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CHITOSAN ENCAPSULATED CLOVE OIL MITIGATES NUTRIENT DETERIORATION AND PRESERVES PHYSIOCHEMICAL ATTRIBUTES OF *Cucumis Sativa* and *Musa Acuminate*

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ABSTRACT

This research evaluated the use of chitosan loaded with clove oil to prevent nutrient deterioration, quality and extend the shelf-life of cucumbers and plantains. The study involved the synthesis as well as characterisation of chitosan (CTS) from crab shells and afterward loaded with clove oil (C.oil) synthesised from clove seed. The process yielded 45.7% CTs and 14.0% C.oil. The synthesised CTS was 60% pure and had a 78% deacetylation level. The chitosan-loaded clove oil was applied to cucumbers and plantains to assess their efficacy in preservation and shelf life extension. Application of chitosan-loaded clove oil reduced weight loss in cucumbers from 30.14% to 17.54% and in plantains from 48.89% to 28.63%. The untreated normal control (NC) samples had a significant increase in pH and a decrease in total titratable acidity (TTA), while a slight change in pH and TTA was observed in the cucumbers and plantain treated with chitosan-loaded clove oil. Cucumbers and plantains treated with chitosan-loaded clove oil show no significant reduction in the total antioxidant capacity when in comparison to the control group. Also, there is the retention of the epicarp green colouration for a longer period in the fruits coated with CTS +1% C.oil and CTS solution than in untreated samples. In conclusion, chitosan-loaded clove oil demonstrated significant potential in extending the shelf-life and preserving the quality of cucumbers by an additional 15 days and plantains by an additional 4 days.

Keywords: Chitosan, Clove Oil, Cucumber, Plantain, Shelf-Life

INTRODUCTION

There has been an escalation in recent years in the demand for natural and sustainable approaches to food preservation. Consumers are seeking replacements to synthetic preservatives for perishable crops due to concerns about their potential health risks and environmental impact (Silvennoinen *et al*., 2014).

Perishable crops like cucumbers and plantains are widely consumed produce with unique sensory characteristics and nutritional profiles. However, vulnerability to microbial spoilage and enzymatic browning poses significant challenges during the storage of cucumber and plantain, with 51% and 10% postharvest loss, respectively (Adeniyi and Ayandiji, 2014; Rahman, 2020). Biodegradable polymer like chitosan, derived from chitin, offers numerous advantages in the sustenance of perishable crops (He *et al*., 2020).

Chitosan provides a high surface area for efficient encapsulation, protection, and controlled discharge of essential oils. This encapsulation technique improves the solubility and stability of the oil, ensuring its sustained antioxidant and antimicrobial effects during storage (Negi and Kesari, 2022). Moreover, chitosan itself exhibits inherent antimicrobial properties and forms a protective barrier on the surfaces of fruits and vegetables. This antimicrobial activity of chitosan can help prevent microbial contamination and spoilage, further contributing to the preservation of fruits like cucumber and plantain (Kumar *et al*., 2020). Additionally, chitosan contributes to reduced moisture loss, maintaining optimal quality and minimising enzymatic browning, thus conserving the visual appeal and nutritive quality of the produce. Natural compounds like clove oil have also demonstrated promising antimicrobial and antioxidant properties that can address these challenges He *et al*., 2020).

Clove oil is rich in bioactive compounds, including eugenol, which shows a strong antimicrobial activity against a variety of pathogens, including yeasts, fungi, and bacteria. Its antibacterial effectiveness can help prolong the shelf life of the fruits and vegetables by reducing or inhibiting the proliferation of decomposing microorganisms (Núñez *et al.*, 2001). Furthermore, clove oil possesses potent antioxidant properties, which can mitigate oxidative reactions, such as lipid oxidation and enzymatic browning that lead to the deterioration of produce during storage (Aguilar-Gonzalez *et al*., 2015). Therefore, to improve proper coating of crops, the delivery and effectiveness of chitosan and clove oil, among other essential oils, can be employed (Atarés and Chiralt, 2016). Few details are available regarding the impact of chitosan-loaded clove oil on the shelf-life and quality of some other perishable crops, such as cucumber and plantain. Hence, this research is aimed at investigating the potential of chitosan-loaded clove oil towards quality preservation and shelf-life extension of cucumber and plantain.

MATERIALS AND METHODS

Purchase of Crabs, Clove Seed, Cucumber and Plantain

Crabs (*Scylla serrata* (Forskål)) were purchased from Ojoo market, Epe (coastal region), Lagos State. The crabs were killed by boiling them for 20 minutes in hot water, after which the shells (exoskeletons) were manually separated from the crabs. With the aid of a grinding machine, the crab shells were pulverised into powder after drying. Also, clove seeds were purchased for the extraction of clove oil from Bodija market, within Ibadan metropolis, Oyo State.

Eighty (80) pieces of freshly harvested matured unripe cucumber and plantain from a field plot at the Nigerian Horticulture Research Institute, Ibadan, were purchased and sorted based on colour, size and absence of external injuries.

Sanitisation of the washed fruits was done by dipping in 0.1% (v/v) Sodium hypochlorite (NaOCl) solution for 30 s and afterwards air-dried with the aid of an electric fan before the commencement of treatment.

Synthesis of Chitosan from Crab Waste

The methods of Burrows et al. (2007), which were described by Ogungbemi et al. (2020), were used for chitosan extraction from the powdered carapace samples. Briefly, deproteinization of the crab's exoskeleton was done for 2 hours using 4% NaOH, after which demineralisation was carried out with 1% v/v HCl for 24 hours. Deacetylation with 50% NaOH was done for 2 hours at a boiling temperature on the resultant crude chitin to obtain the chitosan.

Characterisation of Chitosan

Purification of Chitosan

Chitosan was purified using the precipitation technique by dissolving 10 g of the material in 1 L solution of 2% acetic acid in a conical flask, which was shaken for 4 hours. Following that, precipitation was allowed to occur after adding 0.5 mol.dm^{-3} NaOH. The resultant precipitates were cleaned, oven-dried for one hour at 103 °C and filtered (Hussain et al., 2013).

Degree of Deacetylation

The method of Czechowska-Biskup *et al*. (2012) was used to determine the degree of deacetylation (DD). A 25 cm^3 aqueous solution of 0.1 mol.dm^{-3} hydrochloric acid was used to dissolve 0.125 g of chitosan, and the mixture was swirled for about 30 minutes to ensure the chitosan was fully dissolved. Titration using 0.1 mol.dm^{-3} NaOH was then carried out on the solution. The degree of deacetylation was determined using the formula stated below:

$$DD(\%) = NH_2\%/9.94\%$$

$$NH_2\% = \frac{(C1\times V1 - C2\times V2)\times 0.016}{G\times(100-WW)} \times 100$$

Oil Extraction and Quantification from Clove Seed

The essential oil was extracted from a fine powder of ground clove seeds, after which 50 g was introduced into the Soxhlet extractor for the extraction of the oil. The oil was transferred into a bottle and stored at room temperature. About 3ml of the extracted clove oil was then quantified using GC-MS (Guan et al., 2007).

Preparation of Chitosan-loaded Clove Oil

The method of Hasheminejad et al. (2019) was used to prepare chitosan loaded with clove oil. 1% (v/v) acetic acid was used to produce 0.3% (w/v) chitosan solution with continuous overnight stirring at 25oC. Büchner funnel and Whatman 42 filter paper were used to filter the solution after the pH was adjusted using 9 N NaOH to 4.6. For 30 minutes, the 0.3% Chitosan solution was agitated at 25 °C with Tween 80 (HLB 15.9, 1% w/v) added as a surfactant. Oil was gently dropped into the resulting solution to get an emulsion with a 1:1 (v/v) ratio of chitosan to clove oil.

Application of Chitosan-Loaded Clove Oil

36 pieces of cucumber and plantain were divided into 6 groups (each group containing 6 pieces of cucumber and Plantain). The control group, Chitosan group, clove oil group and the Chitosan-loaded with clove oil at 0.5%, 1% and 1.5% respectively. The coating of the crops was carried out through immersion of crops into their corresponding coating materials for 3 seconds, after which the samples were placed in a stackable crate at room temperature for monitoring of physical, chemical and microbial activities for 4 weeks (analysis was carried out once per week for 4 weeks).

Physical Observation

Physical observation to be observed includes: weight loss and observable physical change of colour.

Weight loss

During the four weeks of storage, the percentage of weight loss in the control and coated groups was determined with the aid of an electronic balance (GE812, Sartorius, Germany). All groups were represented in triplicate. Percentage weight loss calculation for the control and each treatment group was according to Fawole and Opara (2013).

Colour

The epicarp colour change of cucumber and plantain was estimated using the description of Oyewole et al. (2023) in Table 1 below.

Table 1: Plantain and Cucumber Fruits Epicarp Colour Description

Ripening Stage	Depiction of Peel Colour	Physiological Phase of Ripening
1	Green	Pre-climacteric
2	Pale green	Pre-climacteric
3	Pale green with yellow tip	Onset to climacteric
4	Yellow: Green – 1: 1	Climacteric
5	More yellow than green	Climacteric
6	Pure yellow (purely ripe)	Climacteric
7	Yellow with black specks	Onset of senescence (senescent spot developing)
8	Yellow: Black – 1:1	Senescence
9	More black than yellow	Senescence

Source: Oyewole et al., 2023.

Microbiological Analysis

The procedure described by Graça *et al*. (2015) was used for the microbial analysis with slight modifications. The population of yeast and moulds, mesophilic and psychrophilic bacteria, were evaluated in the samples (including control, Chitosan, clove oil and chitosan-loaded clove oil) during the weekly analysis of the stored crops. The mixture was serially diluted (10−1 to 10−9) and added to the medium culture. Plate count agar (PCA) was used to evaluate the total aerobic mesophilic and psychrophilic bacteria after they were incubated at 35 ± 1 °C for two days and 4 °C for seven days, respectively. Evaluation of yeast and moulds was done on a potato dextrose agar (PDA) after they were incubated at 28 ± 1 °C for 7 days.

Chemical Analysis

Moisture Content/Dry Matter Determination

The moisture content was determined according to the method of AOAC (2010). Crucibles were oven-dried after washing to eliminate any form of dirt. They were placed in the desiccator for cooling and afterwards weighed. A sample of a known weight was placed in the crucible and then weighed before transferring it to the oven for drying at a temperature between 103-105 ^{o}C. The dried samples were cooled in a desiccator and afterwards weighed. Oven drying continued until a constant weight was obtained.

Calculation:

$$Moisture\ Content\ \% = \frac{(Weight\ Loss)}{(Weight\ of\ Sample)} X\ 100$$

Dry matter = 100- Moisture content

Total Soluble Solid, Titratable Acidity and pH

Total Soluble Solid (TSS), TTA and pH values were evaluated using the method described by Ghasemnezhad et al. (2015). A refractometer (Refractometer Abbe, Bellingham & Stanley Ltd, UK) was used to evaluate the TSS in control and treatment groups, and data are expressed as °Brix. The pH was measured using a pH meter. Titration volume from the titration of 5 ml of juice to the endpoint of pH 8.2 with 0.1 N NaOH was used to calculate the TTA. The results were expressed as a citric acid percentage.

Total Sugar

The total sugar was determined using the Lane-Eynon method for total sugar described (dos Santos et al., 2023). Reducing sugar concentration in the samples based on their reducing power towards certain metallic salts is determined through the titration method. In this process, copper sulfate in an alkaline tartrate system (Fehling's solution) is reduced by the sample.

Total Antioxidant Activity

The process described by Apek et al. (2008) was used to estimate the total antioxidant capacity. The procedure commenced by using 300 mmol/L acetate buffer, a 10 mmol/L TPTZ/HCL solution, and a 20 mmol/L ferric chloride solution for the preparation of FRAP reagent. The standard curve was established by adding 〖〖Fe〗_2〗^+ to the FRAP reagent. Samples were introduced into cuvettes and appropriately diluted. Following this, FRAP reagent was added to each cuvette containing the sample. Absorbance readings at a wavelength of 620 nm were directly taken from the cuvettes, thereby utilising a spectrophotometer. The concentrations of 〖〖Fe〗_2〗^+in the samples were calculated based on the standard curve. These determined concentrations of 〖〖Fe〗_2〗^+ aided in establishing the antioxidant capacity of the samples.

Statistical Analysis

One-way analysis of variance (ANOVA) with the aid of IBM SPSS Statistics version 23 (IBM Corporation, USA) was used to analyse the results. Duncan's multiple range tests were used to estimate the statistical differences among mean values of the various treatments at a 95% significant level.

RESULTS AND DISCUSSION

Effects of Chitosan-Loaded Clove Oil on the Weight Performance of Plantain and Cucumber

In Table 2 below, the weight of the plantain in the clove oil control group, chitosan + 0.5% clove oil and chitosan + 1.5 % clove oil treated groups significantly decreased than the normal control group while the chitosan control group and the chitosan + 1% clove oil treated group has a significant increase weight when compared to normal control.

Table 2: Effects of Chitosan-loaded Clove Oil on the Weight Performance of Plantain for Fifteen Days (15)

Treatment	Baseline	Day 3	Day 6	Day 9	Day 12	Day 15
Normal Control	236.08±0.54^{a}	206±8.50^{a}	174 ± 5.42^{b}	139.89 ± 8.51^{a}	129.52 ± 6.62^{a}	120.66 ±11.18^{b}
CTS control	220.81±15.32^{a}	215±9.21^{a}	192 ± 6.31^{c}	178.46 ± 7.54^{b}	168.5 ± 7.79^{c}	159.18 ± 6.17^{c}
Clove Oil Control	250.05±16.54^{a}	221±13.14^{a}	148 ± 7.52^{a}	121.01 ± 9.16^{a}	111.34 ± 5.17^{a}	101.67 ± 2.46^{a}
CTS + 0.5% C.oil	240.51 ± 15.31^{a}	206±14.23^{a}	148 ± 10.54^{a}	132.97 ± 8.17^{a}	123.50 ± 5.23^{b}	115.40 ± 6.99^{ab}
CTS + 1% C.oil	220.85 ± 18.11^{a}	210±7.15^{a}	194 ± 11.52^{c}	184.54 ± 8.17^{b}	165.89 ± 6.61^{c}	157.25 ± 8.14^{c}
CTS + 1.5% C.oil	230.41 ± 11.12^{a}	210±7.44^{a}	156 ± 6.28^{a}	114.53± 13.67^{a}	110.5 ± 7.58^{a}	107.56 ± 2.45^{a}

Expression of values is in mean ± standard error of mean (n=3)

Table 3 below shows that the percentage loss in weight of plantain is significantly higher in the clove oil control group, chitosan + 0.5% clove oil and chitosan + 1.5% clove oil treatment group in comparison to that of the normal control group while the weight loss significantly decreases in the chitosan + 1% clove oil treatment group when compared to the normal control.

Table 3: Effects of Chitosan-loaded Clove Oil on the Weight Performance of Plantain for Fifteen Days (15)

Treatment	Baseline	Day 15	Weight loss (%)
Normal Control	236.08 ± 10.54^{a}	120.66 ± 7.18^{b}	48.89
CTS control	220.81 ± 15.32^{a}	159.18 ± 6.17^{c}	27.91
Clove Oil Control	250.05 ± 16.54^{a}	101.67 ± 2.46^{a}	59.34
CTS + 0.5% C.oil	240.51 ± 15.31^{a}	115.40 ± 6.99^{ab}	52.02
CTS + 1% C.oil	220.85 ± 18.11^{a}	157.25 ± 8.14^{c}	28.63
CTS + 1.5% C.oil	230.41 ± 11.12^{a}	107.56 ± 2.45^{a}	53.32

Expression of values is in mean ± standard error of mean (n=3)

In Table 4 below, the weight of the cucumber in the clove oil control group has no significant difference when compared to the normal control group at day 25, while the chitosan + 1% clove oil treated group and chitosan + 0.5% clove oil treated group has a significant increase in weight when compared to the normal control after 25 days.

Table 4: Effect of Chitosan-loaded Clove Oil on the Weight Performance of Cucumber Shelf-life for Twenty-five Days (25)

Treatment	Baseline	Day 5	Day 10	Day 15	Day 20	Day 25
Normal Control	328.51±25.42^{a}	307.15±8.46^{a}	287.45±8.67^{a}	268.31±11.14^{a}	248.32±10.12^{a}	229.50±12.61^{a}
CTS Control	337.76±16.14^{a}	328.26±8.71^{b}	309.53±13.54^{a}	291.34±8.69^{b}	272.36±9.68^{b}	254.21±4.22^{b}
Clove Oil Control	378.43±29.21^{ab}	360.11±9.82^{c}	326.54±11.53^{b}	292.32±17.71^{b}	258.39±14.64^{ab}	224.45±8.87^{a}
CTS + 0.5% C.oil	345.33±18.77^{a}	321.15±6.62^{b}	307.32±12.61^{a}	293.57±6.42^{b}	279.24±8.82^{b}	265.61±8.49^{b}
CTS + 1% C.oil	394.38±11.24^{b}	382.42±14.26^{c}	367.80±7.62^{c}	353.50±14.54^{c}	339.54±12.66^{c}	325.22±11.63^{c}
CTS + 1.5% C.oil	380.47±21.98^{b}	360.22±12.44^{c}	328.43±16.55^{b}	296.64±14.62^{b}	264.38±11.62^{a}	232.14±10.77^{a}

Expression of values is in mean ± standard error of mean (n=3)

From Table 5 below, the percentage weight loss in the clove oil control group has no significant change when compared with the normal control group, but a significant decrease is seen in the chitosan + 1% clove treatment group when in comparison with the normal control and other treatment groups. This shows that chitosan + 1% clove oil prevented weight loss of cucumber better than chitosan + 0.5% clove oil.

Table 5: Effects of Chitosan-loaded Clove Oil on the Weight Loss of Cucumber Shelf-life for Twenty-five Days (25)

Treatment	Baseline	Day 25	Weight loss (%)
Normal Control	328.51 ± 15.42^{a}	229.50 ± 12.61^{a}	30.14
CTS Control	337.76. ± 11.14^{a}	254.21 ± 14.22^{b}	24.74
Clove Oil Control	378.43 ± 16.21^{ab}	224.45 ± 8.87^{a}	40.69
CTS + 0.5% C.oil	345.33 ± 18.77^{a}	265.61 ± 4.49^{b}	23.09
CTS + 1% C.oil	394.38 ± 21.24^{b}	325.22 ± 11.63^{c}	17.54
CTS + 1.5% C.oil	380.47 ± 21.98^{b}	232.14 ± 10.77^{a}	38.99

Expression of values is in mean ± standard error of mean (n=3)

Effects of Chitosan-Loaded Clove Oil on the Dry Matter of Plantain and Cucumber

In Table 6 below, the dry matter of plantain in the clove oil control group after 15 days of treatment significantly increases when in comparison with the normal control group. No significant difference is seen in the chitosan + 0.5% clove oil and chitosan + 1.5 % clove oil treatment groups when in comparison to the normal control group. However, a significant increase was seen in the dry matter of the chitosan + 1% clove oil treatment group when compared to the normal control group.

Table 6: Effects of Chitosan-Loaded Clove Oil On the Dry Matter of Plantain

Treatments	Baseline	Day 15	Dry Matter loss (%)
Normal Control	41.28 ±1.23^{a}	20.45 ± 1.45^{a}	50.46
CTS Control	40.34 ± 1.86^{a}	28.28 ± 2.63^{b}	29.89
Clove Oil Control	41.73 ± 0.98^{a}	24.25 ± 1.90^{ab}	41.88
CTS+0.5% C.oil	40.06 ±2.65^{a}	21.25 ± 1.29^{a}	46.95
CTS+1% C.oil	41.40 ± 1.52^{a}	29.19 ± 2.15^{b}	29.49
CTS+1.5% C.oil	43.39 ± 2.73^{a}	25.99 ± 2.32^{ab}	40.10

Expression of values is in mean ± standard error of mean (n=3)

In Table 7, the dry matter of cucumber in the clove oil control group shows a significant decrease when compared to the normal control group. No significant difference was seen in the chitosan +0.5% clove oil and chitosan + 1% clove oil treatment groups when compared to the normal control group, but a significant decrease in the dry matter was seen in the chitosan + 1.5% clove oil treatment group when compared to the normal control group.

Table 7: Effects of Chitosan-Loaded Clove Oil On the Dry Matter of Cucumber

Treatments	Baseline	Day 25	Dry Matter loss (%)
Normal Control	7.32 ±0.73^{a}	6.21 ± 0.45^{b}	15.16
CTS Control	6.92 ± 0.68^{a}	6.21 ± 0.63^{b}	10.26
Clove Oil Control	7.03± 0.83^{a}	4.47± 0.60a	36.41
CTS+0.5% C.oil	7.09 ±1.05^{a}	5.09± 0.29^{ab}	28.20
CTS+1% C.oil	6.93 ± 0.52^{a}	5.63± 0.15^{ab}	18.75
CTS+1.5% C.oil	7.48 ± 1.73^{a}	4.99 ± 0.32^{a}	33.29

Expression of values is in mean ± standard error of mean (n=3)

Effects of Chitosan-Loaded Clove Oil on the Physico-Chemical Properties of Cucumber and Plantain

Effect of Chitosan-Loaded Clove Oil on the pH and Total Titratable Acid of Cucumber and Plantain

The pH value of cucumber in Figure 1 below shows that the normal control significantly increases at day 5 of treatment and decreases further as treatment continues till day 25. The chitosan + 1% clove oil-treated group shows a higher pH value than the normal control and other treated groups at 25 days of treatment.

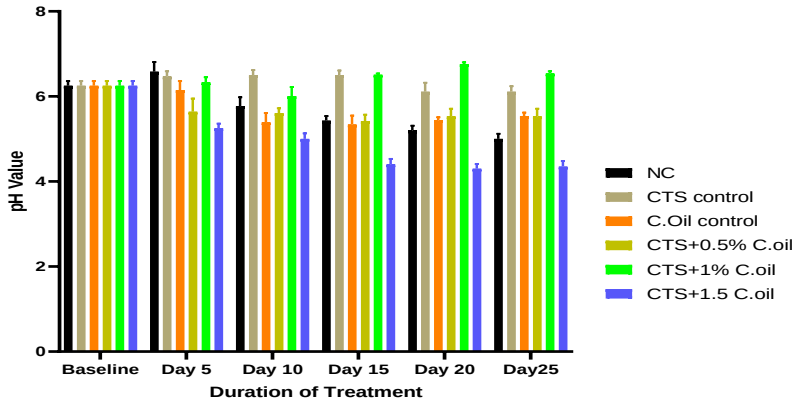

Figure 1: Effect of Chitosan-Loaded Clove Oil on the pH Value of Cucumber Expression of Values is in mean ± Standard Error of Mean (n=3)

The pH value of plantain in Figure 2 shows a significant decrease in the clove oil control group when in comparison to the normal control, while the groups exposed to chitosan + 1% clove oil showed higher pH values than the normal control throughout the 4 days of treatment.

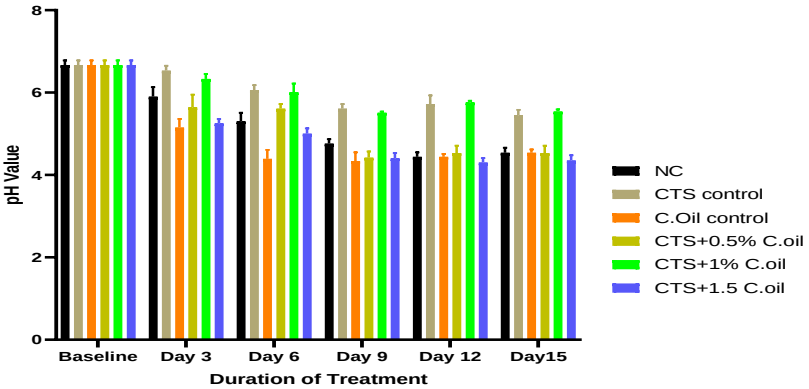

Figure 2: Effect of Chitosan-loaded Clove Oil on the pH Value of PlantainExpression of Values is in mean ± Standard error of mean (n=3)

The Total titratable acid of cucumber, Figure 3, shows a significant decrease in the clove oil control group when compared to the normal control at day 5 of treatment which continues to decrease as treatment progresses until day 25 while the chitosan + 1% clove oil shows a mild reduction in the TTA value when in comparison to the normal control.

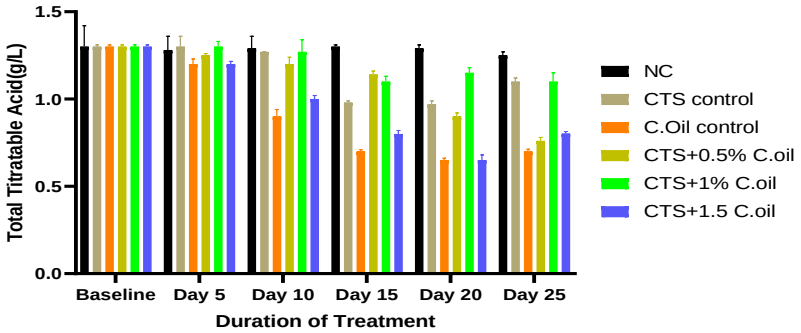

Figure 3: Effect of Chitosan-loaded Clove Oil on the Total Titratable Acid of Cucumber Expression of Values is in mean ± Standard error of mean (n=3)

The total titratable acid in Figure 4 shows that there is a significant decrease in the clove oil control group when in comparison to the normal control, while the group exposed to chitosan + 1% clove oil increases significantly in comparison to that of the normal control group.

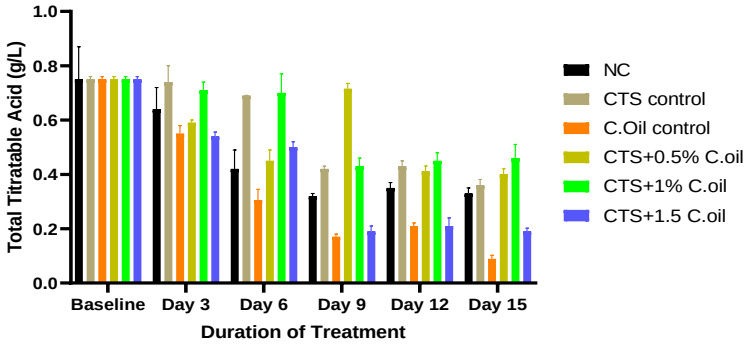

Figure 4: Effect of Chitosan-loaded Clove Oil on the Total Titratable Acid of Plantain Expression of Values is in mean ± Standard Error of mean (n=3)

Effects of Chitosan-Loaded Clove Oil on the Total Soluble Solids and Total Sugar of Cucumber and Plantain

The total soluble solid of cucumber, Figure 5 significantly increases in the clove oil control group, chitosan + 0.5% clove oil and chitosan + 1.5% clove oil treatment group in comparison to the normal control throughout the 25 days of treatment while no significant difference is seen in the chitosan + 1% clove oil treatment group when compared to the normal control.

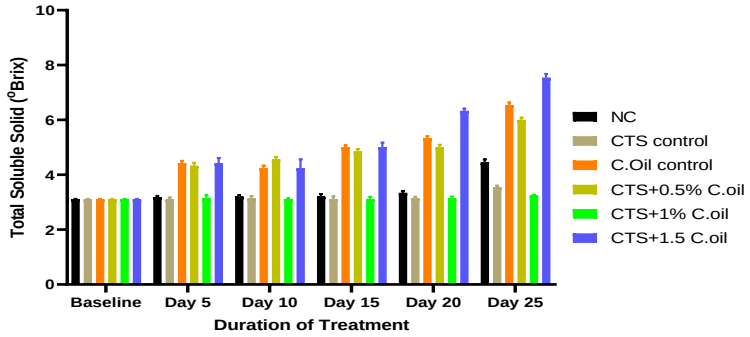

Figure 5: Effect of Chitosan-loaded Clove Oil on the Total Soluble Solid of Cucumber Expression of Values is in mean ± Standard Error of mean (n=3)

The total soluble solid of plantain, Figure 6, significantly increases in the clove oil control group and significantly decrease in the chitosan control group when compared to the normal control. The chitosan + 1 % clove oil treated group significantly decreases when compared to the normal control, as against the chitosan + 0.5% clove oil and chitosan + 1.5% clove oil treatment group, which significantly increases in comparison with the normal control group.

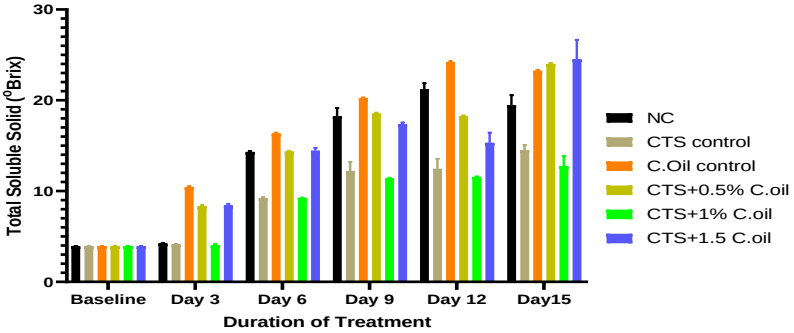

Figure 6: Effect of Chitosan-loaded Clove Oil on the Total Soluble Solid of Plantain Values are Expressed in mean ± Standard Error of mean (n=3)

The total sugar in Figure 7 shows that there is a significant increase in the clove oil control group, chitosan + 0.5 % clove oil and chitosan + 1.5 % treatment groups in comparison to the normal control throughout the treatment. The chitosan + 1% clove oil treatment group shows no increase in total sugar when compared to the normal control.

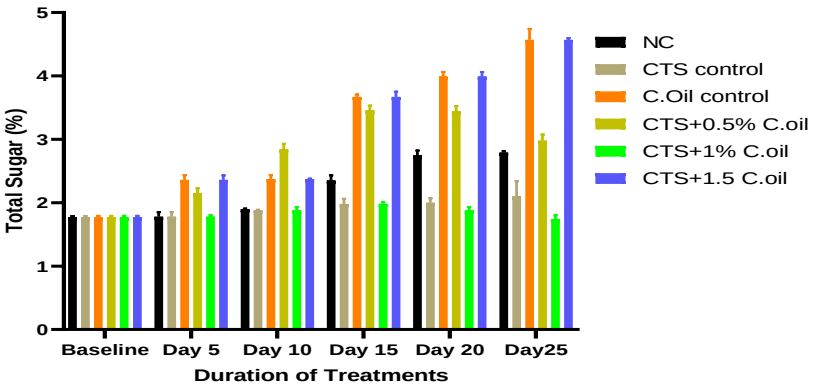

Figure 7: Effect of Chitosan-loaded Clove Oil on the Total Sugar of Cucumber
Values are Expressed in mean ± standard Error of mean (n=3)

The total sugar of plantain in Figure 8 shows that there is a significant increase in the clove oil control group and chitosan control group when in comparison with the normal control group, while the group treated with chitosan + 1% clove oil significantly decreases when compared to the normal control.

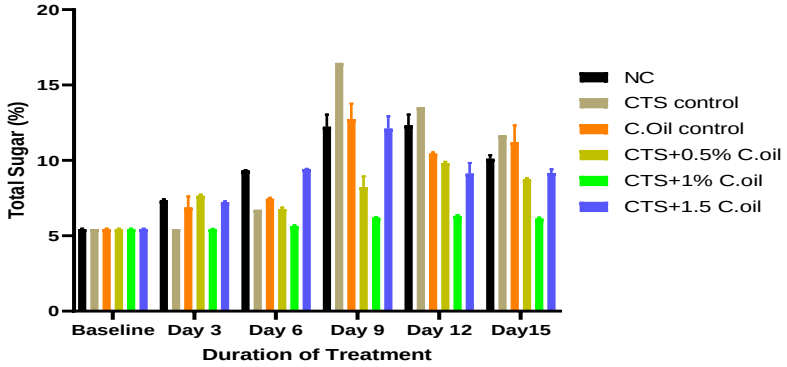

Figure 8: Effect of Chitosan-loaded clove Oil on the Total Sugar of Plantain
Expression of Values is in mean ± Standard Error of mean (n=3)

Effects of Chitosan-Loaded Clove Oil on the Total Antioxidant Capacity of Cucumber and Plantain

Figure 9 shows that there was no significant decrease in the cucumber's total antioxidant capacity in the clove oil and chitosan control groups, respectively, and the groups exposed to chitosan-loaded clove oil after completion of treatment, when compared to the normal control.

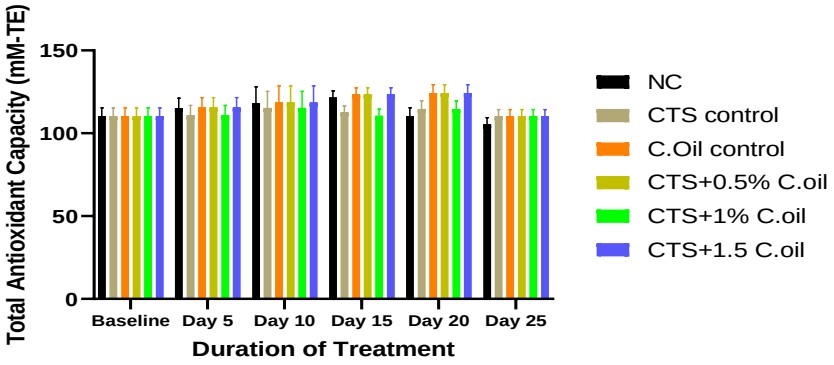

Figure 9: Effect of Chitosan-loaded Clove Oil on Total Antioxidant Capacity of Cucumber
Expression of Values is in mean ± Standard Error of mean (n=3)

Figure 10 shows a significant increase in the plantain's total antioxidant capacity in the clove oil control group and groups exposed to chitosan-loaded clove oil upon completion of the treatment when compared to the normal control.

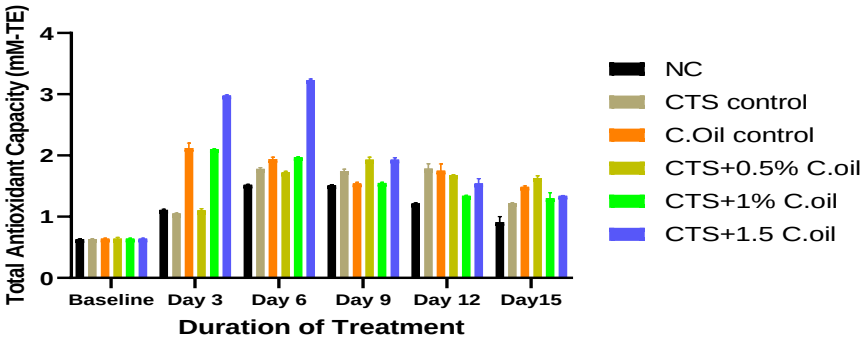

Figure 10: Effect of Chitosan-loaded Clove Oil on the Total Antioxidant Capacity of Plantain
Expression of Values is in mean ± Standard Error of mean (n=3)

Effects of Chitosan-Loaded Clove Oil on the Epicarp Colour Change of Plantain and Cucumber

Table 8 and figure 11 below shows the effect of the chitosan-loaded clove oil on the epicarp colour change of plantain. It was observed that on the 9th day of treatment, the epicarp of the clove oil control group, chitosan + 0.5% clove oil and chitosan + 1.5% clove oil were at the spoil colour which is similar to that of the normal control but the chitosan control group and the chitosan + 1% clove oil treated group still maintain its ripening colour.

Table 8: Plantain Epicarp Colour Change

Treatments	Baseline	Day 3	Day 6	Day 9	Day 12	Day 15
Normal Control	1	2	6	9	9	9
CTS Control	1	1	2	5	7	9
C.oil Control	1	4	9	9	9	9
CTS + 0.5% C.oil	1	2	8	8	9	9
CTS + 1% C.oil	1	1	3	5	7	9
CTS + 1.5% C.oil	1	2	6	9	9	9

1-Green; 2-Pale green; 3-Pale green and yellow tips; 4-Yellow: Green 1:1 5-More yellow than green
6-completely yellow 7-Yellow with black spot 8-Yellow: black 1:1 9-More black than yellow
Expression of values is in mean ± standard error of mean (n=3)

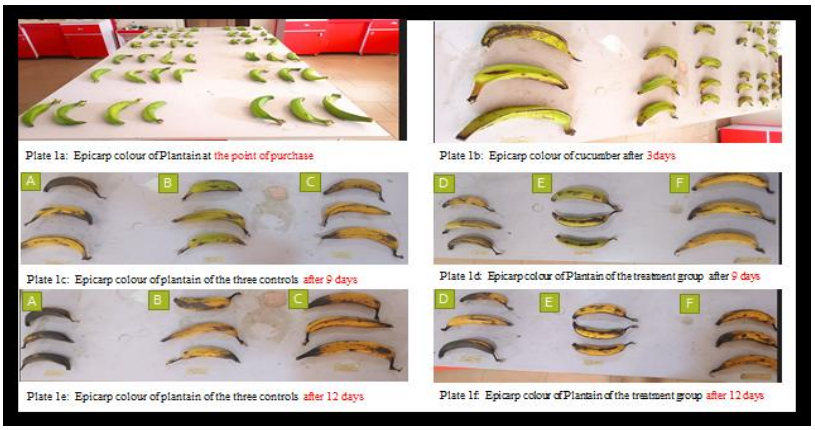

Figure 11: Pictorial Representation of the Epicarp Colour Change of Plantain

Table 9 and figure 12 below shows the effect of the chitosan-loaded clove oil on the epicarp colour change of cucumber. It was observed that on the 25th day of treatment, the epicarp of the clove oil control group was at the spoil colour stage when compared to the normal control, while the chitosan + 1% clove oil maintained its unripe colour when compared to the normal control.

Table 9: Cucumber Epicarp Colour Change

Treatments	Baseline	Day 5	Day 10	Day 15	Day 20	Day 25
Normal Control	1	1	3	4	4	5
CTS Control	1	1	1	2	2	2
C.oil Control	1	3	5	5	7	9
CTS + 0.5% C.oil	1	1	4	5	5	7
CTS + 1% C.oil	1	1	1	1	2	2
CTS + 1.5% C.oil	1	2	3	3	5	7

1-Green; 2-Pale green; 3-Pale green and yellow tips; 4-Yellow: Green 1:1 5-More yellow than green

6-completely yellow 7-Yellow with microbial attack 8-Yellow with severe microbial injury 1:1 9-More microbial injuries
Expression of values is in mean ± standard error of mean (n=3)

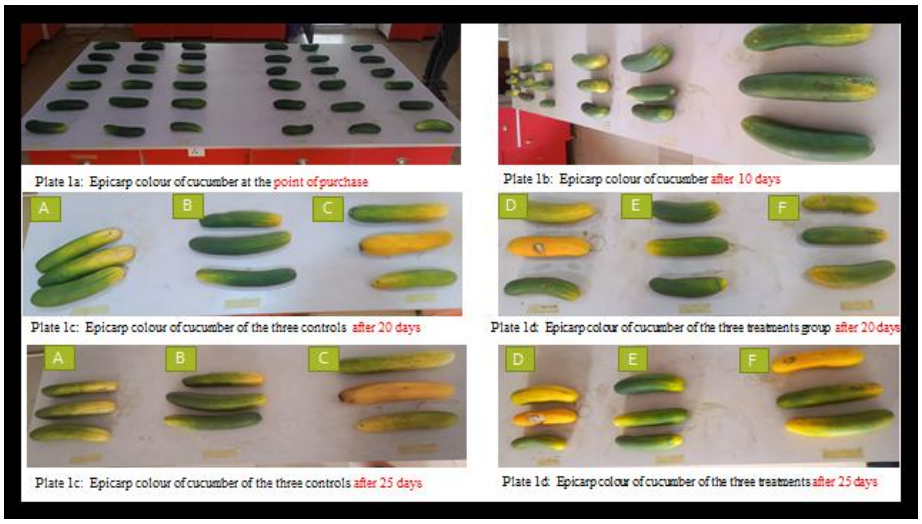

Figure 12: Pictorial Representation of the Epicarp Colour Change of Cucumber

Effects of Chitosan-Loaded Clove Oil on the Microbial Analysis of Plantain and Cucumber

Table 10 below shows that the total plate count and total coliform count of plantain have a countable plate and coliform count when compared to that of the control at both dilution factors of 10^3 and 10^6, respectively. However, the total mould count of plantain in the chitosan + 1% clove oil treatment group at the dilution factor of 10^3 has a mould count that is too numerous to count when compared to that of the control.

Table 10: Total Plate Count, Total Coliform Count and Total Mould Count for Plantain at Day 10

	Total Plate Count		**Total Coliform Count**		**Total Mould Count**	
	10^3	10^6	10^3	10^6	10^3	10^6
Normal Control	120	0	20	0	TNTC	1
Clove oil Control	52	0	5	0	77	1
Chitosan Control	TNTC	TNTC	210	7	TNTC	TNTC
0.5% C.oil + CTS	TNTC	TNTC	TNTC	1	TNTC	TNTC
1.0% C.oil + CTS	113	0	12	0	TNTC	0
1.5% C.oil + CTS	231	12	78	0	52	0

At day 15, the total plate count, total coliform count and total mould count of plantain in the chitosan + 1% clove oil and chitosan + 1.5% clove oil treatment group as shown in Table 11 are countable at both the dilution factor of 10^3 and 10^6 when compared to that of the normal control except for the total plate count of chitosan + 0.5% clove oil at both dilution factor and the chitosan + 1% clove oil at dilution factor 10^3 which are too numerous to count when compared to the normal control.

Table 11: Total Plate Count, Total Coliform Count and Total Mould Count for Plantain at Day 15

	Total Plate Count		**Total Coliform Count**		**Total Mould Count**	
	10^3	10^6	10^3	10^6	10^3	10^6
Normal Control	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
Clove oil Control	TNTC	TNTC	0	0	TNTC	TNTC
Chitosan Control	TNTC	TNTC	341	23	302	29
0.5% C.oil + CTS	TNTC	TNTC	0	0	244	42
1.0% C.oil + CTS	TNTC	12	0	0	111	1
1.5% C.oil + CTS	132	0	110	0	334	12

Table 12 shows that the plate count of cucumber at day 10 is too numerous to count at both dilution factors 10^3 and 10^6, and in the chitosan + 0.5 clove oil treatment group, which is similar to the control group. The same also goes for the total mould count at the dilution factor of 10^3. The chitosan + 1% clove oil group and the chitosan + 1.5% clove oil group have countable total coliform count, total plate count, and total mould count when in comparison to that of the normal control group.

Table 12: Total Plate Count, Total Coliform Count and Total Mould Count for Cucumber at Day 10

	Total Plate Count		**Total Coliform Count**		**Total Mould Count**	
	10^3	10^6	10^3	10^6	10^3	10^6
Normal Control	TNTC	TNTC	TNTC	0	TNTC	12
Clove oil Control	158	2	0	0	48	0
Chitosan Control	TNTC	45	54	0	56	0
0.5% C.oil + CTS	TNTC	TNTC	84	0	TNTC	26
1.0% C.oil + CTS	243	201	34	0	50	0
1.5% C.oil + CTS	67	0	1	0	21	0

Table 13 shows that the total plate count of cucumber at day 25 was too numerous to count at both dilution factors 10^3 and 10^6. Also, in the chitosan + 0.5% clove oil and chitosan + 1% clove oil treatment groups, the total plate count was too numerous to count, which is similar to the control group. The same also goes for the total mould count at the dilution factor of 10^3. The chitosan + 1% clove oil group and the chitosan + 1.5% clove oil group have countable total coliform count and total mould count when in comparison to that of the normal control group.

Table 13: Total Plate Count, Total Coliform Count and Total Mould Count for Cucumber at Day 25

	Total Plate Count		**Total Coliform Count**		**Total Mould Count**	
	10^3	10^6	10^3	10^6	10^3	10^6
Normal Control	TNTC	TNTC	TNTC	217	2	12
Clove oil Control	TNTC	29	88	0	72	0
Chitosan Control	TNTC	TNTC	156	0	219	23
0.5% C.oil + CTS	TNTC	TNTC	TNTC	23	TNTC	26
1.0% C.oil + CTS	TNTC	TNTC	201	12	300	13
1.5% C.oil + CTS	89	0	0	0	0	0

Discussion

One interesting way to preserve quality and prolong shelf life is to mix clove oil with chitosan when coating plantains and cucumbers (Yousuf *et al*., 2021). Applying chitosan, a naturally occurring biopolymer with film-forming and antibacterial qualities, on fruits and vegetables creates a barrier of defence. Eugenol-rich clove oil fortifies these qualities with its strong antibacterial and antioxidant effects. (Yuan *et al*., 2016). From the results obtained in Tables 3 and 5, the main causes of weight loss in plantains and cucumbers during storage are respiration and moisture evaporation. Chitosan coatings considerably diminish this weight loss by generating a semi-permeable barrier that inhibits the transport of water vapour, especially when laden with clove oil. This result was in agreement with Pereira dos Santos *et al*. (2019), who proposed that the moisture-retaining properties of chitosan and essential oils preserve the firmness and general physical integrity of the fruit. Furthermore, the produce's dry matter, or solid component, maintains its stability after being treated with chitosan and clove oil. This stability is explained by the decreased metabolic activity brought on by clove oil's antibacterial properties, which inhibit microbial development, spoiling and preserving the dry matter (Chaudhary *et al*., 2020). The pH of cucumber and plantain, as seen and Figures 1 and 2, showed a mild change when it was compared to the normal control. This is in line with the report of Hasheminejad and Khodaiyan, (2020). Microbial activity and enzymatic reactions during storage have been shown to cause changes in pH. Coatings containing chitosan and clove oil serve to keep the pH steady by reducing microbial growth, which frequently results in the production of acid. Delays in spoiling and prolonged freshness are correlated with this pH stability (Yu *et al*., 2017). Total titratable acidity, representing the total acid content, is an important quality parameter. The growth of acid-producing microbes is inhibited by the antimicrobial qualities of clove oil in the chitosan matrix, which slows the rise in total titratable acidity (Ajibola *et al*., 2023). This control aids in the prolongation of natural flavor profile of cucumber and plantain for a prolonged period of time. TSS, which is made up of sugars, organic acid, and other soluble constituents, tends to increase as fruits and vegetables ripens. To acquire balance in the TSS, the chitosan-clove oil coating slows the metabolic processes that transform starches into sugars. Preservation of this balance is needed to preserve the sensory attributes of the produce (Beckles, 2012). Total sugar content is a crucial parameter for spoilage and ripening. Together, clove oil's antibacterial effect and chitosan's barrier effect prevent the degradation of sugar and formation of spoilage microorganisms. As a result, the concentration of sugar changes more gradually and is under control (Bhardwaj *et al*., 2019).

Microbial analysis is crucial in determining the effectiveness of preservation methods. Chitosan and clove oil possess extremely high antimicrobial activity, and microbial load on cucumber and plantain is greatly reduced. Microbial cell membranes break down through the action of eugenol in clove oil, and natural antibacterial activity in chitosan helps initiate this decline. Low microbial count is therefore linked with extended shelf life and enhanced quality maintenance (Perumal *et al*., 2022)

In conclusion, the use of chitosan with clove oil as a coating for plantain and cucumber effectively prolongs shelf life and retains quality by reducing weight loss, stabilizing dry matter content, maintaining pH and titratable acidity, controlling total soluble solids and sugar content, and significantly minimizing microbial contamination. The approach offers a natural and safe alternative to synthetic preservatives that is in line with consumer demands for cleaner labels and environmentally friendly techniques in food preservation.

CONCLUSION

The study demonstrated that the application of chitosan and a combination of chitosan and 1% clove oil effectively maintained the weight of plantain and cucumber, providing significant protection against weight loss. Notably, the epicarp colour of both plantain and cucumber was best

preserved under the chitosan with 1% clove oil treatment after 9 and 20 days, respectively. This suggests that this specific coating concentration effectively delays the senescence process in these fruits. Microbial analysis further indicated that the chitosan with 1% clove oil treatment resulted in the most substantial reduction in microbial growth, highlighting the antimicrobial efficacy of this coating. In conclusion, chitosan combined with 1% clove oil exhibits great potential in the extension of shelf life of cucumbers and plantains by at least 15 days and 4 days, respectively. This research underscores the effectiveness of this treatment in enhancing post-harvest quality and longevity of these fruits. It is therefore recommended that the exoskeleton of crustaceans that has been termed a waste for so many years should be a means to convert waste to wealth and more essential oils should be considered for the development of coating materials, as clove oil shows limitations in epicarp colour retention at high concentrations.

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