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Biopreservation of Minced Meat with Encapsulated *Lactobacillus acidophilus* and Green Tea with Nano Selenium in Chitosan Alginate Double Layer Shell

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ABSTRACT

The main contributing factors in deteriorating meat products' quality are lipid oxidation and microbial growth. The main objectives here were the employment of prebiotic [green tea water extract (GTWE)] as a mediator for biosynthesizing selenium nanoparticles (SeNPs), the conjugation of probiotic cells (*Lactobacillus acidophilus*) with the former mixture and the encapsulation of this composite inside alginate/chitosan double-layer capsules to evaluate their antimicrobial/antioxidant potentialities in meat products.The infrared analysis, UV-vis spectroscopy and transmission microscopy confirmed the biosynthesis of SeNPs using GTWE, with mean diameter of 9.13±2.69 nm, well dispersion and spherical shapes. The GTWE/SeNPs exhibited potent antibacterial activities against *Escherichia coli* and *Staphylococcus aureus*; the *S. aureus* was more sensitive, and the nanocomposite action exceeded the action of ampicillin. The GTWE/SeNPs also had an antioxidant activity of 88.9 %. The encapsulation of *L. acidophilus* in alginate/chitosan double layer resulted in capsules with 3.6±0.4 mm diameter and could protect probiotic cells from viability loss in acidic pH values, and high bile salt concentrations. The treatment of minced meat by blending with fabricated double layered capsules leading to significant decrease in microbial quality (total count, yeast and molds, psychrophilic bacteria) and spoilage chemical parameters (TBARS and TVB-N values) after 7 days of refrigerated storage. The formulated GTWE/SeNPs nanocomposites and their encapsulation with *L. acidophilus* in alginate/chitosan double-layer capsules provided effectual biopreservative and quality keeping agents for protecting minced meat.

Keywords: Bio-preservation**,** Probiotic, Chitosan, Green tea, Selenium

1. Introduction

Minced meatpossess high levels of protein, moisture, and fat in food, it is prone to microbial development and lipid oxidation (Andrade *et al.,* 2023). A variety of techniques can be used to slow down microbial growth and lipid oxidation in order to retain the nutritional value and safety of food (Vaithiyanathan *et al.,* 2011) such asdrying (Gausman *et al.,* 1952), ionizing radiations (Monk *et al*., 1995), high pressure processing (Yordanov and Angelova, 2010), and chemicals (Davidson *et al*., 2013). Since the safety and impact on health of chemical preservatives are under discussion, the use of natural compounds is considered a safe alternative (Bondi *et al.,* 2017). Probiotics are one of the most recent natural preservatives (Udayakumar *et al.,* 2022 and Vvas and Rana, 2017).

Probiotics known as live microbial feed supplements benefit the host animal (human consumption) by restoring a healthy microbial balance. (Kechagia *et al.,* 2013). Probiotics have beneficial effects such as lowering lactose intolerance, anti-cancer, hypotensive, hypocholesterolemic, andimmunostimulatory activities (Aghebati *et al.,* 2021). Probiotics were reported to have antimicrobial activities (Masoumi *et al.,* 2022). They can reduce the development of pathogens and increase the food's

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shelf life (Kazemi , 2014). Probiotic bacteria have a number of vital properties, including resistance to bile salt and gastric acidity, adherence to intestinal epithelial cells and antimicrobial activity against pathogenic microbes by producing some secondary metabolites (Shokryazdan *et al.,* 2017). Lactic acid bacteria (LAB) and bifidobacteria are the two types of probiotics that are most frequently employed (Gaggia *et al.,* 2011).

Probiotics that are administered must withstand the challenging gastric environment and go to the colon in sufficient quantities (Rokka and Rantamäki, 2010). The primary problems with probiotics are, the free bacteria's inability to thrive in big numbers when a functional food product is on the shelf and exposed to oxygen and the host's GI tract environment (Champagne and Kailasapathy, 2008). Their efficiency, viability during storage, and product shelf-life are hence tightly related (Dimitrellou *et al.,* 2016). Encapsulation technologies are postulated to be a favorable physical barrier for protection of probiotics against harsh environmental conditions such as freezing and gastric juice (Arena *et al.,* 2015).

Alginate is the most often used biopolymer for encapsulation (Chávarri *et al.,* 2010). A notable food additive that can be used in foods without harm is alginate. (Bi *et al.,* 2022). Some of the advantages of ALG as an encapsulating agent are its non-toxicity, capacity to make gentle matrices with calcium chloride to entrap microbial cells, simplicity of usage, and low cost (Wang *et al.,* 2022). Application of alginate is constrained by its poor stability in the presence of chelating agents and in acidic conditions below pH 2.0 (Roquero *et al.,* 2022). Alginate beads' stability was increased by chitosan coating, which also increased the survivability of the probiotics that were encapsulated in them (Krasaekoopt *et al.,* 2004). Hejazi and Amiji reported that chitosan is hydrolyzed by microflora available in colon, while alginate is solubilized by sequestering calcium ions (Hejazi and Amiji, 2003).

Green tea contains many polyphenols which are composed mainly of several kinds of catechins (Jin *et al.,* 2012). Green tea may balance the human intestinal flora, which would be beneficial for the host's health (Tzounis *et al.,* 2008). Taylor stated that administration of catechins caused significant increases in the amounts of bifidobacteria and lactobacilli, so act as a prebiotic (Taylor *et al.,* 2005). Green tea has been reported to have antioxidant activity due to polyphenolic content (Kopjar *et al.,* 2015). Reygaertstated that catechin components of green tea are responsible for the observed antibacterial activity (Wanda, 2014). Presence of antioxidants in green tea favoring its action as reducing agent in the green synthesis of selenium nanoparticles (Sentkowska *et al.,* 2023). Selenium nanoparticles (SeNPs) have been reported to be potent antibacterial agents (Han *et al.,* 2021). SeNPs were found to have higher antimicrobial activity than inorganic selenium (Hariharan *et al.,* 2012). Sentkowska also stated that SeNPs have potent antioxidant activities (Sentkowska *et al.,* 2023).

The study's objectives were to improve the meat quality and its shelf life by assessing encapsulation alginate and chitosan coating throughevaluating their capacity to increase *Lactobacillus acidophilus* survival when combined with green tea (GT) and SeNPs during exposure to conditions resembling those in the gastro-intestinal tract.

2. Material and methods

Bacterial strain and culture condition

A glycerol stock culture of *Lactobacillus acidophilus* isolated in Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat University, Sadat City, Egypt, was utilized in MRS broth at 37°C for 15 hours and then reactivated for 12 hours. The reactivated cells were centrifuged at 4,000 g for 20 min at 4 °C, followed by physiological saline solution wash. After two washes, the bacterial suspension was centrifuged under the same conditions. The pellets were re suspended in saline water with a final cell concentration of 9–10 log CFU/ml. The vitality of the cells was examined by serial dilution and plating on MRS agar, Hi media, India. The cells were then incubated at 37°C for 48 hours (Zanjani *et al.,* 2014).

The challenged pathogens, e.g. *Staphylococcus aureus* (ATCC 12599), and *Escherichia coli* O157:H7 (ATCC 43895), were grown and screened in TSA/TSB "Tryptic Soy Agar/Broth, respectively", Difco, Cockeysville, Md.

Prebiotic extraction

Extraction of green tea (GT)

Aqueous extract from green tea bags by Lipton (Unilever, UK) was dipped into sterile water that had been heated to a rolling boil and then allowed to macerate. After ten minutes, the tea bags were taken out. The infusion was then filtered (Pyrzynska and Sentkowska 2021).

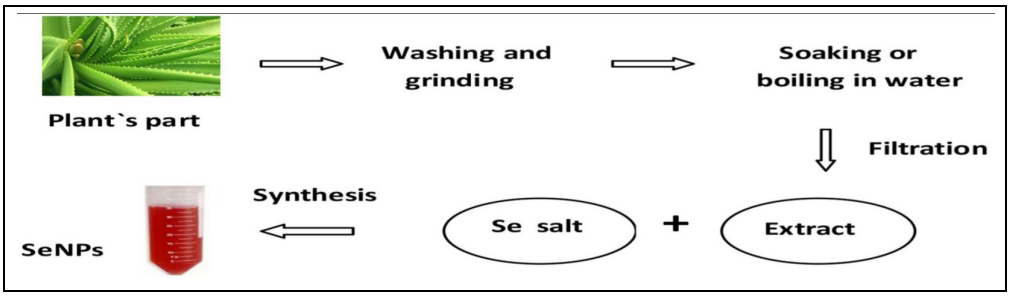

Fig. 1: Selenium nano-particle bio-synthesized from extraction of green tea

Green synthesis of GT/SeNPs using green tea extract

In an equivalent volume, green tea extract and a 50 mm Na_2SeO_3 solution was swirled in the dark for 10 minutes at 25°C. Then, to the earlier solutions, drops of a 50mM aqueous solution of citric acid were added. The reaction mixtures were agitated with a magnetic stirrer for 60 minutes to complete the reduction process. It was clear that GT/SeNPs had been created when the solution's colour changed from pale yellow to red. The resulting suspension was centrifuged at 8,000 rpm for 10 minutes. It was then lyophilized and maintained at a low temperature to characterize it (El-Saied *et al.,* 2021).

Characterization of SeNPs with GTW

- UV-Visible spectroscopy studies

A spectrophotometer was used to record the UV-Visible spectra of GT/SeNPs (Jasco V-650, Tokyo, Japan). The absorbance was determined to be between 200 and 400 nm (Youssef *et al.,* 2022).

- Fourier transform infrared spectroscopy (FTIR) analysis.

The produced compounds GT/SeNPs were dried and thoroughly mixed with KBr powder (for example, samples were well mixed with 1% (w/w) potassium bromide before analysis). The (4000-500 cm1) region's FTIR spectra were obtained with a resolution of 1 cm1 utilizing (IR Tracer-100 Shimadzu, Kyoto, Japan) (Youssef *et al.,* 2022).

-Transmission electron microscopy (TEM) examination

TEM used to assess the size and form of GT/SeNPs. Samples were sonicated for 10 minutes after being diluted with distilled water. The sonicated sample was dropped onto copper grids that had been coated with carbon before being vacuum dried for 30 minutes and studied with a 200 kV transmission electron microscope (Leo 0430; Leica, Cambridge, UK) (Youssef *et al.,* 2022).

- Scanning electron microscopy

The sample's morphological features, including shape and size, were examined by SEM (Carl Zeiss Evo 18, Oberkochen, Germany) (Youssef *et al.,* 2022).

Antibacterial activity of GT/SeNPs and CH

Bacteria Cultures.

The qualitative and quantitative antibacterial activity of GT/SeNPs was assessed using Escherichia coli, Staphylococcus aureus, and standardized isolates of virulent food-borne microorganisms. The cultures were propagated and studied using nutrient broth (NB) and nutrient agar (NA) medium at a temperature of 37 °C.

Qualitative assay: Inhibition zone (ZOI)

The qualitative experiment (using the disc diffusion method) was mostly conducted in the dark to rule out any potential effects of light on the activity of NPs. After spreading 24 hour-old bacterial cultures onto NA plates, the surfaces of the inoculants were covered with sterile discs (6 mm in diameter, constructed of Whatman No. 4 filter paper), which were filled with 30 L of GT/SeNPs (concentration: 100 g/mL). The diameters of the formed ZOI were measured following incubation (for 24 hours at 37°C), and their triplicates mean was computed. The standard antibiotic disc for the comparative antibacterial analysis was ampicillin (Elert and Jüttner, 1996).

Quantitative assay: minimum inhibitory concentration (MIC)

To ascertain the MICs of GT/SeNPs and against the investigated foodborne bacteria, the microdilution broth method, as described by Tayel *et al.* (2016) was applied. The tested compounds were serially diluted between 10 and 75 g/mL in 96-well microplates to challenge the bacterial cultures (2x107 CFU/mL). Following the aforementioned incubation period, the chromogenic indicator p-iodonitrotetrazolium violet aqueous solution (4% w/v), which gives red-formazan colour in active living cells, was used to assess the vitality of the cells. There are Parts from wells containing inhibited cells were plated onto fresh NA plates and incubated to confirm the inhibitory efficacy. The lowest concentration at which bacterial growth was suppressed in microplates and on NA plates was identified as the minimum inhibitory concentration (MIC) (Tayel *et al.,* 2016).

Microscopic observation of treated bacterial strains using scanning electron microscopy (SEM) imaging.

After being exposed to SeNPs, SEM imaging was performed to identify morphological changes in E. coli and S. aureus cells to determine the NPs action mode. Using a set technique, the SEM (JSM IT100; JEOL, Tokyo, Japan) bacterial imaging was carried out (Marrie and Costerton, 1984). After being cultured in NB for 24 hours, bacterial cells were exposed to GT/SeNPs for 0 (control), 4, and 8 hours at 37°C.The bacteria were first collected by centrifugation (4,500 g for 30 min), washed with saline buffer, and then centrifuged one more to prepare for SEM. Before being photographed, dehydrated samples were placed on SEM stubs with a gold or palladium covering.

Biopolymer extraction

Extraction of chitosan (CH)

Shrimp shell commercial (Mediterranean Sea, Alexandria), thedeacetylationdegree of 90% was purchased from el Midan Market (Alexandria, Egypt). Preparation of chitosan (2 g/100 ml) solution by 20 gchitosan and mixed with 1000 ml of distilled water and stirred for10 min at 60 C, and then 10 ml of glacial acetic acid were added tothe mixture and kept stirred for 1 h. (Kanatt *et al.,* 2013).

Extraction of sodium alginate (ALG)

Alginate is renowned for its great capacity for hydration and use as a connector or binder (Kanatt *et al.,* 2013). Alginate 2% w/v solutions (Al Gomhorya, Tanta, Egypt) were produced in sterilised distilled water at a set temperature (80°C), agitated, and then cooled. Moreover, distilled water was utilised to make a calcium chloride solution (Sigma-Aldrich, China) at a concentration of 2% w/v, which was then sterilised under autoclaving conditions (121°C, 15 min) while Tween 80 was also used as an emulsifier with continuous stirring for 30 min at 40°c (Raeisi *et al.,* 2020). After that, the finished solution was cooled to room temperature.

Preparation of biopolymer capsules

The beads were produced using the extrusion method. *L. acidophilus* was single-coated with chitosan (CH) and sodium alginate (ALG) at concentrations of 0.6% and 2%, respectively. A 7:1 (v/v) ratio of bacterial supernatant after centrifugation was added by maintaining sterile conditions (polymer to probiotics). With the use of a burette, microbial-polymer-gel solution was extruded and then poured into sterile 0.3 M $CaCl_2$ solution. Immediately after being created, the beads were allowed to harden in $CaCl_2$ while being constantly stirred. The beads were removed through filtering, collected, cleaned with distilled water, and then kept in storage at 4°C (Bozkurt, 2006).

Size and Morphological Analysis

The optical microscope was used to measure the average bead diameter (Master sizer Malvern 2000 UK). Using measurement software, the diameters of 100 randomly chosen capsules were measured (Leica Qwin 550)

Encapsulation Yield

To assess the level of encapsulation, the number of entrapped cells was counted using the pour plate technique on MRS agar, and counts were represented as the number of colonies forming units

(CFU), after being physically broken up in phosphate buffer (pH = 7), the prepared beads were gently shaken at room temperature. The encapsulation yield (EY) was calculated using the equation shown below it evaluates the efficiency of live cell entrapment and survival throughout the microencapsulation process (Elert and Jüttner, 1996).

$$EY = Log_{10}N/Log_{10}No \times 100$$

Where N_0 is the number of free cells put to the biopolymer mixture just before the manufacturing operation, and N is the number of live entrapped cells that were freed from the beads (Vodnar and Socaciu, 2014).

Survivability of Encapsulated Cells in Simulated Gastric Juice

Simulated gastric juice (SGJ) was prepared using hydrochloric acid to correct the pH to 2.0, 9 g/L of sodium chloride, and 3 g/L of pepsin. It was then autoclaved at 121°C for 15 minutes (Ragab *et al.,* 2020) then 10 mL of SGJ and 0.2 g of encapsulated bacteria were combined, and the mixture was continuously stirred at 50 rpm for 120 min at 37 °C. After incubation, beads were broken down in phosphate buffer (pH = 7), and an aliquot of the liquid containing 1.0 mL was then taken out and tested using the pour plate technique, Equation was used (Tayel *et al.,* 2010) to determine the microencapsulated bacteria's survival rate (%).

$$\text{Survival rate (\%)} = Log_{10}N/ Log_{10}N0 \times 100$$

Where N_0 represents the number of viable cells (log CFU/mL) before to exposure to the simulated gastric juice circumstances and N represents the number of viable cells (log CFU/mL) following exposure to the simulated gastric juice conditions (Oberoi *et al.,* 2021).

Survival of encapsulated bacteria in simulated high bile conditions

Bile salts were dissolved in intestinal solution (6.5 g/L NaCl, 0.835 g/L KCl, 0.22 g/L CaCl2, and 1.386 g/L $NaHCO_3$) pH 7.5 to final concentrations of 3.0 g/L to create simulated intestinal juice (SIJ). In 10 mL of SIJ, 0.2 g of encapsulated bacteria were combined, and the mixture was continuously shaken at 50 rpm for 120 min (Ding and Shah, 2009). A 1.0 mL aliquot of the mixture was taken after the beads had been broken down in phosphate buffer (pH = 7), and it was used for the pour plate method analysis. As previously noted, the microencapsulated bacteria's survival rate (%) was calculated.

The encapsulated bacteria's ability to withstand the bile salts in pH adjusted NGYC was examined. The samples were incubated at 378C for 3 hours with a final concentration of 1.0 or 2.0% filter sterilised porcine bile extract (Sigma). At the conclusion of each hour, 100 ml aliquots were obtained to count the cells and were then plated as previously mentioned (Sultana *et al.,* 2000).

Resistance to refrigerated storage

1.8 mL of sterile sodium chloride solution (0.5%, w/v) and 0.2 g of encapsulated bacteria were combined and maintained at 4 ° C in the refrigerator. To ascertain the total number of live cells, beads were destroyed in phosphate buffer (pH = 7) (Vodnar and Socaciu 2012).

Freeze-Drying

1.8 mL of sterile sodium chloride solution (0.5%, w/v) were mixed with 0.2 g of encapsulated bacteria before being lyophilized. In phosphate buffer (pH = 7), lyophilized beads were broken down to count the total number of live cells. To check the vitality of the cells, 10 mg of freeze-dried cells were well mixed with 0.99 ml of 0.85% sodium chloride solution. The approach described in the section on L. acidophilus survival under the conditions of a simulated gastrointestinal tract was then carried out to depolymerize cells and count the number of cell divisions (Lee *et al.,* 2019).

Determination of total phenolic compound content

The Folin-Ciocalteu technique was used to calculate the total phenolic component concentration (Ough and Amarine 1998). The data were given as grammes of gallic acid equivalent (g GAE/kg) per kilogramme of tea leaves. Triplicate measurements were taken.

Antioxidant capacity assay

0.2 mL methanol dilution of the substance followed by 1 ml of a methanolic solution containing 0.1 mM DPPH radical was added. The mixture was well shaken before being incubated at 37°C for 30 min. The absorbance was calculated at 517 nm. Ascorbic acid served as a positive control. The relationship between increased free radical scavenging activity and decreased reaction mixture absorbance was calculated using the equation below:

$$\text{DPPH scavenging activity (\%)} = 100 \times (A_0 - A_1) / (A_0)$$

Where the relative absorbances of the sample and the control are A_0 and A_1, respectively. To demonstrate the results, the average of the three studies is utilized, and both the main values and the standard deviation are given (McCue and Shetty, 2004).

Minced meat quality measurements

pH measurement

In a blender, ten g of sample were blended for 30 seconds with 40 ml of distilled water. After filtering the homogenate, the pH of the filtrate was measured using a digital pH metre (Simion *et al.,* 2014).

Lipid Oxidation

Using the 2-thiobarbituric acid (TBA) test, the rate of lipid oxidation was calculated. To perform the test, 10 g of ground beef was combined with a 200 g/l TCA solution and 135 ml/l phosphoric acid solution, and then homogenised for 30 seconds in a blender, a test tube containing 2 ml of the filtrate and an equivalent volume of TBA aqueous solution (3 g/l) was filled after filtering. The tubes were incubated at room temperature in the dark for 20 hours before the absorbance at 532 nm was measured using a UV-vis spectrophotometer (UV-1200, Shimadzu, Kyoto, Japan). To illustrate TBA value, 10 mg of malonaldehyde per Kg of beef was employed (Jouki and Khazaei 2012).

Total volatile base nitrogen (TVBN)

The mixture was made by combining 200 mL of trichloroacetic acid (TCA) (7.5%), 100 g of minced beef sample, and filter paper according to the procedure given by Malle and Poumeyrol. 25 of the filtrates were added to the distillation unit of the macro-Kjeldahl apparatus, where 5 mL of 10% NaOH was added as a spike. Following this, the distillate was added to 15 mL of 4% boric acid, titrated with 0.05N H2SO4, and the end point was determined using methylene red and bromocresol green. 25 mL of trichloroacetic acid (7.5%) was used as a blank in place of the sample (Malle and Poumeyro 1989).

Color Measurement

A CR-300 colorimeter was used to measure colour. Three measurements of the colour of the minced meat were taken for each analytical point in order to get the scale coordinates for L* (lightness), a* (redness), and b* (brightness) (yellowness). The apparatus was calibrated using a whittaker before each series of readings (El Adab *et al*., 2015).

Microbial Growth

Using a stomacher (Seward Stomacher 400, Norfolk, UK), meat samples were homogenised aseptically with peptone media (LAB-M, Lancashire, UK), 0.1%, w/v, for 1 minute, and then the inoculation plates were plated onto solidified medium to detect bacterial growth, The inoculation plates were incubated at 37 C for two days to estimate the total viable counts, and serial dilutions from meat homogenates were prepared in NB and analysed for microbial counts (Dimitrellou *et al.,* 2016 & Youssef *et al.,* 2022). It is also clear from the established protocols: ISO 4833-1:2013 specifies "Enumeration of total aerobic microorganisms of colony count at 30 °C." [BSI: 2013] London, UK According to ISO 17410:2019, "Enumeration of total microbial psychrotrophic organisms" [BSI: 2019. London, UK]. The definition of "enumeration of yeasts and moulds" is addressed by ISO 21527-1:2008. (BSI: 2008) London, UK.

Sensory Evaluation

From among the employees of the Department of Fish Processing and Biotechnology at Kafr el-Sheikh University in Egypt, ten knowledgeable panellists were selected. The panellists were chosen based on their habits, acquaintance with the minced meat to be evaluated, sensitivity, and capacity to replicate the assessment given. Also, each panellist had a pre-testing session so they could thoroughly explain and define each attribute that will be judged. The panellists conducted a hedonic test in which they assessed a variety of characteristics, including colour, texture, odour, taste, and general acceptability, using non-structured 8-point scales (0 = strongly dislike and 8 = strongly like) **(**Youssef, 2014).

3- Results and Discussion

3.1. Selenium Nanoparticles Biosynthesis Using green tea extract

Moreover, the antibacterial and antioxidant properties of GT-SeNP were evaluated against the Gram-positive *S. aureus* and the Gram-negative *E. coli*, both of which are known to be present in meat and its byproducts (Hariharan *et al.,* 2012). The phenolic component, which is already present in GTWE and is known for its antibacterial properties against infections that are spread by food, such as S. *aureus*, the findings of this investigation indicate that chitosan coatings mixed with GTWE are a possible component that may be employed as a natural preservative to lengthen the shelf life of fresh meats (Montaño-Sánchez *et al.,* 2020).

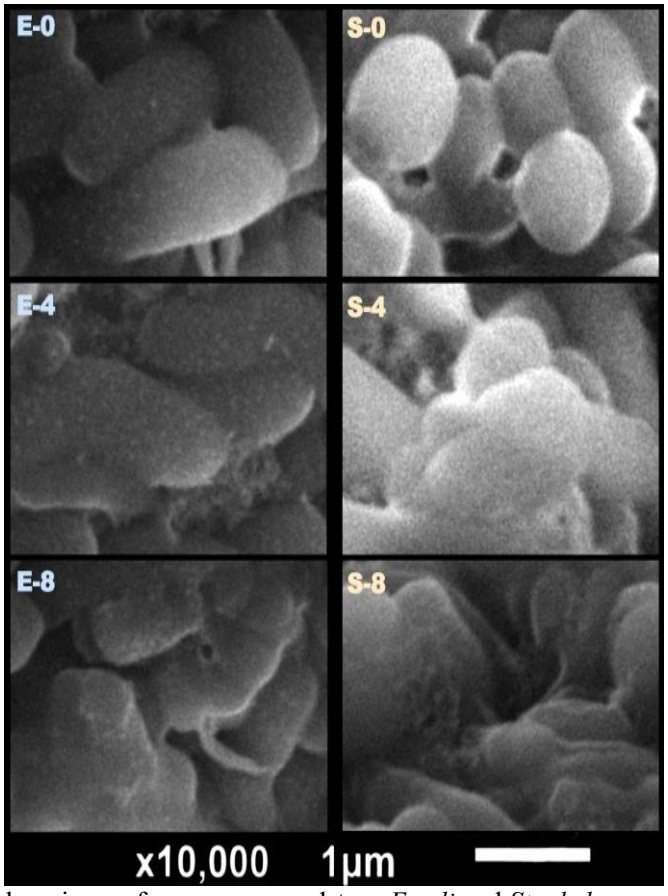

Fig. 2: TEM morphology image for gram –ve and +ve, *E coli* and *Staphylococcus aureus* respectively

Direct visual inspection clearly showed that the colour of the Se solution changed from clear to brownish, proving that the GT extract effectively reduced Se ions to generate SeNPs(Figure), The greatest UV-Vis absorbance (max) of the SeNP solution was also recorded at 267 nm. Due to the higher particle sizes, the redshift in the SeNPs' absorption spectra signals their synthesis as the reaction mixture's colour changes from transparent to reddish.

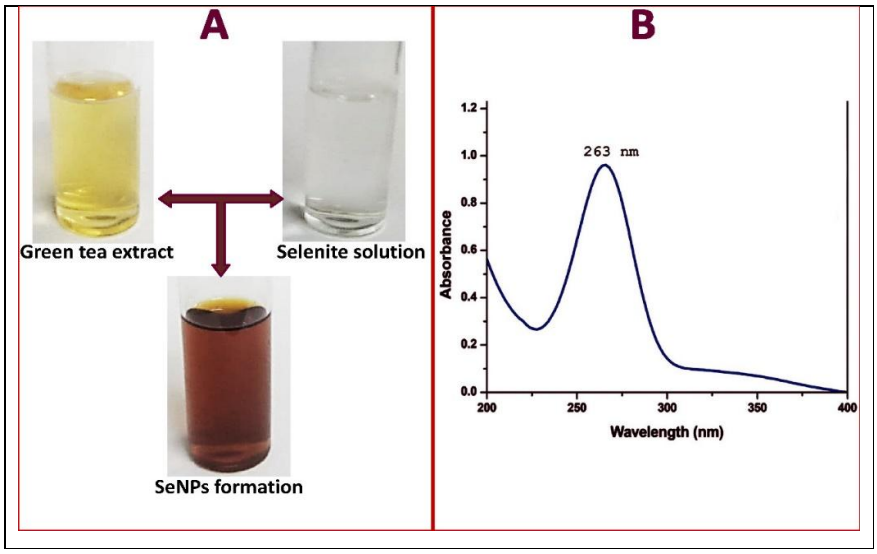

Fig. 3: Visual appearance and UV-Vis spectrum of biosynthesized SeNPs using green tea extract

The counts of coliforms, yeasts and moulds, *E. coli*, and *S. aureus* significantly dropped during the first seven days of storage of treated samples, while the counts of total aerobic bacteria and *Enterobacteriaceae* groups gradually decreased over the course of the subsequent 14 days. After 10 days of preservation, no S. aureus viable cells could be found in the chitosan preserved samples. For the biopreservation of minced beef, the addition of microbial chitosan to the product significantly improved its organoleptic qualities. For a 14-day storage period at 4 °C, chitosan with alginate binding material was able to keep the sensory quality of meat intact. After storage, all the evaluated characteristics improved in comparison to the untreated samples, particularly the odour and taste characteristic (Han *et al.,* 2021).

3.2. Structures of Synthesized Molecules

3.2.1. FTIR Analysis

Fourier transform infrared (FTIR) spectroscopy revealed that O-H, N-H, C=O, and C-O functional groups were involved in the synthesis of SeNPs, which were connected to bioactive compounds that coated their surface following the green synthesis of SeNPs, phenolic OH, aromatic in-plane C-H bending, asymmetric C-H bending (in CH3 and -CH2-), and secondary OH, respectively, may be found at peaks at 1375 cm ^{1}, 1030 cm ^{1}, 1462 cm ^{1}, and 1250 cm ^{1}. The presence of the peaks at 2840 and 2930 cm^{1}, which denote ether-methoxy-OCH3 groups, can be used to identify the biopolymer lignin coupled with SeNPs (Pyrzynska *et al.,* 2021).

3.2.2. Analysis of the ultrastructure of artificial nanoparticles

By using electron microscope imaging, the physiologies of synthetic nanoparticles and nanocomposites (GTWE produced SeNPs) were assessed (Figure 6) Pro-synthesized the morphologies of SeNPs were spherical and semi-spherical, and they had a homogeneous size range, according to the ultrastructure imaging of the NPs by TEM (Figure5).

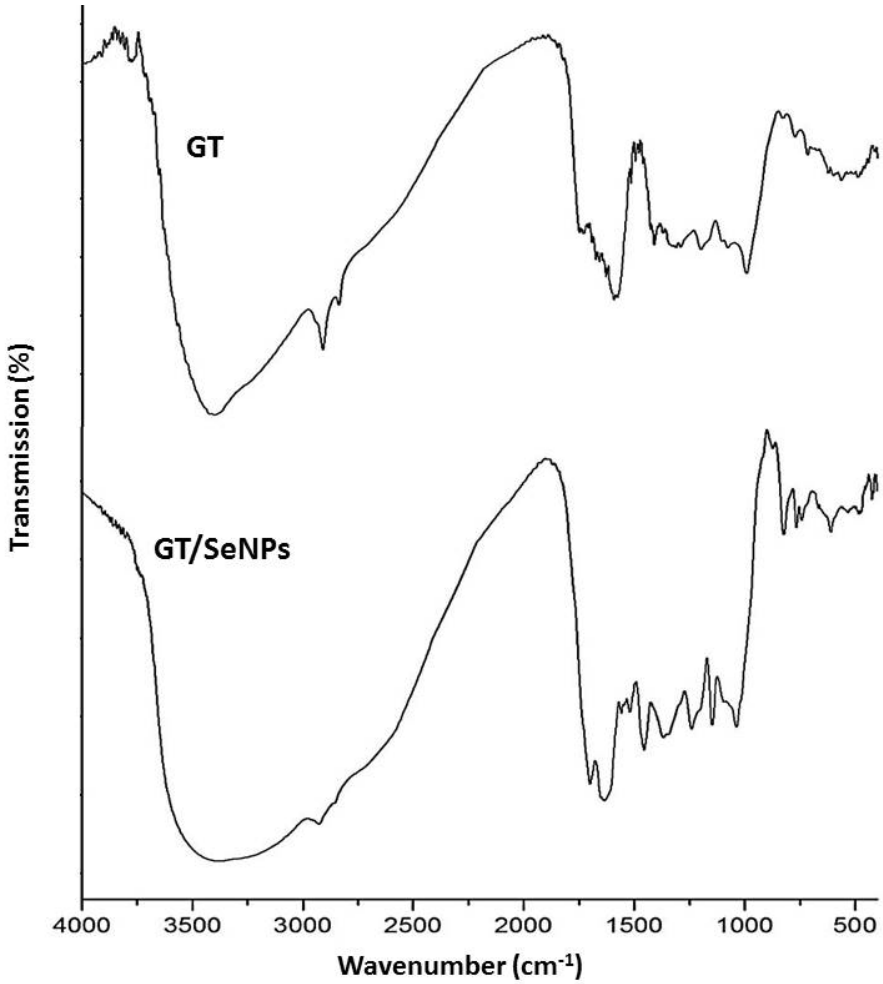

Fig. 4: FTIR analysis spectra for green tea extract (GT) and it's composite with selenium nanoparticles (GT/SeNPs)

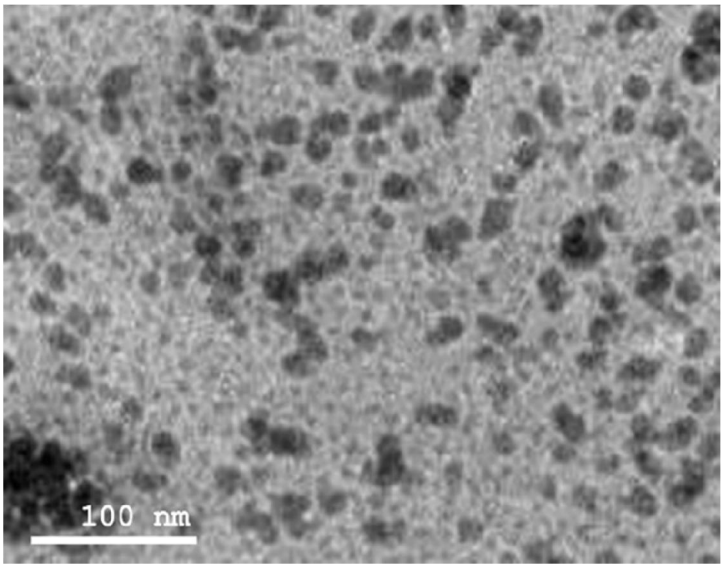

Fig. 5: Transmission picture shows SeNPs made from green tea that have an ultrastructure of synthesized nanoparticles.

3.3. Nanocomposites have antibacterial properties.

3.3.1. Potentials for Antibacterial Activity in Vitro

The screened bacterial pathogens provided quantitative and qualitative evidence of the antibacterial activity that occurred from the application of natural compounds (Table 1). The tested agents were completely effective against the tested bacterial strains, and the total compounds/composites, as GT-SeNPs, demonstrated exceptional antibacterial Gram-positive bacteria (*S. aureus* and *E. coli*), in that order, are more susceptible to antimicrobial treatments than Gram-negative bacteria. The antibacterial efficiency of the GT-SeNPs composite was much higher than that of the typical antibiotic (ampicillin) in terms of ZOIs and MICs. (Table 1).

Table 1: Antibacterial Activity of GT-SeNPs against Staphylococcus aureus and *Escherichia coli* measured in terms of the minimal inhibitory concentration (MIC, lg/mL) and the zone of inhibition's (ZOI) diameter (mm).

	Antimicrobial Assay			
Antimicrobial	***Escherichia coli***		***Staphylococcus aureus***	
Agents	**ZOI ****	**MIC *****	**ZOI**	**MIC**
GT	11.7±0.8[a]	57.5	13.6±0.7[a]	52.5
GT-SeNPs	23.4±1.9[b]	22.5	27.2±2.1[b]	17.5
Ampicillin	20.6±1.1[c]	27.5	22.3±1.2[c]	22.5

***MIC: minimal inhibitory concentrations (mg/L), * Different superscript letters in one column show significant difference at p 0.05. ** ZOI: mean diameter of inhibition zones in mm standard deviation.

3.4. Chitosan and sodium alginate:

The physiochemical properties of A. *brasiliensis* produced microbial chitosan. Its solubility in neutral water or in acetic acid solution (1%, pH 5.0) was increased by the fungal chitosan's low molecular weight (27 KDa) and high deacetylation degree (90%). Using generated microbial chitosan as a preservative and antibacterial coating agent in minced beef led to a significant decrease in the number of analyzed microbial groups during the treatment's 14-day cold storage. While all microbial groups in control samples tended to become more numerous with increased storage time, in samples treated with chitosan, the microbial count was drastically decreased in a time-dependent way. Chitosan's antibacterial action mostly manifests itself as the suppression of nutrient adsorption against S. *aureus*. Whereas the molecular weight of chitosan, which is dependent on the concentration of the polymer's -NH2 groups, is linked to its inhibitory activity against *E. coli*, as is the degree of deacetylation and its concentration in the solution (dependent upon the -NH3+ group) (Liu *et al.,* 2000).

3.5. Shape and size of macrocapsules

The yield of entrapped capsules and their sizes are shown in Table 2. There were no discernible differences in encapsulation yield between coated and uncoated beads ($P > 0.05$), The results also shown that probiotics were effectively encapsulated and that Lactobacillus acidophilus did not suffer a substantial ($P > 0.05$) loss of vitality throughout the bio encapsulation process (Table 2), Our statistics indicate that the average yield of all samples after encapsulation and coating was around 95.5%. These outcomes are in line with and comparable to those of Mokarram *et al.* (2009) who showed that the mean encapsulation yield in the emulsion technique was 99.8% in 2009. Additionally, probiotics' ability to endure peptone water at 4 °C and sterile water (0.5%, w/v) revealed that the quantity of bacteria in all of the samples (bio encapsulated forms) remained noticeably constant.

Table 2: Encapsulation yield of capsules and their sizes as shown in average:

Probiotic	Capsules type	Size of Capsules (mm)	Encapsulation yield (%)
Lactobacillus acidophilus	Alginate – chitosan coating double layer	3.6±0.4mm	95.5%

*Means in a column with a different letter are statistically different (P 0.05).

Light microscopy revealed that all capsules were uniformly and typically spherical in shape. In Figure 6. Chitosan coating altered the surface of alginate beads and impacted the morphology and form of capsules (Figure 6).

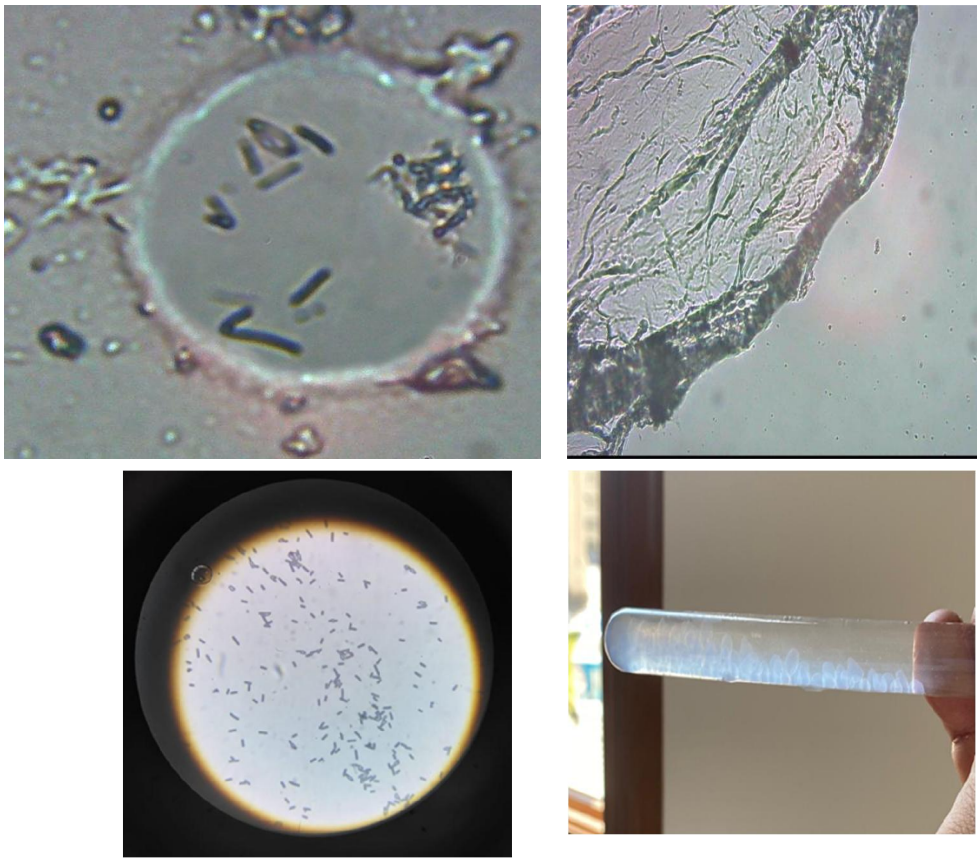

Fig. 6: Light microscope photograph showing calcium alginate- chitosan coating containing *Lactobacillus acidophilus*

Chitosan-coated capsules had an average diameter of 3.6± 0.4 mm. Using this method, when the capsules are introduced to the food product, they are distributed in macro range size and have a soft texture. A study in 2009 (Mokarram *et al.,* 2009) revealed usage of double-coated sodium alginate capsules both used a variety of micron sizes (75.339 0.209 m). According to Krasaekoopt *et al.* (2004) a bigger chitosan coated capsule size of 1.89 mm was produced in 2004 using the extrusion method. Furthermore, (Koo *et al.,* 2001) found that adding chitosan to alginate beads did not alter the form or size of the beads. Numerous studies have demonstrated that shrinking the size of the capsules to less than 100 m would not significantly improve the probiotics' survival rate under conditions of gastric secretion (Ragab *et al.,* 2020).

3.6. *In vitro* total phenolic Content in GT extract

The primary polyphenolic components in tea leaves and tea infusions are catechins (flavan-3-ols), with green tea infusion appearing to be a greater source of catechins. The main polyphenolic component of green tea is epigallocatechin-3-gallate (EGCG), which was present in concentrations ranging from 169 to 90.1 mg g. The benefits of EGCG for antioxidants and health promotion are well documented. The overall amount of phenol in free extracts obtained is approximately 331.19 ± 6.00 [mg GAE per g extract], according to the GT extracts' total phenol concentrations (Baldemir *et al.,* 2017; Sentkowska and Pyrzyńska, 2023).

3.7. Tolerance to Bile Salts and Acid

L. acidophilus was encapsulated showed good probiotic viability and excellent resistance to acid and bile environments without suffering any appreciable cell count reduction. When exposed to various pH conditions, the viable count of free cells decreased by about 4-6 log cycles in comparison to the control groupwhich was maintained for 4 hours in sterile distilled water without bile (pH 7), as indicated in Table 4. The survival rate of the encapsulated cells in the 2% bile concentration with alginate and

chitosan was similarly proportionately lower by the time the cells were exposed to bile salt solutions in this experiment, it was completely consistent with the literature, which used chitosan and calcium alginate as the encapsulating materials. In the past, Ding and Shah, (2007) found that increasing the growth mixture's bile salt content by 2.0% led to the highest possible survival rate. The present investigation showed that the encapsulated beads trapped with alginate and chitosan created organic acid and decreased pH when compared to free cells, Table 3 reveals that throughout the 2 hours, there was a 1-2 log decline in cfu mL^{-1} when compared to pH 2 and pH 1. The encapsulated cells survived with a pH of 3.0, although the pH of 3, which was used in this study to select prospective probiotic bacteria, is a typical pH for the human stomach, it made sure that acid-resistant strains were isolated. Additionally, there were no statistically significant (p 0.05) differences in cell viability between different incubation times. These findings supported those from previous research. The researchers also discovered that the encapsulated L. acidophilus effectively displayed improved vitality at various pH values in comparison to the non-encapsulated group. According to other data for pH 1.2, the non-encapsulated L. *acidophilus* was completely killed after 1 hour of incubation, however the encapsulated *L. acidophilus* remained above at pH 1.5 after 2 hours (Ding and Shah, 2007).

Table 3: Effect of low pH (2) on the survival of probiotic bacteria in both free form and in chitosan-alginate capsules

	Viable cell count (log CFU/mL)				
Time (hr)	**0**	**1\2**	**1**	**1 1\2**	**2**
Viable Free Cells	9.75± 0.07	8.10 ± 0.08	6.30± 0.35	6.41 ± 0.05	6.43 ± 0.53
Encapsulated with Ch.+ alg.	10.73 ± 0.18	9.13 ± 0.34	7.97 ± 0.15	7.42 ± 0.01	7.19 ± 0.67

Table 4: Effect of bile salt on the survival of probiotic bacteria that are free to grow and probiotic bacteria that are enclosed with chitosan-alginate

	Viable cell count (log CFU/mL)		
Time (hr)	**2**	**4**	**8**
Viable Free Cells	10.52-log	6.35-log	4.01-log
Encapsulated with Ch.+ alg.	10.55-log	8.65- log	7.19-log

Protecting probiotic cells in the stomach environment during low-pH exposure is one of the key goals of encapsulation. Alginate chitosan capsules improved cell survival in the current study's setting, which was akin to the actual gastrointestinal situation. Probiotic bacteria were most effectively shielded from bile salt by chitosan-coated alginate spheres chitosan coating provides the best protection in bile salt solutions because an ion exchange process takes place when the beads absorb bile salt, as a result, there could be a limit to how much bile salt diffuses into the beads. By doing this, interactions between bile salt and probiotics in capsules will be avoided. It was also stated that *Bifidobacteria*and *L. acidophilus* with chitosan-containing alginate beads showed better viability than the absence of chitosan in alginate and well-known Alginate, a negatively charged polymer, is surrounded by a semipermeable membrane made of the positively charged polyamine chitosan. This membrane prevents cell release and promotes gel stability since it does not disintegrate in the presence of Ca^{2+} chelators or antigelling chemicals. Our findings agree with those of other research using bile salt concentrations that were comparable to ours. For instance, research indicates that probiotic bacteria in capsules can survive longer than probiotic cells in free form (Alsaiqali *et al.,* 2016 and Chávarri *et al.,* 2010).

3.8. Antioxidant capacity assay

The DPPH experiment results revealed that green tea extract and manufactured selenium nanoparticles effectively inhibited free radicals **[**Vyas, J., & Rana, S., 2017**]**, with the latter showing a far greater capacity to scavenge hydroxyl radicals than any other extracts that were employed in their synthesis. The maximum antioxidant capacity was demonstrated by nanoparticles produced by combining the chemicals in a 1:1 ratio (88.9 %) in this investigation of selenium nanoparticles made utilizing green tea (Lipton Label). Any of the used reagents' activity was reduced when their concentration was raised. Nonetheless, in this instance, the greatest ability to scavenge OH radicals were close to 100% (Sentkowska and Pyrzyńska 2023).

3.9. Minced meat quality treatment

While the treatments were stored in the refrigerator, every therapy had been evaluated.

3.9.1 pH measurement

The effects of the storage period on the pH levels of meat were also analyzed. According to a study conducted by Biswas and Kandeepan in 2007, the pH value of the meat increased significantly during the frozen condition. The denaturation of proteins during frozen storage may be the cause of the increase in pH after 14 days, as well as endogenous enzymes and microbes that break down meat proteins to create ammonia, organic sulphides, and amines that raise pH (Azad and Akter, 2005). Meat undergoes autolysis, which may account for the considerable pH rise with extended freezer storage (Abdulgadir *et al.,* 2015).

3.9.2. Lipid Oxidation (TBARS)

It was previously reported that some raw meat products may contain malonaldehyde, which is produced by lipid oxidation. The TBARS values revealed that the levels of malondialdehyde in the samples during the storage period affected the formation of the TBARS. The observed increase in the levels of malondialdehyde during the storage period was also significant. On the 18th day, the total amount of malondialdehyde in the samples reached 0.82 mg/kg, which indicates that the level of lipid oxidation in the meat was low (Jouki and Khazaei 2012).

3.9.3. Total volatile base nitrogen (TVBN)

The total TVBN of meat samples was significantly increased by 4C. This is because the protein breakdown process is triggered by the microorganisms that produce proteolytic enzymes. These enzymes then produce volatile compounds, which can lead to various negative effects, such as odour and drip losses (Simion *et al.,* 2014 and Jouki *et al.,* 2012).

3.9.4. Microbial Growth

The effects of coating minced meat with the developed antimicrobial compounds/composites (Pro, GTWE/SeNPs, and CHI) on the chemical and microbiological parameters of meat samples are shown in Table 4. Comparing the control (water-dipped) samples to the zero time control group (TVB-N and TBARS), the control samples showed remarkably high microbial group counts (such as total bacterial count, psychrophilic bacteria, yeast, and moulds) and chemical degradation characteristics. The chemical and microbiological characteristics of the meat samples treated with antimicrobial agents may be largely maintained. The nanomaterials (SeNPs) showed noticeably greater effectiveness for preserving a meat's characteristics. After 7 days of storage, the encapsulated cells covered by a double layer of chitosan and alginate were the formulation that considerably reduced microbial counts and chemical parameter increases when compared to the control (Youssef *et al*., 2022).

Table 5: Microbial and chemical characteristics of minced beef after seven days of cold storage at four degrees Celsius

Coating material	Evaluation Qualities				
	Microbial Quality (log CFU/g)			Chemical Quality (mg/kg)	
	Total Count	Psychrophilic Bacterial	Yeast and Molds	TVB-N	TBARS
Control	4.82 ± 0.65	3.83 ± 0.62	2.26 ± 0.63	35.507 ± 0.538	0.410 ± 0.026
Encapsulated cells	1.41 ± 0.17	1.16± 0.38	10 CFU<	21.656± 0.217	0.241± 0.019

3.9.5. Sensory Evaluation

Using a seven-point hedonic scale (1, strongly dislike to 7, extremely like), the sensory attributes of meat samples were assessed in the current study in terms of appearance, colour, odour, flavour, texture, and overall acceptability. These results revealed that samples coated with chitosan and alginate did not produce any off flavours that would cause consumers to reject the items. By the third day, uncoated samples' appearance, colour, odour, texture, and general acceptance had received "unacceptable" rankings. The findings of the sensory evaluation and the microbiological and chemical investigations agree rather well, and the uncoated samples quickly went bad due to microbial

development, developing a slimy look and unpleasant odour after just three days. Therefore, the alginate and chitosan coating's antioxidant and antibacterial properties reduced the oxidative effects, extending the product's shelf life while retaining quality (Kanatt *et al.,* 2013 and Chandramohan *et al.,* 2019).

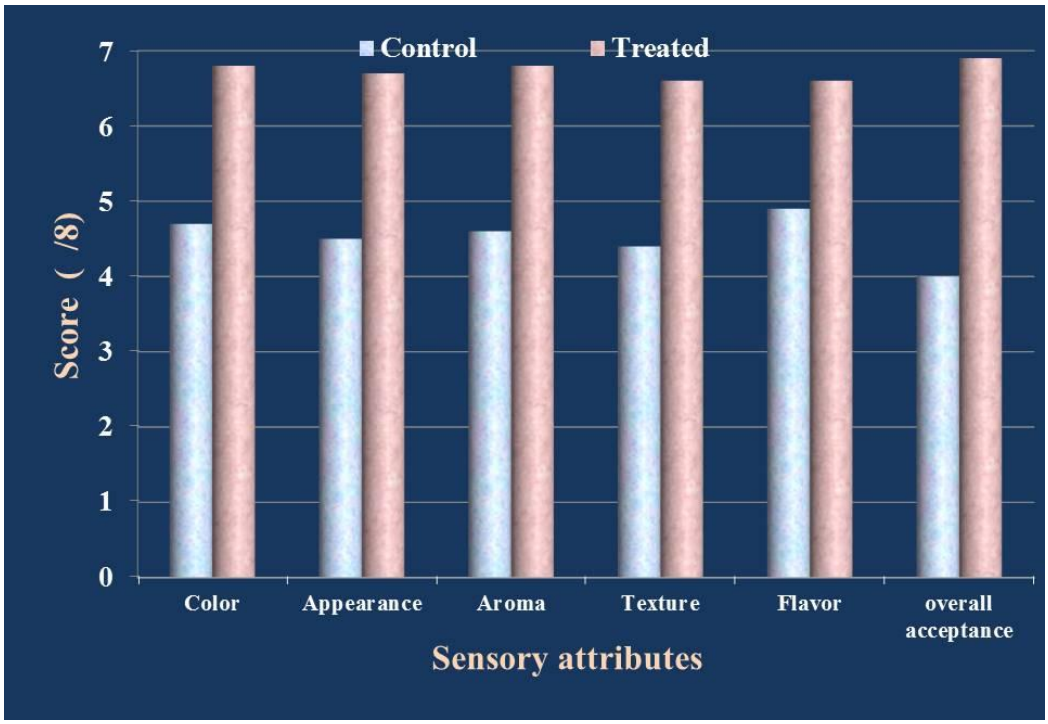

Fig 7: Sensory attributes of control minced meat, compared with treated samples after holding in low temperature (4±1 °C) for 7 days.

4. Conclusion

Microbiological, chemical, and sensory evaluation tests revealed that adding lactobacillus probiotic and GTWE to minced beef products may boost microbiological safety, keep high quality characteristics, and extend shelf life during chilled storage. The persistence of dangerous bacteria in meat products meant there could be a risk of contamination during post-processing and the growth of these bacteria in meat products was greatly inhibited by the chitosan + alginate covering.

During the storage durations utilized here, chitosan alginate coating combined with GTWE and probiotic enhances the physicochemical (pH, colour, and lipid oxidation) and microbiological properties of minced meat samples. Their significance results from the delays in oxidative reactions, colour changes, and sensory attribute modifications. For short- or long-term storage, plant extracts can be employed as an ingredient. As the use of plant extracts becomes more widespread and reaches an industrial level, it is crucial to combine them with other healthier strategies like reducing or replacing fat, salt, and nitrite, using cutting-edge processing techniques, and coordinating the production of meat with sustainable practices.

Finally, it is advised to look for practical and efficient ways to use these extracts in active packaging, as this might spark tremendous interest in the integration of plant extracts into the future preservation and health-related qualities of meat and meat products.

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