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Development And Application of Gelatin Based Edible Coating Containing Cellulose Nanocrystal in the Storage of Fresh Cucumber

***[1]Habeeb K. Saka, [1]Ibrahim Bala, [1]Oladimeji O. Olaleye, [1]Farida U. Ahmad, [1]Ahmad K. Yerima, [1]Abdullah T. Ibrahim, [1]Ahmad A. Adamu, [1]Hauwa M. Abdullahi, [1]Mardiya S. Ayuba, [1]Abdulqadir B. Ibrahim, [1]Binta R. Isa, [1]Fatima J. Babba.**

[1]Perishable Crops Research Department,
Nigerian Stored Products Research Institute,
Kano

Email: saka.habeeb@yahoo.com

Abstract

Cellulose nanocrystal (CNC) has attracted attention in recent times because of its unique properties. In this study, the ability of gelatin based edible coating containing CNC supplemented with glycerol was investigated to extend the shelf life of fresh cucumber (Cucumis sativum) for a storage period of 14 days. CNC was produced using chemical method and characterised using X-ray diffraction (XRD) and Fourier transform-infrared spectroscopy (FTIR). Filmogenic solution of gelatin was also produced by dissolving 10 g of gelatin powder in 100 ml of distilled water with 0, 1 and 4 g of CNC/10 g gel. The effect of two treatments alongside the control experiment was studied on some physicochemical and microbial assay were conducted. The result obtained indicate that the Derby Schrerer's formula employed to calculate crystallite size of CNC yielded 5.9 nm. CNC- coated cucumber retained the green colour of cucumber in the first 7 days of storage. For each treatment, there were differences between the physicochemical parameters and the storage time. Weight loss, total soluble solids and titratable acidity effects were in direct correlation with the level of concentrations of the gelatin-based CNC. However, the level of retentions in ascorbic acid, flavonoids and alkaloids were higher in low treatment concentration (10000 ppm) than in the high concentration (40000 ppm) of the filmogenic coating material used. Results emanated from microbiological assays revealed that E. coli was found to be the predominant bacterial species identified in cucumber samples. No Salmonella sp. or Shigella sp. was isolated from the samples. Aspergillus sp. was found to be the most occurring fungal specie in the coated cucumber sample. Associated edible coating such as the gelatin-based CNC produced in this present research has provided reliable data for the shelf-life extension of fresh cucumber fruits at low concentration for a storage period of 2 weeks which could be used to extend the conservation period of fresh cucumber to support long period of time between harvest and transportation to distant markets.

Keywords: Cellulose nanocrystal, Cucumber, Filmogenic solution, Gelatin, Edible coating

INTRODUCTION

Nanotechnology is gaining recognition as a tool for food security, through the production of food with outstanding quality (Neme *et al*., 2021). Nanotechnology, having characteristics of nanoparticles is emerging to be useful in elongation of shelf life of fruits and vegetables also proving to be inhibitory to the proliferation of microorganisms (Upadhyay *et al.,* 2022). Edible

**Author for Correspondence*

coating is simply a layer of edible material formed around food or placed between its components (Fakhouri *et al*., 2014). Acceptable sensorial characteristics, appropriate barrier properties, good mechanical strength, microbial, biochemical and physiochemical stability, safety, low cost and simple production technology are qualities of interest in choice of coating materials (Vaishali *et al*., 2019; Fakhouri *et al*., 2014). Fresh cucumber, due to its high moisture content, has high affinity to fast deterioration shortly after harvest. The deterioration may be connected to improper handling practices which could result to physical damage or pathogenic microorganism's infestation. FAO (2019) estimated about 2 billion people in the world experienced moderate to severe food insecurity, lacking access to quality food predisposes them to malnutrition (Neme *et al*., 2021). About 33% of the food delivery worldwide for human consumption is lost after harvesting according to Food and Agriculture Organization (Upadhyay *et al*., 2022). Pathogenic organisms were culpable in food wastage globally, minimizing wastage will increase the supply and cost of food (Neme *et al*., 2021).

Fresh cucumber is one of the most preferred fruit vegetables used for culinary purposes aside its therapeutic medicinal properties and beauty culture applications (Ugwu & Suru, 2021). It is highly consumed fruit crop in Nigeria and the world at large. It is generally eaten raw either alone or garnished with other fruits and vegetables (Uthpala *et al*., 2020). Cucumber is a source of phytonutrients such as flavonoids and a host of antioxidants which are of pharmacological importance (Guan *et al*., 2023). Owing to the larger population of Nigerian, a case study of Kano state, did reveal that the production of fresh cucumber is highly significant as food source as well as economic buffer for the farmers and traders (Okafor & Yaduma, 2021). Therefore, the need to minimize postharvest losses of fresh cucumber through the use of biodegradable packing material cannot be over emphasized. This research was aimed at the development and application of gelatin-based edible coating containing cellulose nanocrystal in the storage of fresh cucumber.

MATERIALS AND METHODS

Collection of Rice Husk and Fresh Cucumbers

Rice husk for the production of cellulose nanocrystal was obtained from Alhamsad rice mills and transported to Nigerian Stored Products Research Institute (NSPRI), Kano Zonal office for further analysis. Fresh cucumbers were purchased from Yankaba market, Kano and transported to NSPRI Kano Laboratory. All chemicals and reagents employed for the analyses were of analytical grades.

Production of Cellulose Nanocrystals

The method described by Fakhouri *et al*. (2014) was employed for the production of cellulose nanocrystal. Briefly, rice husk was milled to fine powder and 10 g of rice husk powder was submerged in 200 ml of NaOH solution (10% w/w) for 4 hours at 60 ^{o}C. The solution was filtered and washed with distilled water to achieve 7.0 pH and the filtrate was dried at 40 ^{o}C. Combined solution (300 ml) of NaOH (5% w/w) + $CaO(Cl)_2$ (2.5% w/w) was added to the dried rice husk and the mixture was allowed to stay for 4 hours at 45 ^{o}C. The solution was filtered and washed to achieve 7.0 pH and the filtrate was dried at 40 ^{o}C. The rice husk was then treated with 250 ml of 60% sulphuric acid (with vigorous stirring) for 30 minutes at 55 ^{o}C. The mixture was then centrifuged until CNC was suspended in solution and the suspended solution went through dialysis for 72 hours.

Characterization of Cellulose Nanocrystal

The crystallographic structure of CNC was determined using X-ray diffraction analysis which was carried out by irradiating the CNC with indirect X-ray and measuring the intensities and scattering angle of which are as a result of the CNC. X-ray diffraction is, a non-destructive technique, widely used for the identification of crystalline structure of unknown powdered materials (Callister & Rethwisch, 2018). It delivers information related to unit cell dimensions, crystal phase, crystal orientation and crystallinity of the materials. X-ray Diffraction analysis was made in a PANalytical X'pert Pro Multipurpose X-ray Diffractometer.

Development of Gelatin-Based CNC Filmogenic Solution

The filmogenic solution of gelatin was produced by dissolving 10 g of gelatin powder in 100 ml of distilled water with 0, 1 and 4 g of Cellulose nanocrystals/10 g gel (to produce 0.0000 ppm, 10000 ppm and 40000 ppm concentration). The mixture was heated at 70 ^{o}C for 10 minutes. Afterwards, glycerol was added under smooth mechanical agitation for workability and firmness of the film (Fakhouri *et al*., 2014).

Coating of Cucumbers

The cucumbers were selected for homogeneity in size, consistence and ripening. The selected cucumbers were washed with vinegar to get rid of potential spoilage organisms and then washed with clean water. The cucumbers were coated with filmogenic solutions in 3 groups; Control (0.0000 ppm nanocrystal), group 2 (10000 ppm nanocrystal) and group 3 (40000 ppm nanocrystal).

Physiochemical and Phytonutrients Analysis

Determination of Weight Loss

The weight loss of cucumber was carried out using the method described by Shah & Hashmi (2020) using the following equation: Weight loss (%) = ((W1 - W2)/W1) x 100 %

Determination of Total Soluble Solids (TSS) and Titratable Acidity (TA)

Cucumber juice was extracted from each of the treated sample for TA according to the method described by Khaliq *et al.* (2015). TSS concentrations were measured using (WY-25 Digital) Abbe Refractometer. The T.A was measured by titrating a 1:10 mL aliquot (1 mL of juice with 9 mL of distilled water) with 0.1M sodium hydroxide using phenolphthalein indicator and was expressed as percentage of citric acid equivalent in the basis of fresh weight.

Determination of Total Sugar Content

The total sugar content (TS) was determined using the phenol–sulfuric acid method according to the method described by Dubois *et al.* (1956). Approximately 0.25 g of cucumber pulps was homogenized in 20 mL of 70 % ethanol (v/v) and then filtered. Aliquot of 1 mL of the filtrate was treated with 1 mL 5 % phenol (v/v) and 5 mL of 98 % sulfuric acid (v/v). After 1 hour of extraction, the coloured solution was allowed to cool followed by absorbance reading at 490 nm using UV/Visible spectrophotometer (PG Instrument). A standard curve was generated using a standard glucose solution and TS was expressed as mg of glucose equivalents per gram of fresh weight.

Determination of Flavonoid Content

Flavonoid determination was done by the method reported by Ejikeme *et al*. (2014) and Boham & Kocipai (2016). Exactly 50 mL of 80 % aqueous methanol added was added to 2.50 g of blended sample in a 250 mL beaker, covered and allowed to stand for 24 hours at room

temperature. After discarding the supernatant, the residue was re-extracted (three times) with the same volume of ethanol. Whatman filter paper number 1 was used to obtain the filtrate which was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until constant weight was obtained. The percentage of flavonoid was calculated as: % Flavonoid = Weight of flavonoid filtrate/ Weight of sample × 100 %.

Determination of Alkaloids Content

The alkaloid content was evaluated according to the method described by Harborne (2003). Exactly 200 mL of 10 % acetic acid in ethanol was added to 2.50 g of cucumber pulp in a 250 mL beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume and then followed by addition of 15 drops of concentrated ammonium hydroxide dropwise to the extract until the precipitation was complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 20 mL of 0.1 M of ammonium hydroxide and then filtered using Whatman filter paper. The residue was dried in an oven and the percentage of alkaloid is expressed as: % Alkaloid = Weight of alkaloid /Weight of sample × 100 %.

Determination of Ascorbic Acid Content

Vitamin C content was determined according to titration method reported by AOAC (2010). About 2.0 g each cucumber pulp was weighed into 50 mL distilled water. Equal volume of extraction solution containing 15 g Metaphosphoric acid, 40 mL acetic acid glacial in 500 mL distilled water was dispensed to sample aliquot. Three drops of thymol blue indicator (0.1 g thymol blue in 10.75 mL of 0.03M NaOH diluted in 250 mL water) was added to aliquot and then titrated with indophenol standard solution to rosy pink at end-point. Standard ascorbic acid solution prepared by dissolving 0.05 g in 50 mL in extraction solution was then titrated in similar ways to samples.

Estimation of Bacterial and Fungal Load

The samples were prepared according to the method described by Arienzo *et al*. (2020). Briefly, each cucumber blended in 100 mL of sterile distilled water under sterile conditions. Aliquot of 1 mL from the homogenate was dispensed into 9 mL of sterile distilled water to make the stock solution. Aliquot of 1 mL from the stock solution was serially diluted in 9 mL of sterile distilled water up to 10^{-6}. The bottle containing dilution factor of 10^{-3} were plated onto sterile Nutrient agar (Hi-Media, India), incubated aerobically at 37 °C for 18-24 hours to determine the total aerobic bacterial count. Enumeration of bacterial species was carried out by plating aliquot of 1 mL from 10^{-3} tube onto plate of sterile MacConckey agar (Hi-Media, India), Salmonella Shigella agar (Hi-Media, India) and were incubated aerobically at 37 ^{o}C for 18-24 hours. Distinct colonies from overnight incubation were sub-cultured onto a fresh plate of sterile MacConkey agar and incubated at 37 ^{o}C for 18-24hour. Identification of bacteria was carried out by subjecting the distinct bacterial growth to Gram staining, catalase test, coagulase test, urease test, oxidase test, Indole production, citrate utilization test, triple sugar iron agar (TSI), oxidase and methyl-red and voges-proskauer`s test (MRVP) to determine the identity of the bacteria. The bacteria were identified using Koneman Chart (Procop *et al*., 2017). Fungi load determination was carried out according to the method described by Feroz *et al*. (2016). Briefly, 1 mL aliquot from 10^{-3} tube was plated onto potato dextrose agar (Hi-Media, India) and subsequently incubated at room temperature. The growth on potato dextrose agar (PDA) was counted after 2 days of incubation and expressed as mould count per millilitre (mc/g). The fungi species were identified after seven (7) days of incubation using physical

observation and Lactophenol cotton blue staining technique and interpreted with the aid of Atlas of Mycology.

Statistical Analysis of Data

Experimental analysis was carried out in triplicate and data expressed as means ± standard deviation. Physico-chemical properties were analysed using one-way analysis of variance (SPSS 16.0 version 2007). Descriptive statistics was uses used to calculate mean bacterial and fungal load**.**

RESULTS AND DISCUSSION

Cellulose nanocrystals are unique nanomaterials derived from the most abundant and inexhaustible natural polymers (Thompson *et al*., 2019). CNC primarily obtained from naturally occurring cellulose fibres are biodegradable and renewable in nature, hence serves as environmentally friendly materials with wide range of applications (Yang *et al*., 2019). XRD analysis of CNC provides the crystallographic information of the CNC, the diffractogram (Figure 1), which presented a well-defined diffraction peaks. The sharp and narrow peaks observed at lattice plane between 20°-30° 2θ value indicate the formation of an ordered layered material which characterized a crystalline material, thus, reflecting that CNC was formed. With respect to the 23.7 lattice plane, the crystallite interlayer spacing was found to be 3.8Å and using the Derby Schrerer's Formula the crystallite size was calculated to be 5.9 nm.

FTIR gives the main functional groups present in a material or sample (Nandiyanto *et al*., 2019). The adsorption peak (Figure 2) at 3223 cm^{-1} – 2341 cm^{-1} were attributed to O-H and C-H stretching vibration respectively. O-H vibration of absorbed water is responsible for the peak absorption at 1622 cm^{-1}. The peak around 1424 was due to the C-H and C-O vibration contained in the polysaccharide rings of cellulose.

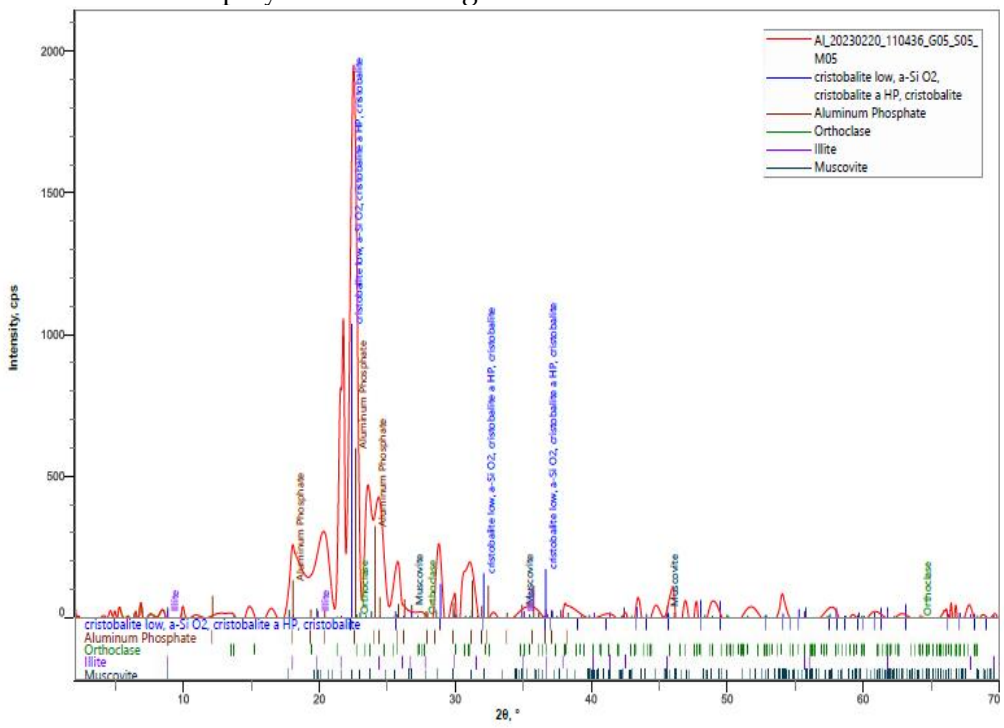

Figure 1: XRD of Cellulose Nanocrystal

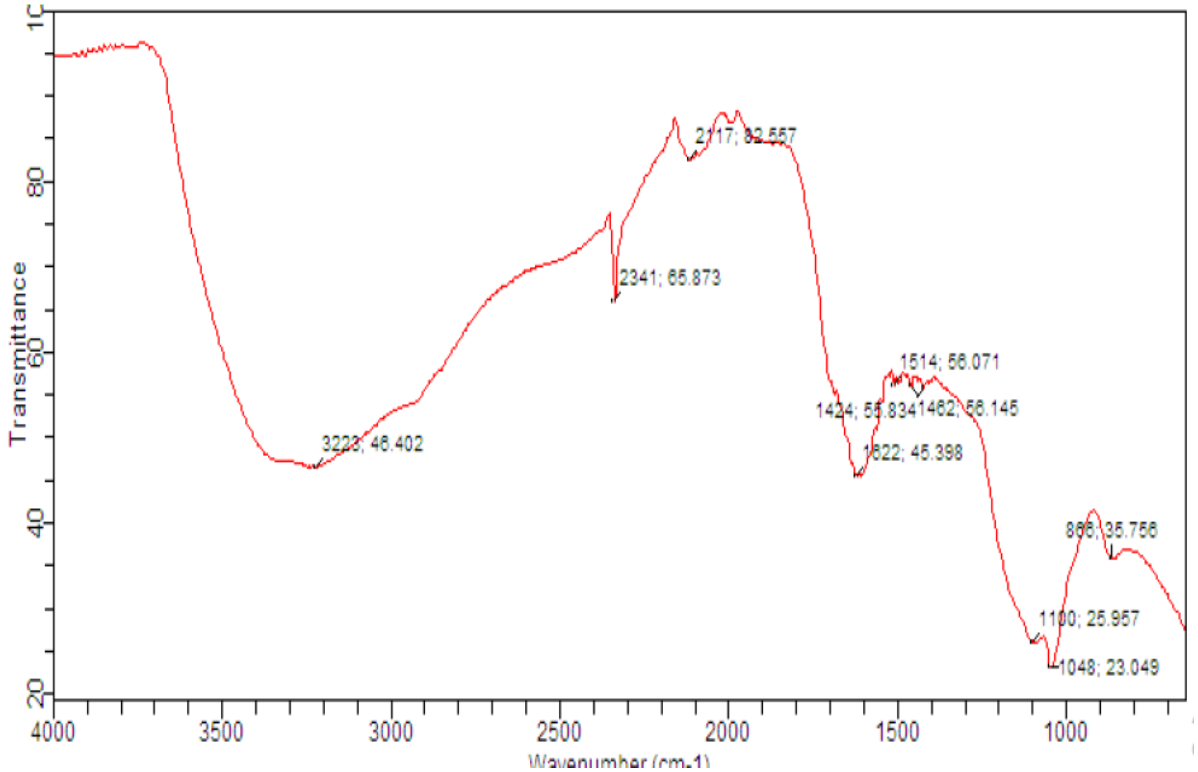

Figure 2: FTIR of Spectrum of Cellulose Nanocrystal

Physical observation of gelatin-CNC nanoparticles treated cucumber fruits showed that the cellulose nanocrystal supplemented with glycerol adhered uniformly to the cucumber without rupture. The coating enhanced the natural look of the cucumber in the first instance (Table 1). It was observed that the control sample without CNC showed evidence of discoloration after 7 days of storage while those with CNC retained the green colour of the cucumber. The control sample showed evidence of gross deterioration at 14 days of storage, while the cucumber sample with 40000 ppm CNC had evidence of deterioration at the 14th day of the experiment.

Table 1: Physical Observation of Gelatin-CNC Nanoparticles Treated Cucumber Fruits in Storage

CNC conc.	Day 0	Day 7	Day 14
0.0000 ppm			
10000 ppm			
40000 ppm			

Ppm= Part per Million CNC conc- Cellulose Nanocrystal Concentration

The results of physicochemical properties of the coated and uncoated cucumbers with respect to concentrations of the filmogenic material used are presented in Table 2. WL (%) increased throughout the entire period of 14 days during which the highest WL within the ambient storage conditions (29±3°C; 58±6 RH) was 30.28% of the uncoated cucumber fruits followed by the coated cucumber of 10000 ppm (9.19%); an implication that showed the 40000 ppm coated having the least WL (7.08%) during the storage period. The disparities in the WL of cucumber fruits have shown that concentrations of the CNC filmogenic material had direct impact on water loss prevention during transpiration. Hmmam *et al.* (2021) recorded a significant decline in coated and uncoated mango fruits stored for 28 days. There was a gradual increase in TSS as storage time progressed in all treatments (Table 2). The increase was lower in the 10000 ppm CNC-coated cucumbers than the 0.0000 ppm cucumbers during storage. Cucumbers coated with the highest concentration (40000 ppm) recorded slightly higher (2.28%) TSS than the 10000 ppm CNC nanoparticles coated cucumber fruits with 1.99% TSS value at the end of 14 days storage time. During storage period, the variation in TSS could be due to alterations of the cell wall and hydrolysis of complex carbohydrates by the activities of hydrolytic enzymes (Kittur *et al*., 2001). In previous study by Gardesh *et al*. (2017) on coated climacteric fruits, similar findings were observed with nanoparticles coatings' showing ability to decelerate the increase in TSS of fruits in storage.

Similarly, there was a gradual decline in TA for all treatments over the storage period of 14 days as depicted also in Table 2. The TA results showed that the CNC coating properties had reduced the deterioration of cucumber fruits compared to the control during the storage period. Cucumbers coated with low concentration of CNC practically had the least decline (0.39 %) in TAs than the high concentrated (40000 ppm) which had a 0.36 % TA at the end of storage period. The TS of both experimental and control samples increased throughout the storage timeline with a sharp increase (from 422.09 to 435.81 mg/100 g) in uncoated cucumber fruits was observed. It was observed in this study that increase in TS was in direct relationship with the physical parameter (WL) and indirectly with the effect of TA which agree with the research findings of Hmmam *et al*. (2021) on Seddik" Mango coating with nanoparticles. The initial ascorbic acid estimate of 5.02 mg/100 g before coating and storage, there was a progressive decline in the level of ascorbic acid during storage in both coated and uncoated cucumber fruits. Highest decline was observed with the uncoated (control) sample with about over 60% loss at the end of storage time. Although, CNC coating material was impactful in retaining ascorbic acid during storage, however, the highly concentrated cellulose nanocrystal material retained about 60% of the ascorbic acid while >80% retention was estimated for the 10000 ppm (lowest CNC concentration used). There was a decline in flavonoids in the coated and uncoated cucumber cucumbers throughout storage time. Just like the ascorbic acid level, the retention level of flavonoids was least (39%) in the control cucumbers followed by the much 40000 ppm coated experimental cucumbers (85%) and 97.7% in the least 10000 ppm coated cucumber at the end of the storage period. Flavonoids is one of many bioactive properties of cucumber fruit which contributes to protection of cellular inflammation, proliferation and platelet aggregation (Ejikeme *et al*., 2014). In our experiment, it has shown that coating of cucumbers with CNC coating materials can retain the level of flavonoids than vitamin C. In this study, prior to coating and storage, alkaloid content was estimated to be 3.02 mg/100 g. There was a steady decline in the level of alkaloids during storage in both coated and uncoated cucumber fruits. The rate of decline between the start of the experiment till the end of storage period ranged from initial 3.02 mg/100 g to 1.27 mg/100 g, 2.84 mg/100 g and 1.66 mg/100 g in the 0.0000 ppm, 10000 ppm and 40000 ppm treatment samples respectively. Alkaloids have been associated with some important pharmacological effect especially of analgesic potency (Sotiroudis *et al*., 2010).

Table 2. Shelf-Life Effect on Physico-chemical Parameters of Fresh CNC Nanoparticles Treated Cucumber Fruits

CNC concentration: 0.0000 ppm							
Storage Time(Days)	WL (%)	TSS (°Brix)	TTA (%)	TS (mg/100 g)	AA (mg/100 g)	FLV (mg/100 g)	ALK (mg/100 g)
0	0.00	3.51	0.44	422.09	5.02	2.61	3.02
7	26.47	3.59	0.39	429.12	3.88	2.00	2.95
14	30.28	4.21	0.33	435.81	1.99	1.02	1.27
CNC Concentration: 10000 ppm							
Storage Time (Days)	WL (%)	TSS (°Brix)	TTA (%)	TS (mg/100 g)	AA (mg/100 g)	FLV (mg/100 g)	ALK (mg/100 g)
0	0.00	3.51	0.44	422.09	5.02	2.61	3.02
7	5.08	3.53	0.43	425.79	4.83	2.59	2.99
14	9.19	3.58	0.39	428.09	4.51	2.55	2.84

CNC Concentration: 40000 ppm							
Storage Time (Days)	WL (%)	TSS (°Brix)	TTA (%)	TS (mg/100 g)	AA (mg/100 g)	FLV (mg/100 g)	ALK (mg/100 g)
0	0.00	3.51	0.44	422.09	5.02	2.61	3.02
7	3.14	3.55	0.39	422.92	4.36	2.32	2.98
14	7.08	3.59	0.36	426.81	3.03	2.21	1.66

CNC= Cellulose Nanocrystal., WL=Weight Loss., TSS= Total Soluble Solids., TTA= Titratable Acidity., TS= Total Sugar., AA= Ascorbic Acid., FLV=Flavonoids., ALK=Alkaloids. Ppm= Part per Million

The bacterial loads shown in Table 3 of coated cucumber in storage show that the highest bacterial load was observed in the control sample with 0.0000 ppm CNC ($3.4x10^4$ CFU/ml). Relatively lower bacterial load recorded in cucumber coated with CNC may be as a result of antimicrobial property of CNC. *E. coli* was found to be the predominant bacterium identified in the cucumber samples. No *Salmonella* sp. or *Shigella* sp. was isolated from the samples. The fungal load of coated cucumber samples in storage (Table 3) shows no fungal growth in the 0th day of fungal culture of cucumber. A higher fungal load was observed in the control group ($2.4x10^4$ mc/g). Aspergillus sp. was found to be the most occurring fungus in cucumber samples.

Table 3: Microbiological Assay of Gelatin-CNC Nanoparticles Treated Cucumber Fruits

Storage Time CNC conc.	Day 0 CFU/ml	Day 7 CFU/ml	Day 14 CFU/ml	Identified Bacteria
0.0000 ppm	$1.0x10^2$	$3.4x10^4$	$2.6x10^7$	*E. coli*, *S. aureus*, *Bacillus* sp.
10000 ppm	$1.0x10^2$	$1.8x10^3$	$1.2x10^4$	*E. coli*
40000 ppm	$1.0x10^2$	$3.9x10^3$	$1.6x10^5$	*E. coli*

Storage Time CNC conc.	Day 0 mc/g	Day 7 mc/g	Day 14 mc/g	Identified Fungi
0.0000 ppm	NG	$1.3x10^2$	$2.4x10^4$	*Aspergillus niger*, *Aspergillus terreus*,
10000 ppm	NG	$1x10^2$	$1.9x10^2$	*Penicillium* sp., *A. niger*
40000 ppm	NG	$2.1x10^2$	$3.4x10^3$	*A. niger*

Ppm= Part per Million CNC- Cellulose Nanocrystal, CNC conc- Cellulose Nanocrystal Concentration CFU/ml- colony forming unit per millilitre, mc/g – Mould count per gram, NG- No growth

CONCLUSION

Cellulose nanocrystal can be obtained from rice husk using acid hydrolysis. Synthesized edible gelatin-based cellulose nanoparticle from rice husk has proved to be effective alternative treatments to enhance the postharvest life of fresh cucumber fruits. The overall findings from physicochemical and microbial assays in this research did suggested that the application of CNC gelatin-based coatings can efficiently be used at low concentration (10000 ppm) for shelf-life extension of fresh cucumber fruits. Gelatin-CNC could be used to extend the conservation period of fresh cucumbers with a view to supporting long period of time between harvest and transportation to distant markets.

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