <sup>bB</sup>	30.77 ± 0.09 <sup>aA</sup>
	5	28.84 ± 0.09 <sup>cB</sup>	30.01 ± 0.06 <sup>bA</sup>	30.13 ± 0.31 <sup>bA</sup>
	7	28.18 ± 0.22 <sup>dB</sup>	29.63 ± 0.16 <sup>cA</sup>	29.91 ± 0.11 <sup>bA</sup>
	9	27.37 ± 0.06 <sup>eC</sup>	29.09 ± 0.05 <sup>dB</sup>	29.73 ± 0.09 <sup>bA</sup>
	12	26.11 ± 0.19 <sup>fC</sup>	28.45 ± 0.09 <sup>eB</sup>	29.22 ± 0.15 <sup>cA</sup>

Means of 3 repetitions ± standard deviation, followed by the same lowercase letter in the column and uppercase in the line do not differ from each other by the Tukey test ( $p < 0.05$ ). (T1) control (uncoated strawberries); (T2) 2% chitosan + 1% clove essential oil; (T3) 2% chitosan + 1.5% clove essential oil.

The L\* parameter is an indicator of fruit darkening. As can be observed in Table 3, all the strawberries showed decreasing L\* values with storage time. Analyzing parameter L\*, from the fifth day, all treatments showed a significant difference between them. All days, treated strawberries were also significantly brighter than control samples after of the 12 days (29.63±0.21 vs 31.6±0.05 and 31.54±0.09, respectively), which might be considered a positive result, considering that decrease in brightness is usually due to the formation of dark tissues and brown spots, that may be related to inappropriate storage conditions as well as yeast infection [9]. Basaglia and coauthors [32] also found a decrease in L\* values when they studied chitosan and cinnamon essential oil coatings in minimally processed pineapples.

a\* values of all samples slightly decreased over time, showing the tendency to have a less saturated red color. However, significant reductions were detected after 5 days for control and after 7 days for treated fruit. Clove oil had a significant effect on red color of strawberries all days of storage, showing more saturated color than control samples.

Explanations for this result may be found in the among red color is relation of a anthocyanin on present in the strawberry fruit. Anthocyanins are considered the compounds responsible for red color of strawberries [33], and discoloration of the fruit may be due to anthocyanin degradation by the action of hydrolytic enzymes that, by breaking down the linkage of the glycosidic substituent in the moieties, lead to loss of color during postharvest storage [34,9].

Measurements of b\* values, representing the chromaticity of blue (negative value) and yellow (positive value) colors, showed a significant ( $p \leq 0.05$ ) reduction b\* values for treated strawberries which ranged from the initial and final value of 30.88±0.26 to 26.11±0.19 vs 30.89±0.14 to 28.45±0.09 and 30.91±0.08 to 29.22±0.15 for control and treated strawberries, respectively, at the days of storage time. Indeed significant differences were detected from the beginning to the end of storage time, although fruit developed a significantly less vivid coloration, as evidenced by lower values of Chroma after five days in the treated strawberries.

Coating treatments showed a better firmness than control treatment during storage time (Table 4).

**Table 4.** Firmness (N) of strawberries coated with chitosan and different concentration of clove essential oil at 5±1°C, for 12 days.

Days	Treatments		
	T1	T2	T3
0	5.23 ± 0.19 <sup>aA</sup>	5.31 ± 0.12 <sup>aA</sup>	5.33 ± 0.09 <sup>aA</sup>
1	5.20 ± 0.11 <sup>aA</sup>	5.30 ± 0.11 <sup>aA</sup>	5.33 ± 0.11 <sup>aA</sup>
3	5.03 ± 0.36 <sup>abA</sup>	5.27 ± 0.09 <sup>aA</sup>	5.29 ± 0.09 <sup>aA</sup>
5	4.89 ± 0.12 <sup>abA</sup>	5.24 ± 0.20 <sup>aA</sup>	5.21 ± 0.23 <sup>aA</sup>
7	4.47 ± 0.26 <sup>bB</sup>	5.04 ± 0.09 <sup>abA</sup>	5.12 ± 0.06 <sup>aA</sup>
9	4.11 ± 0.12 <sup>bcB</sup>	4.96 ± 0.07 <sup>bA</sup>	5.01 ± 0.17 <sup>aA</sup>
12	3.87 ± 0.09 <sup>cB</sup>	4.81 ± 0.21 <sup>bA</sup>	4.97 ± 0.13 <sup>aA</sup>

Means of 3 repetitions ± standard deviation, followed by the same lowercase letter in the column and uppercase in the line do not differ from each other by the Tukey test ( $p < 0.05$ ). (T1) control (uncoated strawberries); (T2) 2% chitosan + 1% clove essential oil; (T3) 2% chitosan + 1.5% clove essential oil.

After 12 days, strawberries coated with chitosan 2% + clove essential oil 1% and with chitosan 2% + clove essential oil 1.5% showed the greatest firmness with values of 4.81 and 4.97 N respectively; while the control treatment recorded a firmness of 3.87 N at the end of the storage time.

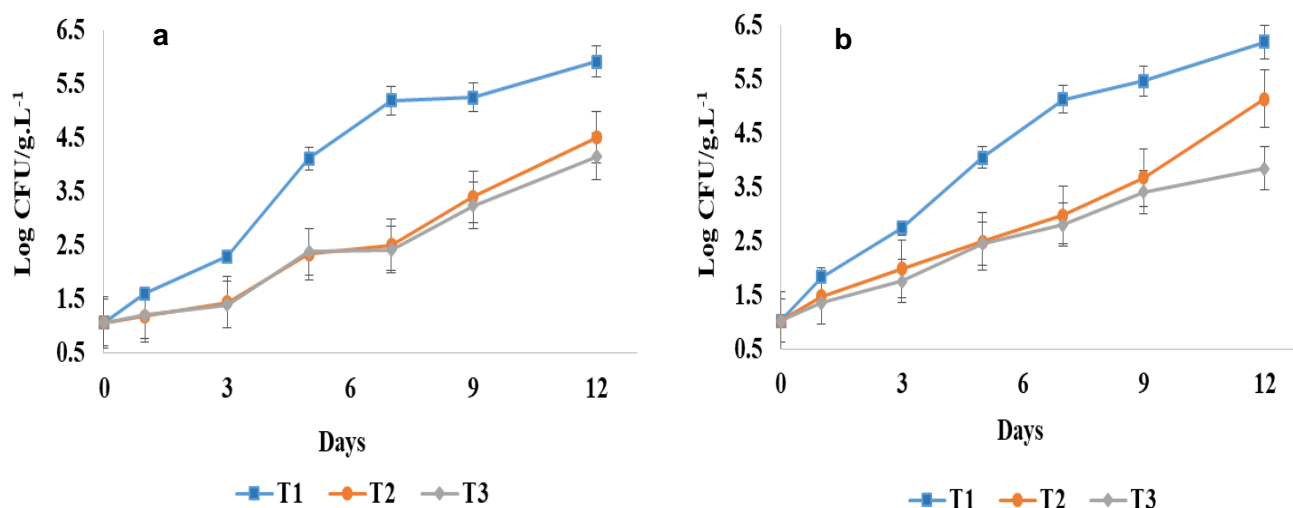
The loss of firmness on strawberries is associated to degradation of the middle lamella of the cell wall by depolymerization of the xyloglucan-cellulose matrix and the solubilization of pectins [35,11]. In this study show that strawberries coated with chitosan 2% and clove essential oil 1.5% showed the best firmness; similar results were reported by Martínez-González and coauthors [24], which recorded a best firmness for strawberries coated with nanostructured chitosan-propolis than non-coated strawberries.

Chu and coauthors [28] also showed similar results in the pullulan-coated strawberry incorporated with cinnamon essential oil, decreasing about 12% of its initial firmness at the end of storage compared to uncoated strawberries that had a greater loss of firmness. The coating with essential oil help to maintain the firmness by antioxidant activity and anti-microorganism activity [36].

The presence of *Escherichia coli* ( $< 10^2$  CFU  $g^{-1}$ ) and *Salmonella sp.* (absence in 25 g) was not detected in minimally processed strawberry samples, confirming the efficiency of good manufacturing practices and the action of organic chlorine in the disinfection of the samples.

Microorganisms rapidly colonized when the storage went beyond 5 days, especially in control strawberries. According to Fan and coauthors [37], coatings applied on fresh fruit produce an internal modified micro-atmosphere that generally affects the growth rate of spoilage and pathogenic microorganisms.

Figure 1 (a) shows statistical difference the growth of psychrotrophic organisms during the 12 days storage period.



**Figure 1.** Psychrotrophic (a) and mold and yeast (b) found in strawberries coated with chitosan and different proportions of clove essential oil at 5±1 °C, for 12 days. (T1) control (uncoated strawberries); (T2) 2% chitosan + 1% clove essential oil; (T3) 2% chitosan + 1.5% clove essential oil.

As observed, there was an accentuated growth of these microorganisms with the passing of the storage days, and the control treatment (T1) presented a higher growth (5.93±0.34 log/CFU/g), differing statistically

from the others at the end of the experiment. The T2 and T3 treatments did not differ from each other during the evaluated period (4.51 and 4.16 log/CFU/g respectively).

As much as there are no standards in psychrotrophic bacteria in Brazil, it has been suggested that foods with populations above  $10^5$  and  $10^6$  CFU/g [38]. In this study, only the control treatment was above these limits from the 12<sup>th</sup> day of storage, demonstrating that the use of coatings was efficient to reduce the growth of psychrotrophic microorganisms. Chevalier and coauthors [5] observed similar results, when they worked with coatings based on protein isolate of tilapia applied on minimally processed melons, and the control treatment also showed values above  $10^5$  from the 9<sup>th</sup>.

Clove has a high amount of eugenol (45-90%) and is a potent antimicrobial agent, capable of degenerating proteins. The action mechanism of the essential oil on microorganisms is complex and has not yet been fully elucidated. Numerous reports have been made about the antimicrobial action mechanisms of oils, which cause lysis and loss of membrane integrity, due to the output of ions and inhibition of cellular respiration [39].

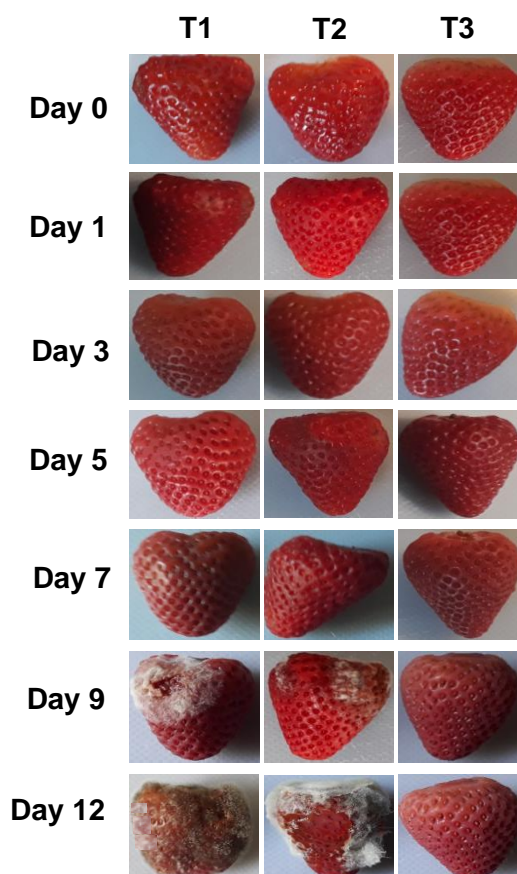
As a typical lipophilic compound, essential oils cross the cell wall and the cytoplasmic membrane, the cytotoxic activity seems to be linked to the disruption of the structures of the different layers of polysaccharides, fatty acids and phospholipids, due to its mechanism of action that reaches several targets to the same time [40].

Figure 1 (b) shows statistical difference the growth of molds and yeasts in strawberries minimally processed during 12 days storage period. The growth of mold and yeast increased over the storage days for all evaluated treatments, with the control treatment (T1) presenting the highest growth ( $6.19 \log \text{CFU g}^{-1}$ ). The growth rate of molds was inhibited in the samples treated with chitosan (2%) and clove essential oil (1 and 1.5%), treatment T2 and T3 ( $5.14$  and  $3.85 \log \text{CFU g}^{-1}$ ) respectively.

Many studies have mentioned the antibacterial and antifungal properties of chitosan. Pizato and coauthors [41], for example, demonstrated the low microbial growth in minimally processed broccoli for up to 12 days in refrigerated storage when 1.5% chitosan coating was used. Tokatli and Demirdöven [42] demonstrated the efficiency of chitosan when working with sweet cherry (*Prunus avium* L.) coated with edible chitosan films. This coating inhibited the growth of yeasts and molds for 25 days in refrigerated storage at 4°C. The antimicrobial action of chitosan is associated with the formation of a polyelectrolyte composition, since its protonated amine groups selectively bind to the cell surface of microorganisms, negatively charged, consequently causing the loss of intracellular components and inhibition of microbial growth [43].

Perdones and coauthors [26] worked with chitosan along with lemon essential oil; they obtained a positive effect in reducing the fungal activity in strawberries stored at 5°C. Also, Hernández-Carrillo and coauthors [44] demonstrated that the combination of pectin with lemon essential oil and reuterine was efficient in reducing the growth of molds and yeasts in strawberries stored under refrigeration, demonstrating a great potential for preserving the fungal deterioration of these fruits. These results agree with the present study, which also found a lower growth of molds and yeasts when coatings based on chitosan and clove essential oil were used, which proves the efficiency of both chitosan and the oil used as an inhibitor of microorganisms.

Figure 2 shows the appearance of strawberries over the days of storage. As we can see, the strawberries developed a darker color (redder) with the passing of the days of storage; besides, the T1 and T2 treatments showed apparent mold growth (days 9 and 12). The use of chitosan (2%) and clove oil (1.5%) were efficient to maintain the quality of the strawberry for longer time. These observations can be confirmed when we evaluate Figure 1 (b), in which there was greater growth of mold and yeast for these treatments.



**Figure 2.** Appearance of strawberries without and with chitosan coatings containing different concentrations of clove essential oil over the days of storage.

## CONCLUSION

The treatments T2 (2% chitosan and 1% CEO) and T3 (2% chitosan and 1.5% CEO) were the most efficient treatments in the conservation of minimally processed strawberries, when compared to the control sample.

The use of chitosan and clove essential oil exhibited a delay in the growth of molds and yeasts, a lower weight loss and firmness. The T2 and T3 treatments proved to have great potential to be applied as coatings as they reduced mass loss by approximately 86%, maintained physical and chemical characteristics, and reduced microbiological changes, especially in mold and yeast growth.

The use of clove oil in its highest concentration (1.5%), kept the minimally processed strawberries safe for consumption for a longer time when stored at  $5\pm 1^{\circ}\text{C}$ .

For future trends in relation to this work, clove oil encapsulated in different coatings may be used.

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

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