



ISSN 1110-0451



(E S N S A)

Nanoparticles and irradiated chitosan impact on the quality of sweet green bell pepper (*Capsicum annuum* L.) under cold storage conditions

Ahmed H. Ewais*^{1,2}, Mai Raslan¹, Ramadan A. Hassanien³, I. T. Zedan⁴

⁽¹⁾ Biotechnology and Life Sciences Department, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Egypt.

⁽²⁾ Quality Assurance and Quality Control Department, Nuclear and Radiological Safety Research Center (NRSRC), Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt.

⁽³⁾ Food Irradiation Departments, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt.

⁽⁴⁾ Renewable Energy Science and Engineering Department, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Egypt.

ARTICLE INFO

Article history:

Received: 13th Dec. 2021

Accepted: 1st Mar. 2022

Keywords:

Gamma radiation;

Edible coating;

Mucilage;

Nanocomposite;

Storage.

ABSTRACT

Biosynthesis of nanoparticles (NPs) is a rapidly expanding topic; the junction of nanotechnology and biotechnology has gained a lot of attention as a result of the growing need for ecologically friendly and effective food safety technologies. Sweet green bell pepper (SGBP) is a perishable vegetable with a short shelf life and a high risk of fungal illness throughout the handling and storage procedure. This study aims to compare the efficacy of different chitosan preparations (irradiated and nanoparticles) in preserving SGBP. Chitosan powder was irradiated by Co⁶⁰ γ -radiation with a total exposure dose of 40 kGy. Six solutions were prepared; chitosan, irradiated chitosan, and nanocomposite (chitosan, mucilage) with two concentrations, 0.5%, 1% for each one. The treatments were applied for the six groups of SGBP fruits by immersion in one solution for two minutes and stored in the refrigerator condition for 28 days. Microbiological and physiochemical analyses were determined weekly. All treatments generally decreased the total microbial count, especially at 1% concentration of the irradiated chitosan treatment. The fungal growth was completely suppressed by treatments with concentration 1%. In addition, the results of chemical analysis showed that the treated fruits retained the acceptable quality characteristics of the consumers compared to the untreated fruits.

1. INTRODUCTION

Sweet green bell pepper (SGBP) (*Capsicum annuum* L.) is one of the annual pepper types from the nightshade family; it has more than one color, including yellow, red, orange, and the green color which was chosen for this study. SGBP is a commercially important vegetable. Despite this, it is a perishable vegetable with a limited shelf life and a high vulnerability to fungi. Quality decline, chilling injury when stored below 7°C, and shriveling coupled with rapid weight loss are all common post-harvest issues for pepper fruits.

Fruit and vegetable losses after harvest are a serious worry for an agriculturally-based country like Egypt. Fruits and vegetables are highly perishable by nature and require careful handling and storage to avoid losses

during post-harvest operations in impoverished nations[1]. SGBP is the most popular and high-value commercial vegetable in Egypt. SGBP has pleasant flavor coupled with the rich content of ascorbic acid and other vitamins and minerals.

Recently, edible films have been developed to extend the shelf life of fruits and vegetables. The technology employs the film to be closely wrapped around the fruits, controlling respiration and transpiration, thus slowing down senescence [2,3]. In this study, SGBP fruits were treated by different chitosan solutions (irradiated & nanoparticles) as edible coating and a modified atmosphere surrounding the product [4]. Chitosan was chosen for this study because it is biocompatible, biodegradable, non-toxic, antimicrobial agent, and eco-friendly natural product [5,6].

Food irradiation is a promising technology that will contribute in achieving food security through preserving food products. This will facilitate food distribution from fertile developed regions to the malnourished peoples of underdeveloped countries [9]. Gamma irradiation on chitosan can improve its antimicrobial properties on fresh vegetables and extend its shelf life [7,8].

Psyllium seed extracts are used in the pharmaceutical industry as a non-toxic binding agent in various pharmaceutical formulations, as it contains polysaccharide which is known for its exceptional mucilaginous properties in an aqueous solution [9, 10].

The use of the gamma irradiated coating material on SGBP fruits for maintaining quality and prolonging shelf life has not been recorded till the present time. Studies on the properties of composite films from chitosan and mucilage are scarce, even though the combinations seem to improve the characteristics of biodegradable films on SGBP fruits. The goal of this research is to evaluate the effective locally made irradiation chitosan coatings on extending the shelf life and improving the quality of SGBP fruits. Thus, psyllium seed extract has been utilized in this study as a novel edible coating used for SGBP fruits preservation.

2. MATERIAL AND METHODS

2.1 Materials:

1. Chemicals

Chitosan powder of low molecular weight (LMW) and Sodium tripolyphosphate (TPP) were obtained from sigma Aldrich Company.

2. Fruits of green bell pepper fruits (*Capsicum annuum* L.)

Approximately 30 Kg. of SGBP (*Capsicum annuum* L.) fruits were obtained from the local market vegetable fruits and divided into seven groups.

3. Psyllium seeds

Psyllium seeds were obtained from the local market for extraction of its mucilage.

2.2 Methodologies

2.2.1 The Experimental Design:

1. Solutions of nanocomposite (chitosan, mucilage), chitosan and irradiated chitosan were prepared at concentrations 0.5 and 1 %.
2. SGBP samples were divided into seven groups. In the first group, the fresh fruits samples were packed on polythene bags with

their codes, closed tightly, and stored under cold storage as given in Table (1).

Table (1): Treatment groups

Group	Treatment
1	Control sample include SGBP fruits without treatment
2	0.5% chitosan low molecular weight (LMW)
3	1% chitosan low molecular weight (LMW)
4	0.5% nanocomposite
5	1% nanocomposite
6	0.5% irradiated chitosan
7	1% irradiated chitosan

3. The other six previous solutions were prepared with two concentrations, 0.5% and 1 % for each solution.
4. SGBP samples were dipped in their solutions and dried in air at room temperature for five minutes, then packed in polythene bags, closed tightly, and preserved under cooling (below 7 °C) for 28 days.
5. Every week, control and replicate samples from each treatment were prepared for microbiological and physiochemical analysis.

2.2.2 Weight loss and decay of SGBP fruits

Weight loss & decay of SGBP fruits measurements have been carried for fruits at zero time and after the cold storage weekly. The visual inspection and weighting were achieved for a defined SGBP fruits from all treatments and the weight loss and fruit decay percentage were registered for statistical analysis. Weight difference was calculated based on the final and initial weight of the samples and was expressed as a percentage. SGBP samples were taken in triplicate and studied from the beginning until the end of the cold storage period.

2.2.3 Preparation of SGBP aqueous extract

The aqueous extract of green bell pepper was prepared to evaluate the antioxidant activity, titratable acidity, PH, total soluble solids

(TSS), phenolic, and carotenes content [11]. A homogenizer was used to homogenize ten grams of SGBP with 40 ml (0.25 g/ml) of distilled water. To get a clear extract, the prepared solutions were filtered using muslin cloths and centrifuged at 10,000 rpm for 10 minutes. The physicochemical estimates were made using a prepared extract of control and treated bell peppers.

2.2.4 Determination of physicochemical analysis

The prepared aqueous extract of SGBP was used to determine the TSS, PH, titratable acidity and ascorbic acid. The TSS (°Brix) was determined by using a digital refractometer, which was calibrated with distilled water prior to taking readings. 10 grams of SGBP tissues were homogenized in a kitchen blender with 40 ml distilled water before being filtered using cotton wool. To acquire the (°Brix) reading, a drop of the filtrate was placed on the prism glass of the refractometer. To get the original TSS (percent) of the SGBP extract, the measurements were multiplied by a dilution factor. SGBP extract, pH was evaluated using a digital pH meter. The titration method was used to determine the titratable acidity (TA). Using a household blender and 40 ml of distilled water, extract tissues (10 g) were homogenized. Cotton wool was used to filter the mixture. 0.1 N NaOH was used to titrate the filtrate (5 ml) with one to two drops of phenolphthalein (0.1 percent) as an indicator to an endpoint pink [12]. The percentage of citric acid per 100 g fresh weight was used to calculate the results. Ascorbic acid was determined by using the method reported by Ranganna et al. [13]. Fruit extract tissues (10 gm) from three fruits were homogenized in a kitchen blender with 90 ml of 3 % metaphosphoric acid (HPO₃). A conventional dye solution (2,6-dichlorophenol-indophenol) was used to filter the mixture, resulting in a pink tint that lasted for 15 seconds. The ascorbic acid concentration of fresh fruit was measured in milligrams per 100 grams.

2.2.4.1 Determination of total phenolic content

The total phenolic content (TPC) has great importance in the nutritional, organoleptic, and commercial properties of fresh vegetables through their contributions to sensory properties such as flavor and color. TPC was determined in the SGBP extract by colorimetric

method Folin-Denis reagent according to Swain and Hillis (1929) [14].

2.2.4.2 Determination of total chlorophyll & carotenoids

Total chlorophyll and carotenoids were determined according to the methods described by Holm, G. et al., [15] and Von Wettstein et al. (1957) [16]. One gram of fruit from each pepper's equator was frozen for 24 hours at -16 °C and then defrosted. Chlorophyll was extracted with 10 mL of 80% acetone, centrifuged, and the supernatant was quantified with a UV-240 visible light spectrophotometer at wavelengths 440, 644, and 662 nm. Chlorophyll a and b concentrations were calculated using the following equations:

$$\text{Chlorophyll a} = 9.84 \times A_{662} - 0.990 \times A_{664}$$

$$\text{Chlorophyll b} = 21.426 \times A_{664} - 4.650 \times A_{662}$$

$$\text{Total Chlorophyll (a+b)} = 5.134 \times A_{662} + 20.436 \times A_{644}$$

$$\text{Carotenoids} = 4.695 \times A_{440} - 0.268(a+b)$$

A = absorbency at corresponding wavelength, values 9.784, 0.990, 21.426, 4.650, and 0.288 is the molar absorptivity coefficient according to Holm [15] and Wettstein [16] for acetone (absorption of 1 cm).

2.2.4.3 DPPH radical scavenging capacity assay

Samples were determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) [17]. Reaction mixtures of samples were prepared by mixing the appropriate amounts of SGBP extract 1.5 ml, of DPPH and completed with methanol to a total volume of 4 ml. The prepared solutions were left in the dark for 60 minutes, and then the absorbance was measured at 515 nm. All measurements were performed in three replicates to express the results in terms of average values. Methanol was used to zero spectrophotometer. Scavenging radical capacity was calculated via Trolox calibration curve and expressed as mg of Trolox equivalent per 10 g of SGBP fruits (mgTE/10 g f.v.).

2.2.5 Mucilage extraction

Psyllium seeds were purchased in Egypt from a local market. Psyllium mucilage was extracted by combining Psyllium seeds with distilled water in a ratio of 0.54 gm per 25 ml distilled water and boiling for 30 minutes to form a mucilaginous gel [18] to separate the pure gel from additional

impurities, the gel was filtered using a muslin cloth. The transparent gel was allowed to cool at room temperature for 1 hour before being dried in a dryer at 40 °C for 24 hours. The resulting dried mucilage was ground into tiny granules. HPLC-RI was used to examine the mucilage granules [19]. A quaternary pump, degasser, and auto injector were included in the chromatographic system that was connected to the refractive index detector. The Agilent program was used to collect the chromatographic data. The samples were analyzed on an Aminex-carbohydrate HPX-87c column in isocratic conditions with deionized water at a flow rate of 0.5 ml/min. The column temperature was kept at 85 °C and the detector temperature was kept at 50 °C. The detection of samples was done by comparing retention time standards.

2.2.6 Synthesis of nanocomposite:

For the synthesis of nanocomposite, the ionic gelation process was used [20]. The nanoparticles were made by gelating a chitosan solution with 85 percent sodium tripolyphosphate (TPP). The interaction between positively charged amino groups in chitosan solution and negatively charged in TPP resulted in ionotropic gelation.

To prepare a nanocomposite of 0.5% w/v, 0.25g of chitosan and 0.25g of mucilage powder were dissolved in 250 mL distilled water and droplets of a 1 % acetic acid solution were added. The solution was stirred using magnetic stirring at room temperature for 20–24 hr until a clear solution was obtained. To prevent particle aggregation, the pH was adjusted to 4.6:4.8 by adding 1N NaOH. sodium tri polyphosphate TPP solution prepared with ration (3:1) (composite: TPP). TPP solution was added drop-wise with a syringe to chitosan solution under magnetic stirring (800 rpm) at room temperature [21,22]. For further crosslinking of nanoparticles, the prepared suspension was kept stirring for 30 min. The CS nanoparticles were collected by centrifugation at 5.000 rpm and kept under freeze drier. Finally; the dried CSNPs were taken for characterization tests and composite nanoparticles formulation.

2.2.7 Antimicrobial effects

Antimicrobial effect of LMW chitosan and mucilage was investigated using the method of Balouiri, M., et al. (2016) [23] against three food

pathogens; *Bacillus cereus*, *Salmonella typhimurium*, and *Candida albican*. Pathogenic strains were obtained from ATCC.

Table (2) indicates the culture medium and different incubation conditions used for antimicrobial analysis.

Table (2): Culture medium and incubation conditions used for each microorganism

Microbial type	Medium	Incubation conditions
<i>Bacillus cereus</i> (ATCC33018)	Mueller–Hinton Agar	37 °C / 24-48 hr.
<i>Salmonella typhimurium</i> (ATCC14022)	Mueller–Hinton Agar	37 °C / 24-48 hr.
<i>Candida albicans</i> (ATCC10231)	Sabaroud Dextrose Agar	25 °C / 24-48 hr.

2.2.8 Instruments

- X-ray Diffraction (XRD) model (Shimadzu XRD-6000)** located in Beni Seuf university was used to measure the structural properties of the synthesized CS materials and nanocomposite.
- Fourier transforms infrared (FT-IR) VERTEX 70** infrared spectrometer (Bruker Optics, Germany) located in Beni Seuf University was use for define functional group in samples (chitosan, mucilage, nanocomposite, irradiated chitosan).
- Transmission Electron Microscope (TEM)** Model JEOL(JEM-1400) located in CURP, Cairo university, was used to record high magnification images of samples; irradiated chitosan, nanocomposite, chitosan LMW, and mucilage.
- Centrifuge model** (Sigma, 3–18, KS, Germany), was used for SGBP extract homogenization.
- Digital pH meter** (Model AD-1030, ADWA), was used to determine PH of SGBP extract.
- Digital refractometer** (Model RX-5000CX, Atago, Japan), was used to determine TSS (°Brix) of SGBP extract.
- HPLC/refractive index detector** (Model series 1200) located in, agricultural research center (FTRI), was used for the determination of sugar percentage in mucilage solution.

8. Spectrophotometer Shimadzu Model UV-240 device was used to determine the total chlorophyll in the SGBP supernatant.

2.2.9 Statistical Analysis

All of the examined parameters were analyzed using factorial experiments with two factors in a randomized full block design with three replicates for each treatment, as described by Snedecor et al. [24]. The significant differences between the means were tested using the Least Significant Differences test (LSD) at a 5% level of probability. The statistical analysis was carried out using the "MSTAT-C" software tool [25].

3. RESULTS AND DISCUSSION

3.1 HPLC RI analysis for mucilage

Optimization of chromatographic separation of the different sugars present in the mucilage, with an adjustable time frame for analysis using water as a mobile phase, proved efficient. Using water as a mobile phase for the separation of sugars is inexpensive and obviously non-toxic and it did not generate a toxic waste as an output of the analysis.

The fractionation of sugars in the mucilage solution was achieved by using an HPLC-RI system connected to the refractive index detector. The psyllium mucilage contains xylose (39.60%), arabinose (10.01%), and traces of other sugars, according to the refractive index signal test. (Fig. 1).

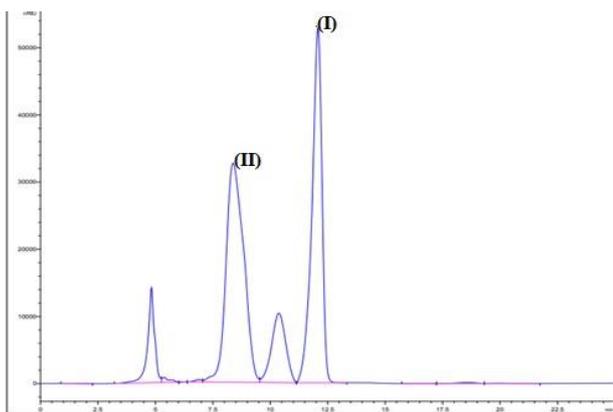


Fig. (1): HPLC-RI analysis of psyllium mucilage (I) xylose, (II) arabinose

3.2 X-ray Diffraction (XRD) Analysis

Figure (2) shows the XRD patterns of irradiated chitosan, nanocomposite, chitosan and mucilage. (No.3) that was acquired. For chitosan

(CS), the peaks appeared at values of 10.45° , 20.2° , and 35.9° , with an average crystallite size of 8 nm, while for nanocomposite (chitosan, mucilage), the XRD peaks appeared at two values: 11.46° and 43.3° , with an average crystallite size of 23.19 nm, which matches well with the literature value [26]. The amorphous structure of the polymer causes the peaks to broaden [27]. The XRD pattern for the mucilage pattern, peak appeared at a value of 59.99° with crystallite size of 91.5 nm. In addition to peaks with values 10.18° , 19.86° , and 35.9° with an average crystallite size of 5.7 nm can be seen in the irradiated chitosan XRD pattern.

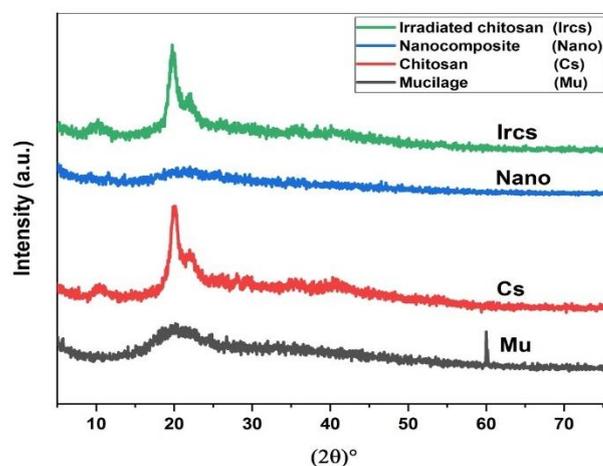


Fig. (2): XRD patterns of irradiated chitosan, nanocomposite, chitosan LMW, and mucilage

3.3 Fourier transforms infrared (FTIR) analyses

The FTIR spectra of TPP, nanocomposite, chitosan LMW and mucilage are shown in Figure (3). The wavenumber range of $350\text{--}4000\text{ cm}^{-1}$ was used to record the spectra. The FTIR spectra of chitosan reveals O–H stretching at 3.445 cm^{-1} , C–H and C–N stretching at 2.881 cm^{-1} , N–H bending at 1.663 cm^{-1} , and C–O–C band stretching at 1.087 cm^{-1} , all of which are consistent with Saraswathy et al. (2001) [28]. The nanocomposite's FTIR spectra exhibits C–H and C–N stretching at 2.919 cm^{-1} , N–H bending at 1.549 cm^{-1} , and C–O–C band stretching at 1.029 cm^{-1} , which is consistent with Saraswathy et al. (2001) [28] and Ali, Mohamed et al. (2018) [29]. The FTIR spectrum of mucilage shows O–H stretching at 3.420 cm^{-1} , C–H and C–N are stretching at 2.923 cm^{-1} , N–H bending at 1.636 cm^{-1} , and C–O–C band stretching at 1.046 cm^{-1} . The FTIR

spectrum of TPP shows O–H stretching at 16.553 cm^{-1} , C–H and C–N are stretching at 11.547 cm^{-1} , N–H bending at 8.985 cm^{-1} , and C–O–C band is stretching at 4.836 cm^{-1} .

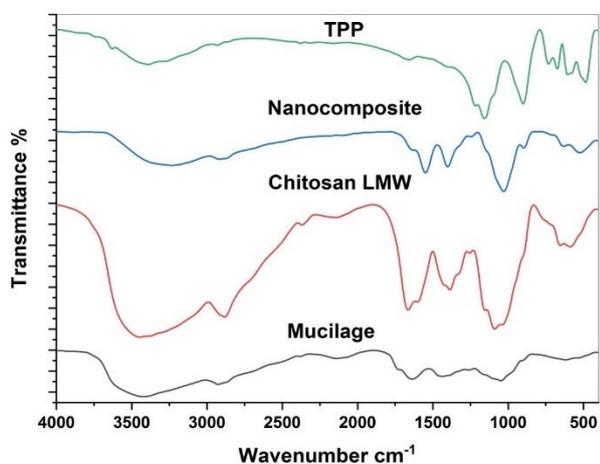


Fig. (3): The FTIR spectrum of mucilage, chitosan (LMW), Nanocomposite, and TPP

3.4 Transmission Electron Microscope (TEM)

High magnification photographs of materials and crystallographic studies are captured using a Transmission Electron Microscope (TEM). A typical TEM image depicts the size and shape of chitosan particles (Fig. 4a) ranging from 67 to 100 nm and (4b) mucilage particles, which appeared as an interconnected network with porous nature probably of poor crystallinity as indicated from the XRD pattern. In this case, nanocomposite (chitosan-mucilage) is used as in Figure (4c). The particles were found to be spherical in shape, with an average particle size of 16 to 26 nm, Figure (4d) with an average particle size of 22 to 48 nm for irradiated chitosan. Transmission electron microscopy (TEM) was used in this study using a JEOL JEM-100Cx microscope operating at 80 kV.

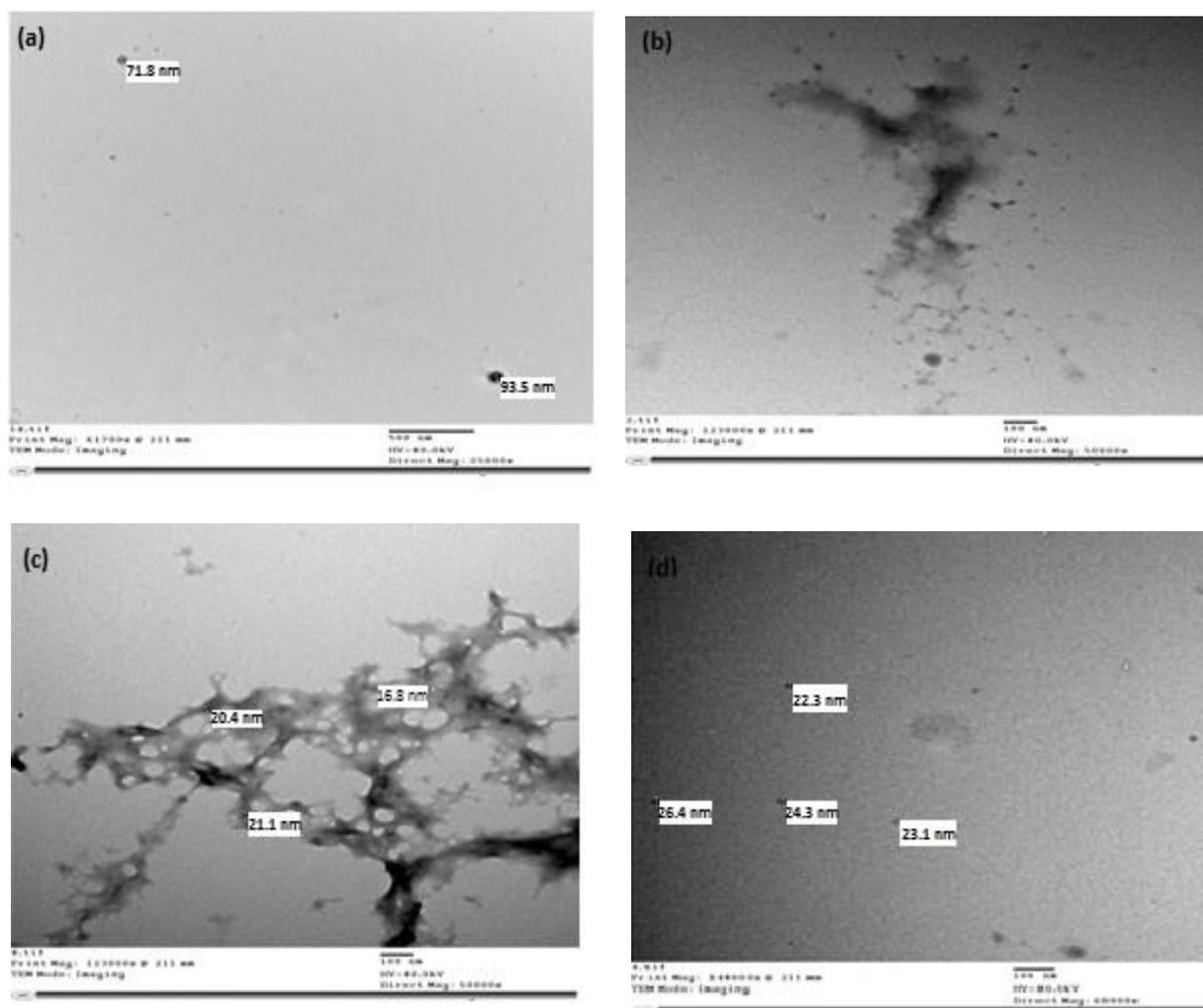


Fig. (4): TEM images of chitosan (4a), mucilage (4b), nanocomposite (4c), and irradiated chitosan (4d)

3.5 Antimicrobial activities of investigated materials

3.5.1 Antimicrobial activities of chitosan

Agar well diffusion assay was used to test the antimicrobial efficacy of LMW chitosan against three food pathogens: *Bacillus cereus*, *Salmonella typhimurium*, and *Candida albicans*, was carried out using agar well diffusion assay. Table (3) reveals a suppression effect against all tested bacteria with a concentration of 1.5 percent from chitosan LMW. The results (in Fig. 5) indicate that *Candida* and *Bacillus* were the most susceptible microorganisms to the chitosan (15 mm) inhibitory zone, followed by *Salmonella* (12 mm). All measurements of inhibitory zone were performed in three replicates to express the results in terms of average values.

Table (3): Antimicrobial effects of chitosan LMW against three microorganisms

Chitosan LMW 1.5%	Diameter of the inhibition zone (mm)
<i>Bacillus cereus</i> (ATCC33018)	15
<i>Salmonella typhimurium</i> (ATCC14022)	12
<i>Candida albicans</i> (ATCC10231)	15

Minimum inhibitory concentration (MIC) of chitosan LMW on the tested pathogenic a considerable

inhibitory impact on the development of the investigated microorganisms *Candida albicans* (0.5 ml/ml) and *Bacillus cereus* and *Salmonella typhimurium* (0.25 ml/ml), according to the results in Table (4). All tests were recorded using culture media under incubation conditions as shown in Table (2).

Table (4): Minimum inhibitory concentration (MIC) of chitosan LMW

Chitosan LMW	MIC ml/ml
<i>Bacillus cereus</i> (ATCC33018)	0.25
<i>Salmonella typhimurium</i> (ATCC14022)	0.25
<i>Candida albicans</i> (ATCC10231)	0.5

3.5.2 Antimicrobial activities of mucilage

Mucilage's antibacterial impact was investigated using the *Bacillus cereus*, *Salmonella typhimurium*, and *Candida albicans*. Analysis was examined by utilizing mucilage and agar well diffusion assays. Results indicate no activity against all tested bacteria at a concentration of 1.5 percent mucilage. The results demonstrated that mucilage has no antimicrobial effect on the growth of the microorganisms examined (Fig.6), and that the results were recorded using culture media under incubation conditions as shown in Table (2).

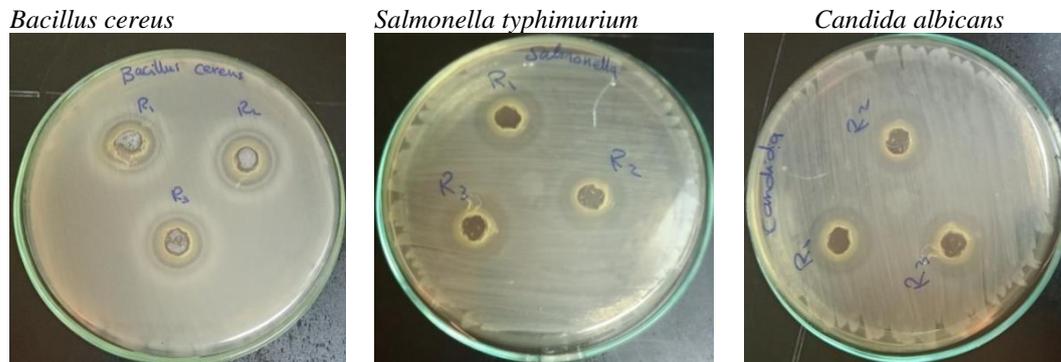


Fig. (5): Inhibition zones showing chitosan effect against three microorganism *Bacillus cereus*, *Salmonella typhimurium*, and *Candida albicans*

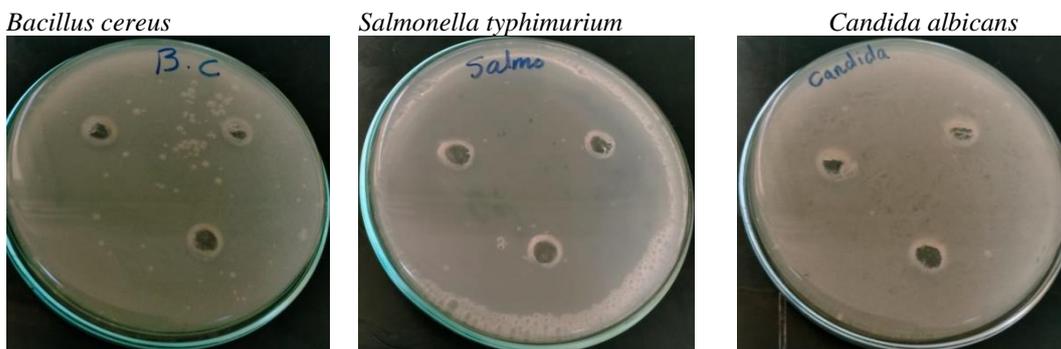


Fig. (6): Mucilage antimicrobial effect against three microorganism *Bacillus cereus*, *Salmonella typhimurium*, and *Candida albicans*

3.6 Microbiological analysis of treated SGBP

3.6.1 Total Bacterial Count (TBC)

Results in Fig.(7) showed that generally, the total bacterial count in fresh fruits under cooling was gradually increased by increasing the storage period. Chitosan have an antimicrobial effect on vegetables under cold storage [30].

The most effective treatment was 1% of the irradiated chitosan decreased the count to 3.24 log cycles after 7 days while, 1% chitosan reduced the count to 3.15 log cycle followed by 1% nanoparticle which reduced the count by 3 log cycle throughout the cold storage period.

The results proved that the most effective treatments for reducing the total bacterial count were solutions with concentrations of 0.5%

irradiated chitosan and 0.5%, nanocomposite within 14 day under cold storage.

3.6.2 Total Fungal Count

The results of total fungal count of SGBP fruits were increased in the untreated fruits from 1.9 to 2.9 log cycle during the cold storage period (Fig.8). In contrast, the treated fruits have low fungal count because of the antifungal effect of chitosan and nanocomposite [31,32].

The treatment 0.5% chitosan decreased the fungal count about one log cycle during 14 days. After 21 days, no fungal count was detected in all treatments. All 1% concentrations of the all treatments could ultimately kill all fungal contaminations from the first day because of the chitosan antifungal effect of irradiated chitosan and nanocomposite.

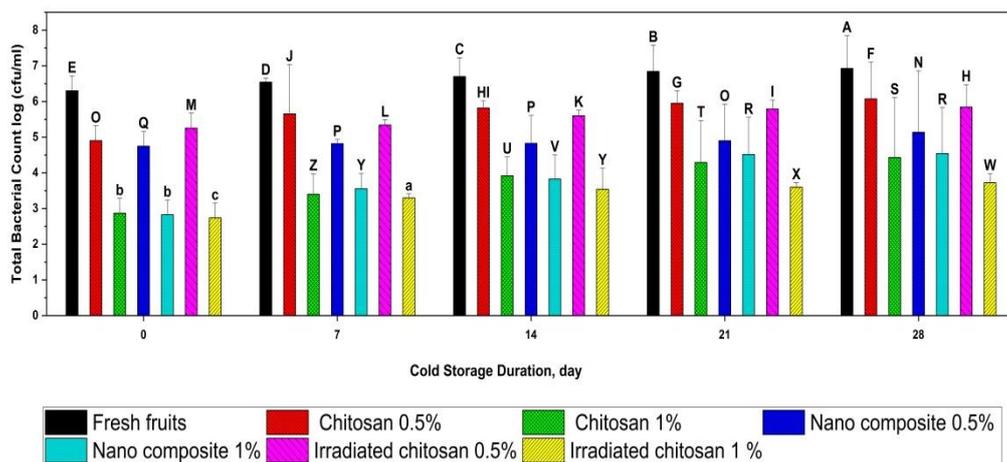


Fig. (7): The effects of chitosan, nanocomposite and irradiated chitosan on total bacterial count of bell peppers stored under cold storage after 28 days

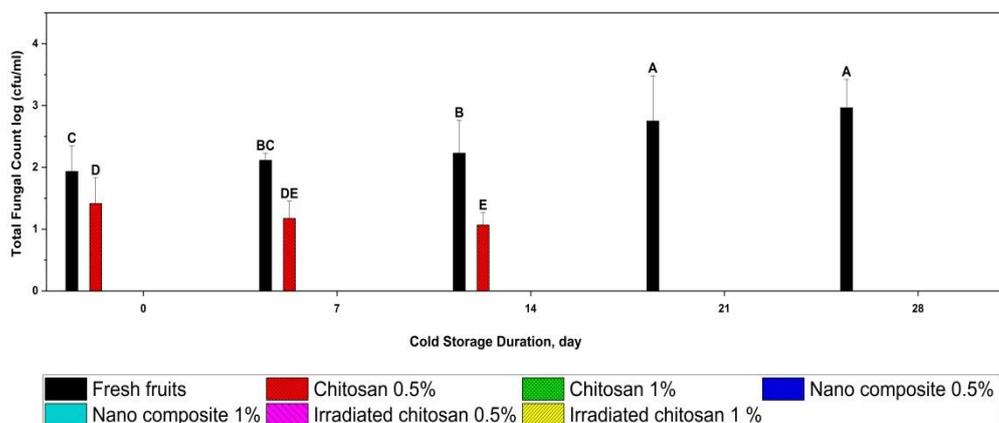


Fig. (8): The effects of chitosan, nanocomposite and irradiated chitosan on total fungal count of bell peppers after 28 days

3.6.3 Total Yeast Count

Chitosan showed a comparable effect on yeast growth as it did on fungal growth. The total count of yeasts on fresh sweet pepper fruits without treatments rose at a two-log cycle rate during the storage period, according to the findings (Fig.9). The highest concentrations, 1% irradiation chitosan, 1% chitosan, and 1% nanoparticle composite, are the most effective for killing yeast contamination during cold storage [33].

3.7 Physiochemical analysis of treated SGBP

3.7.1 Weight loss percentage

Weight loss percentage and fruit firmness increased gradually throughout the storage period irrespective of treatments (Fig.10), which could be due to water loss driven by active metabolic processes, such as transpiration and respiration in

the fruit [34]. However, the coated fruits maintained a higher weight throughout the storage period as compared to uncoated fruit.

Results showed that irradiated chitosan and nanocomposite treatments had the highest effect on reducing weight loss and were followed by chitosan treatment.

3.7.2 Total phenolic content (TPC)

The results show that after the first week under cold storage, TPC of SGBP increased significantly (Fig.11) because of the increased weight loss. Fresh fruits without treatment had a higher total phenol content after 28 days [35]. Treatments with irradiated chitosan had the highest total phenol content, followed by the nanocomposite. Treatments using chitosan, on the other hand, had the lowest overall phenol levels

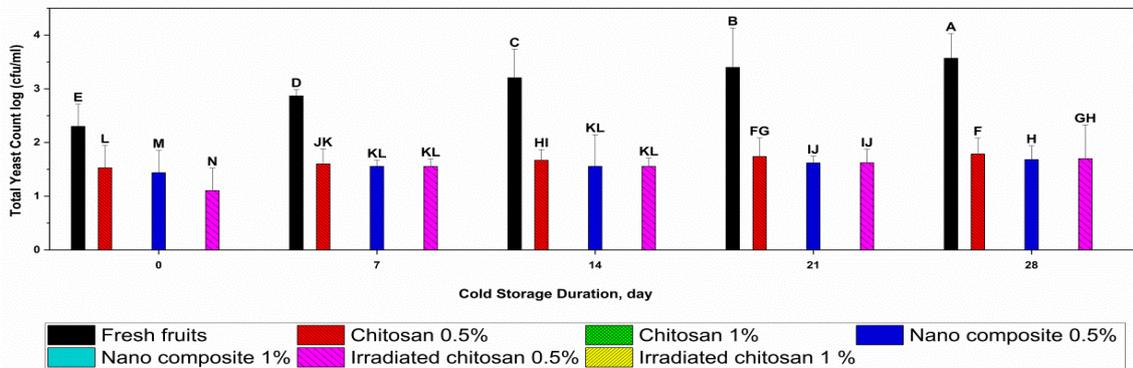


Fig. (9): The effects of chitosan, nanocomposite and irradiated chitosan on total yeast count of bell peppers stored under cold storage after 28 day

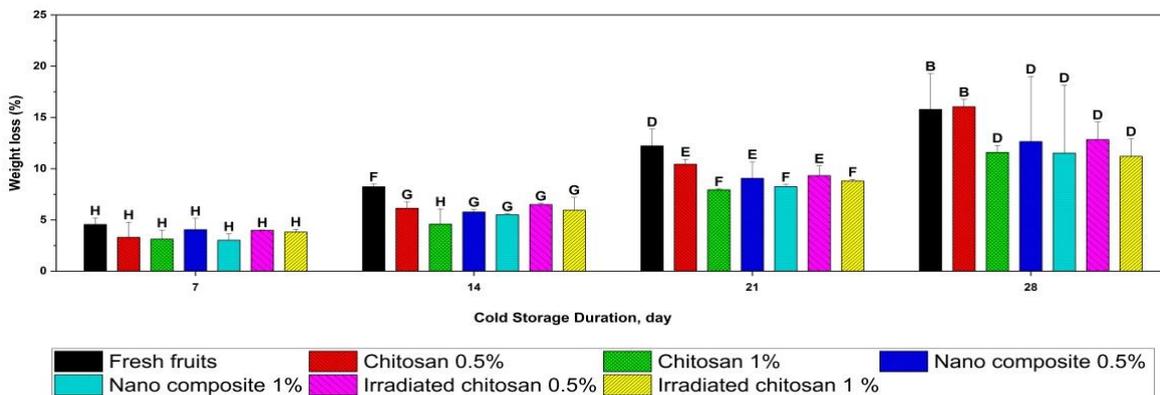


Fig. (10): Effect of different coating treatments on the weight loss (%) of bell peppers stored under cold storage after 28 days

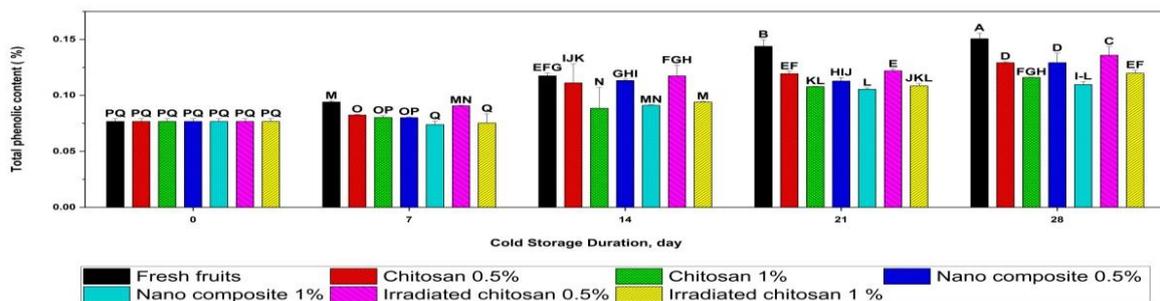


Fig. (11): Effect of different coating treatments on (TPC) of bell peppers stored under cold storage after 28 days

3.7.3 Fruits Decay

Coating treatments and storage time had a substantial impact on SGBP fruit decay. The effects of edible coatings on decay were significant, and indications of decay were significantly reduced in all coated bell pepper fruits. During cold storage, the coated sweet bell pepper fruit with irradiated chitosan showed the least signs of degradation when compared to the nanocomposite coatings. In comparison to uncoated SGBP, all coating treatments delayed the emergence of surface degradation, as illustrated in figure.12. Coated SGBP decayed at a rate of less than 12 ± 2 at the conclusion of the storage period, but uncoated samples decayed at a rate of 30 ± 3 over the cold storage period [36].

3.7.4 Total soluble solids ($^{\circ}$ Brix)

In the present study, the increasing trend of TSS was recorded in bell pepper stored at cold condition

for 28 days (Fig.13). The least increase of TSS was recorded in the fresh bell pepper fruits stored under cold storage conditions (3.45 ± 0.03 $^{\circ}$ Brix) followed by the treatment of chitosan (4.24 ± 0.04 $^{\circ}$ Brix). TSS was increased rapidly in the treated samples as compared to the fresh bell pepper during the storage time. The higher increasing rate of TSS was recorded in bell pepper fruits that were treated by irradiated chitosan (7.32 ± 0.21 $^{\circ}$ Brix) followed by nanocomposite treatments during the cold storage period.

3.7.5 pH measurements

The result of PH measurements shows an increase from 4.6 to 6.13 for the untreated SGBP fruits under cold storage period (Fig.14). On the other hand there was a slight increase for the treated fruits, which increased from 4.9 to 5.8. The best treatments that led to a slight increase in the pH measurements were 1% concentrations for chitosan and nanocomposite.

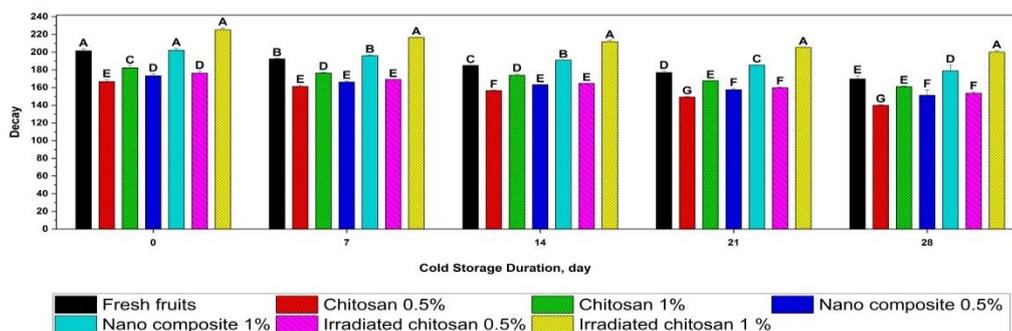


Fig. (12): Effect of different coating treatments on the decay and firmness of bell peppers stored under cold storage after 28 days

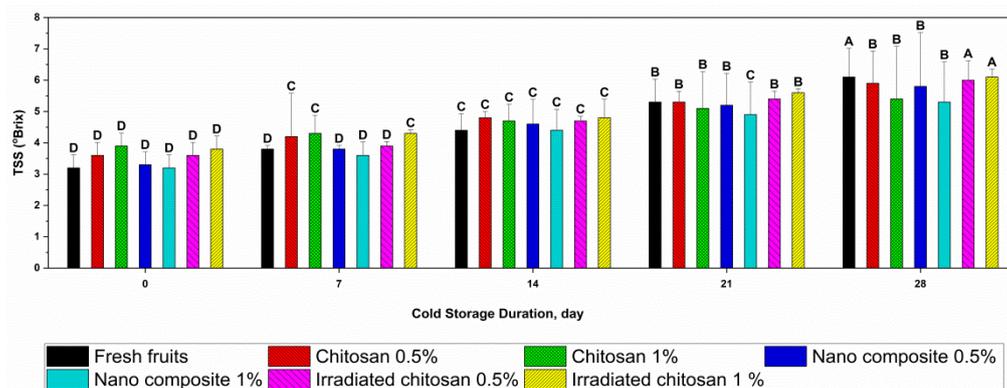


Fig. (13): Effect of different coating treatments on the TSS of bell peppers stored under cold storage after 28 day

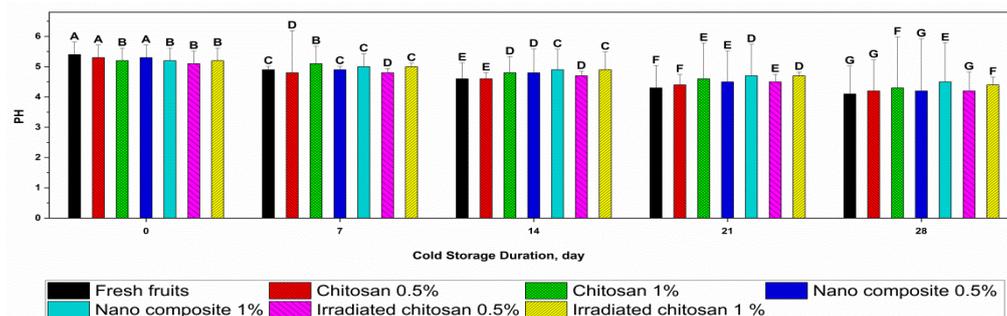


Fig. (14): Effect of different coating treatments on the PH of bell peppers stored under cold storage after 28 days

3.7.6 Total titratable acidity

According to the previous findings, measuring the acidity % (Fig.15) in SGBP fruits extract increased in the untreated SGBP fruits during cold storage for a longer period of time. However there was a small increase in the acidity of the treated fruits. Fruits treated with a concentration of 1% nanocomposite were the most successful treatments, resulting in a modest increase in acidity values.

3.7.7 Antioxidant activity (DPPH assay)

The results of the free radical scavenging (antioxidant) activity in the control and treated bell peppers after 28 days of cold storage are indicated

in figure 16. Antioxidant agents in dietary substances inhibit many of the oxidation events caused by free radicals, reducing the risk of tissue damage and the loss of functional and nutritional characteristics [37]. With a storage term of 0–28 days within a 7-day interval, a declining trend in antioxidant activity was detected in the effect of the edible coating with storage time. The results demonstrated that the composite edible coating of chitosan could help preventing deterioration of bell pepper during the storage period by sustaining phenolic and free radical scavenging activity. The results were in reasonable agreement with the previous findings observed in the chitosan-based edible coating enriched with pomegranate peel extract [38,39]

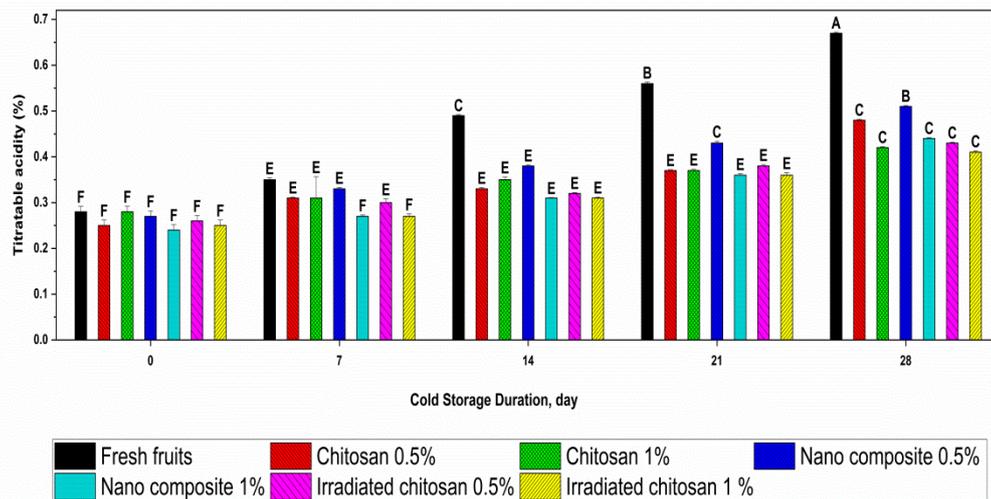


Fig. (15): Effect of different coating treatments on the total acidity percentage of bell peppers stored under cold storage after 28 days

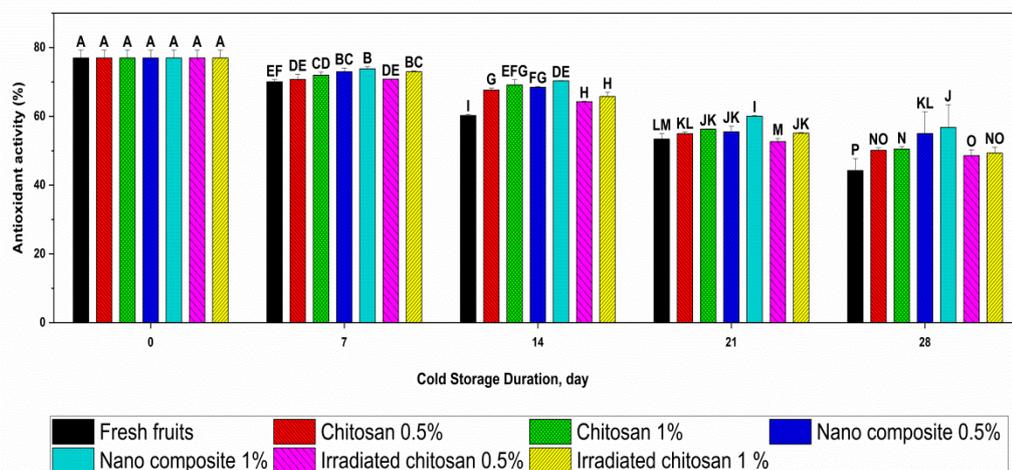


Fig. (16): Effect of different coating treatments on the antioxidant activity percentage of bell peppers stored under cold storage after 28 days

3.7.8 Ascorbic Acid content

During the cold storage period, the ascorbic acid concentration of sweet green bell pepper (SGBP) fruits decreased gradually (Fig.17). Irradiated chitosan and nanocomposite had a greater Ascorbic acid concentration. The ascorbic acid concentration of fresh fruits that had not been treated was lower. The increased respiration and oxidation of acids into sugars could be to blame for the drop in vitamin C concentration. The present findings are similarly consistent with those of Hedayati et al. [40], who found that the coating of SGBP with arabic gum dramatically reduced ascorbic acid loss. Moreover, the linear increase in pH might be ascribed to biochemical, structural, and physiological alterations taking place during respiration. However, the obtained results are also in agreement with the findings of Xing et al.2011 [41] who reported reduced membrane leakage in chitosan coated sweet pepper fruit.

3.7.9 Total chlorophyll content

During the cold storage period, the total chlorophyll concentration in SGBP fruits reduced

substantially. All coated peppers changed color when compared to untreated fresh fruits, and the treated samples remained green at the end of storage. The effect of coating treatments on sweet peppers can be measured objectively by looking at variations in chlorophyll concentration. Peppers had a chlorophyll concentration of 0.10 mg/g when they were first harvested. As demonstrated in (Fig.18), this reference value declined throughout time, stabilizing around 0.01 mg/g in the control treatment towards the conclusion of storage. The rest of the treatments ranged from 0.10 to 0.01 mg/g, with no significant differences.

3.7.10 Total carotenoids (TC)

During the cold storage period, the total carotenoid content of SGBP fruits skyrocketed (Fig.19). The total carotenoid content of sweet pepper fruits rose from the start of storage to 28 days of cold storage. The higher increase in TC during the initial time of storage could be related to the destruction of chlorophyll and accumulation of carotenoid, whereas the decrease in TC during the last period of storage could be due to polyphenol oxidase enzymes gradually destroying the TC.

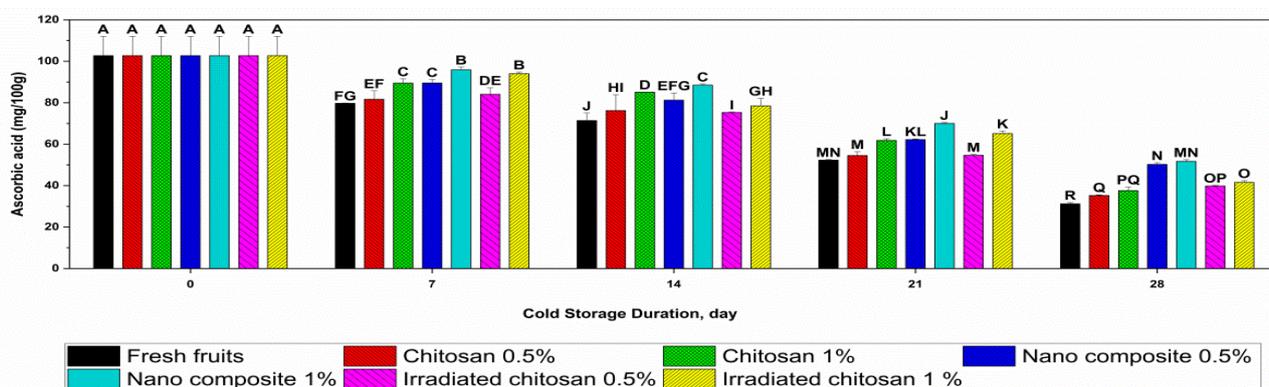


Fig. (17): Effect of different coating treatments on the ascorbic acid content of bell peppers stored under cold storage after 28 days

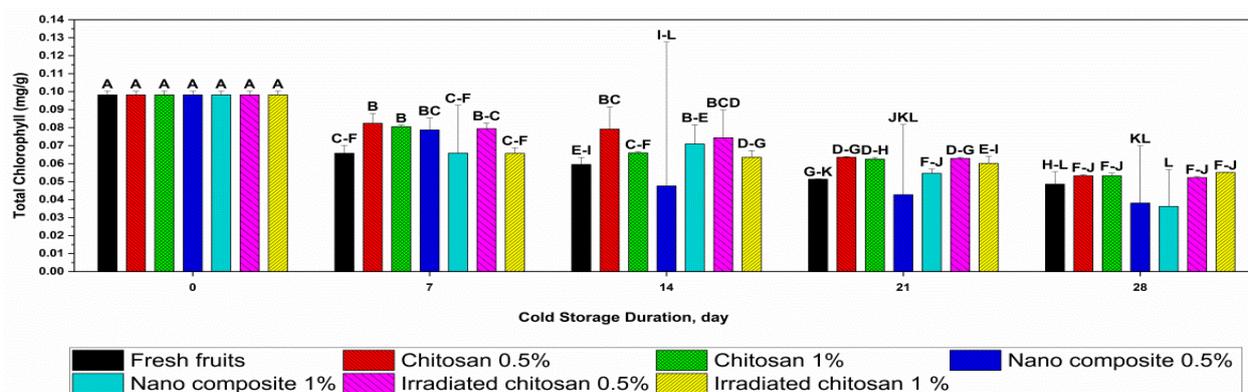


Fig. (18): Effect of different coating treatments on the total chlorophyll of bell peppers stored under cold storage after 28 days

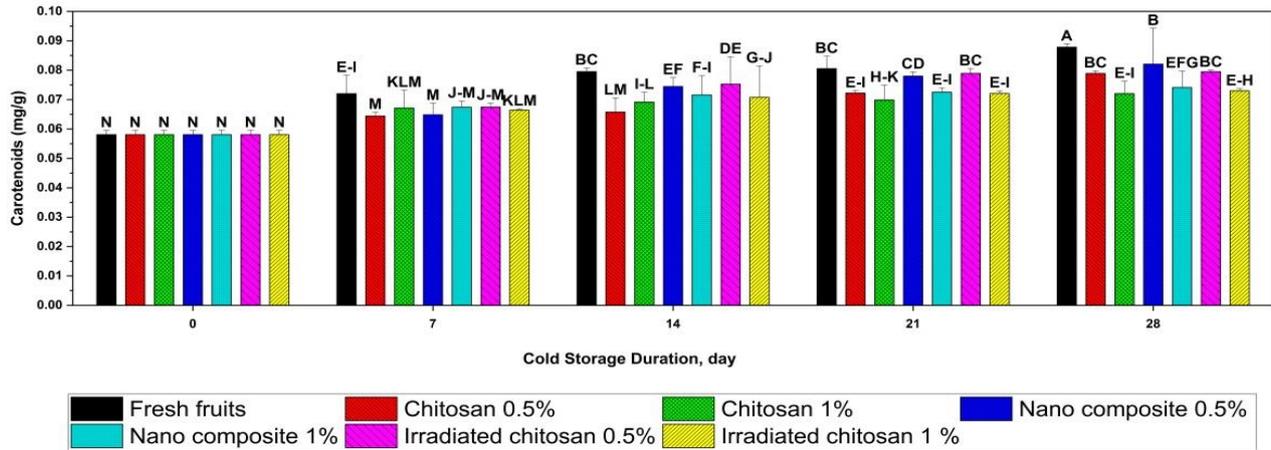


Fig. (19): Effect of different coating treatments on the total carotenoids content of bell peppers stored under cold storage after 28 days

4 CONCLUSION

In this study, we used mucilage from psyllium seeds for the first time as an edible coating for SGBP fruits. Studies on the properties of composite films from chitosan and mucilage are scarce, even though the combinations seem to improve the characteristics of biodegradable films on green bell pepper fruits.

Treatments that showed high efficacy in reducing the total microorganisms were different chitosan solutions with high concentrations; 1% irradiated chitosan followed by 1% chitosan, and 1% nanoparticle composite throughout the cold storage period. This result may be due to the high antimicrobial activity of irradiated chitosan. The proposed best treatments for SGBP for The local markets were solutions treatment with concentrations of 0.5% nanocomposite and 0.5% irradiated chitosan that showed the most effective results in reducing the count of microorganisms within 14 days in cold storage.

In addition, the results of physiochemical analysis showed that the treated fruits by the irradiated chitosan solutions retained the acceptable quality characteristics of the consumers compared to the untreated fresh fruits during the cold storage.

5 ACKNOWLEDGEMENT

The authors are deeply thankful to the Biotechnology and Life Sciences Department, PSAS, Beni-Suef University and National Center for Radiation Research and Technology, Atomic Energy Authority for their support to carry out the study.

6 REFERENCES

- [1] Thakur, M.P., 2018. Advances in post-harvest technology and value additions of edible mushrooms. *Indian Phytopathology*, 71(3), pp.303-315.
- [2] Esyanti, R.R., Zaskia, H. and Amalia, A., 2019, April. Chitosan nanoparticle-based coating as post-harvest technology in banana. In *Journal of Physics: Conference Series* (Vol. 1204, No. 1, p. 012109). IOP Publishing.
- [3] Smith, H.W., Huggins, M.B. and Shaw, K.M., 1987. Factors influencing the survival and multiplication of bacteriophages in calves and in their environment. *Microbiology*, 133(5), pp.1127-1135.
- [4] Duan, C., Meng, X., Meng, J., Khan, M.I.H., Dai, L., Khan, A., An, X., Zhang, J., Huq, T. and Ni, Y., 2019. Chitosan as a preservative for fruits and vegetables: a review on chemistry and antimicrobial properties. *Journal of Bioresources and Bioproducts*, 4(1), pp.11-21.
- [5] Hu, X., Saravanakumar, K., Sathiyaseelan, A. and Wang, M.H., 2020. Chitosan nanoparticles as edible surface coating agent to preserve the fresh-cut bell pepper (*Capsicum annuum* L. var. *grossum* (L.) Sendt). *International Journal of Biological Macromolecules*, 165, pp.948-957.
- [6] Zhang, X., Geng, X., Jiang, H., Li, J. and Huang, J., 2012. Synthesis and characteristics of chitin and chitosan with the (2-hydroxy-3-trimethylammonium) propyl functionality, and evaluation of their antioxidant activity in vitro. *Carbohydrate polymers*, 89(2), pp.486-491.
- [7] Kumar, D., Pandey, J., Kumar, P. and Raj, V., 2017. Psyllium mucilage and its use in pharmaceutical field: An overview. *Curr Synthetic Sys Biol*, 5(134),

- pp.2332-0737.
- [8] Malviya, R., Srivastava, P. and Kulkarni, G.T., 2011. Applications of mucilages in drug delivery-a review. *Advances in Biological Research*, 5(1), pp.1-7.
- [9] Abbasi, N.A., Ashraf, S., Ali, I. and Butt, S.J., 2015. Enhancing storage life of bell pepper by UV-C irradiation and edible coatings. *Pakistan Journal of Agricultural Sciences*, 52(2).
- [10] Abdullah, M.A., Hassanein, R.A. and Mounir, A.M., 2018. Effect of Gamma Irradiated Chitosan and Green Tea on Pod Quality of Fresh-Cut Green Bean under Cold Storage Conditions. *Journal of Horticultural Science & Ornamental Plants* 10 (3): 140-149, 2018.
- [11] Nair, M.S., Saxena, A. and Kaur, C., 2018. Effect of chitosan and alginate based coatings enriched with pomegranate peel extract to extend the postharvest quality of guava (*Psidium guajava* L.). *Food chemistry*, 240, pp.245-252.
- [12] AOAC (1990). Official methods of analysis of the Association of Official Analytical Chemists, 13th ed., Association of Official Analytical Chemists, Washinton D.C.,U.S.A.
- [13] Ranganna, S., 1977. Manual of analysis of fruit and vegetable products.
- [14] Swain, T. and Hillis, W.E., 1959. The phenolic constituents of *Prunus domestica*. I.—The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*, 10(1), pp.63-68.
- [15] Holm, G., 1954. Chlorophyll mutations in barley. *Acta Agriculturae Scandinavica*, 4(1), pp.457-471.
- [16] Von Wettstein, D. "Chlorophyll-letale und der submikroskopische Formwechsel der Plastiden. (1957)" *Experimental cell research* 12.3: 427-506.
- [17] Mitic, V., Jovanovic, V.S., Dimitrijevic, M., Cvetkovic, J. and Stojanovic, G., 2013. Effect of food preparation technique on antioxidant activity and plant pigment content in some vegetables species. *Journal of Food and Nutrition Research*, 1(6), pp.121-127.
- [18] Malviya, R., Srivastava, P. and Kulkarni, G.T., 2011. Applications of mucilages in drug delivery-a review. *Advances in Biological Research*, 5(1), pp.1-7.
- [19] Zielinski, A.A.F., Braga, C.M., Demiate, I.M., Beltrame, F.L., Nogueira, A. and Wosiacki, G., 2014. Development and optimization of a HPLC-RI method for the determination of major sugars in apple juice and evaluation of the effect of the ripening stage. *Food Science and Technology*, 34, pp.38-43.
- [20] Agarwal, M., Agarwal, M.K., Shrivastav, N., Pandey, S., Das, R. and Gaur, P., 2018. Preparation of chitosan nanoparticles and their in-vitro characterization. *Int J Life Sci Scienti Res*, 4(2), pp.1713-1720.
- [21] Hassani, S., Laouini, A., Fessi, H. and Charcosset, C., 2015. Preparation of chitosan-TPP nanoparticles using microengineered membranes—Effect of parameters and encapsulation of tacrine. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 482, pp.34-43.
- [22] Khoerunnisa, F., Nurhayati, M., Dara, F., Rizki, R., Nasir, M., Aziz, H.A., Hendrawan, H., Poh, N.E., Kaewsaneha, C. and Opaprakasit, P., 2021. Physicochemical Properties of TPP-Crosslinked Chitosan Nanoparticles as Potential Antibacterial Agents. *Fibers and Polymers*, 22(11), pp.2954-2964.
- [23] Balouiri, M., Sadiki, M. and Ibnsouda, S.K., 2016. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, 6(2), pp.71-79.
- [24] Snedecor, G. and W. Cochran, Arc sine transformation for proportions. *Statistical Methods*. 8th ed. Iowa State University Press, Ames, 1989: p. 289-290.
- [25] Freed, R., et al., User's guide to MSTAT-C analysis of agronomic research experiments. Michigan State University, USA, 1989.
- [26] Rao, M.R., Sadaphule, P., Khembete, M., Lunawat, H., Thanki, K. and Gabhe, N., 2013. Characterization of *Psyllium* (*Plantago ovata*) Polysaccharide and its Use as a Binder in Tablets. *Ind. J. Pharm. Edu. Res*, 47(2).
- [27] Alarfaj, A.A., 2019. Antibacterial Effect of Chitosan Nanoparticles against Food Spoilage Bacteria. *J Pure Appl Microbiol*, 13(2), pp.1273-1278.
- [28] Saraswathy, G., et al., A novel bio-inorganic bone implant containing deglued bone, chitosan and gelatin. *Bulletin of materials science*, 2001. 24(4): p. 415-420.
- [29] Ali, M.E.A., et al., Chitosan nanoparticles extracted from shrimp shells, application for removal of Fe (II) and Mn (II) from aqueous phases. *Separation Science and Technology*, 2018. 53(18): p. 2870-2881.
- [30] Ali, A., Noh, N.M. and Mustafa, M.A., 2015. Antimicrobial activity of chitosan enriched with lemongrass oil against anthracnose of bell pepper. *Food packaging and shelf life*, 3, pp.56-61.
- [31] Avelelas, F., Horta, A., Pinto, L.F., Cotrim Marques, S., Marques Nunes, P., Pedrosa, R. and Leandro, S.M., 2019. Antifungal and antioxidant properties of chitosan polymers obtained from nontraditional *Polybius henslowii* sources. *Marine drugs*, 17(4), p.239.
- [32] Sathiyaseelan, A., Saravanakumar, K., Mariadoss, A.V.A., Ramachandran, C., Hu, X., Oh, D.H. and Wang, M.H., 2021. Chitosan-tea tree oil nanoemulsion and

- calcium chloride tailored edible coating increase the shelf life of fresh cut red bell pepper. *Progress in Organic Coatings*, 151, p.106010.
- [33] Abebe, Z., Tola, Y.B. and Mohammed, A., 2017. Effects of edible coating materials and stages of maturity at harvest on storage life and quality of tomato (*Lycopersicon Esculentum* Mill.) fruits. *African journal of agricultural research*, 12(8), pp.550-565.
- [34] Kumar, N., Ojha, A., Upadhyay, A., Singh, R. and Kumar, S., 2021. Effect of active chitosan-pullulan composite edible coating enrich with pomegranate peel extract on the storage quality of green bell pepper. *LWT*, 138, p.110435.
- [35] Barzegar, T., Fateh, M. and Razavi, F., 2018. Enhancement of postharvest sensory quality and antioxidant capacity of sweet pepper fruits by foliar applying calcium lactate and ascorbic acid. *Scientia Horticulturae*, 241, pp.293-303.
- [36] Lobo, V., Patil, A., Phatak, A. and Chandra, N., 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews*, 4(8), p.118.
- [37] Nair, M.S., Saxena, A. and Kaur, C., 2018. Characterization and antifungal activity of pomegranate peel extract and its use in polysaccharide-based edible coatings to extend the shelf-life of capsicum (*Capsicum annum* L.). *Food and bioprocess technology*, 11(7), pp.1317-1327.
- [38] Association of Official Analytical Chemists and Association of Official Agricultural Chemists (US), 1925. *Official methods of analysis of the Association of Official Analytical Chemists (Vol. 2)*. The Association.
- [39] Kumar, N., Ojha, A. and Singh, R., 2019. Preparation and characterization of chitosan-pullulan blended edible films enrich with pomegranate peel extract. *Reactive and Functional Polymers*, 144, p.104350.
- [40] Hedayati, S. and Niakousari, M., 2015. Effect of Coatings of Silver Nanoparticles and Gum Arabic on Physicochemical and Microbial Properties of Green Bell Pepper (*Capsicum annum*). *Journal of Food Processing and Preservation*, 39(6), pp.2001-2007.
- [41] Xing, Y., Li, X., Xu, Q., Yun, J., Lu, Y. and Tang, Y., 2011. Effects of chitosan coating enriched with cinnamon oil on qualitative properties of sweet pepper (*Capsicum annum* L.). *Food Chemistry*, 124(4), pp.1443-1450.