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Optimizing *Clarias gariepinus* Fillets Preservation using Mediated Nano- Selenium with *Salvia officinalis* and Nanochitosan: synergism for boosting freshness

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Abstract

AFRICAN catfish (*Clarias gariepinus*) acquires elevated interests with their nutritional importance and propagation easiness, but maintaining their products' quality is challenging. The extract of *Salvia officinalis* petals (SO) was employed for mediating selenium nanoparticles (SeNPs), using direct and facile procedure. Chitosan nanoparticles (CTn) were composited with SO/SeNPs and their nanocomposites (NCs) and parent materials were characterized using spectroscopy and electron microscopy. The SO, with its phenolic contents, effectively mediated SeNPs that had 13.48 nm mean size. The CTn-SO/SeNPs nanoconjugation was achieved and validated using infrared (FTIR) analysis and scanning microscopy. The biopreservation of catfish fillets using fabricated nanomaterials/NCs was very promising, during storage of coated samples for 25 days. The most auspicious treatments were the coating with 0.5% solution of SO/SeNPs and CTn-SO/SeNPs, respectively, for maintaining the fillets' sensory attributes (acceptability, color, odor, flavor and texture) throughout storage. The fillets' coating with NCs could significantly reduce the progress of spoilage parameter (pH, volatile basic nitrogen; TVB-N and reactive thiobarbituric acid; TBARS), compared with control. The SO-mediated SeNPs and their nanoconjugates with CTn provided effectual bases for edible coating to preserve catfish fillets and maintain their quality.

Keywords: Biosynthesis, Catfish, Nanomaterials, Natural products, Seafood preservation.

Introduction

The African catfish (*Clarias gariepinus*) holds immense significance in Egypt, playing a crucial role in both the nation's aquaculture industry and food security. Extensively farmed in the Nile Delta and other aquatic environments, this species has become a fundamental component of the Egyptian diet, serving as a dependable protein source for the growing population[1]. Its ability to thrive in diverse environmental conditions and its rapid growth have established it as a preferred species for aquaculture, thereby enhancing the economic well-being of numerous farmers and fortifying the country's aquacultural production[2]. Furthermore, the nutritional richness and appealing taste of the African catfish have rendered it a popular choice among consumers. Consequently, the sustainable cultivation and utilization of the African catfish in Egypt not only bolster local economies but also significantly contribute to fulfill the protein requirements of the Egyptian populace[3].

The study of African catfish in the form of processed fillet has become pivotal in enhancing the utilization of *C. gariepinus* within the food industry and market. The fillets may be obtained through complex operations to render the raw fish pieces into marketable and tasty products. Further studies seek to develop new ways of improving the fillets time till expiration, in addition to the alimentary aspects of the products made from it due to the consumers' increased demands on higher merits related to food products offered in the market[4]. The study aids in enhancing the method of processing by comprehensively understanding and optimizing the approach, which shares economic, ecological and renewable orientations in aquafarming occupation and the increasing demand for high-quality processed fish products in other countries and the domestic market [5].

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Food preservation using macro selenium (Se) is promising, and a similar potential is anticipated for selenium nanoparticles (SeNPs). It is worth noting that their mechanisms and applications differ. SeNPs, with their size possess a larger surface area and enhanced reactivity; these nanoparticles can be integrated into food packaging solutions to deliver antioxidant benefits that combat spoilage and prolong the freshness of foods [6].

The natural extract from the *Salvia officinalis* plant, commonly known as sage extract (SO), has been acknowledged for its benefits in preserving food thanks to its natural antimicrobial and antioxidant properties [7]. Packaging with compounds such as acids and flavonoids in SO shows effectiveness in inhibiting various harmful microorganisms and pathogens. Its application in food preservation aims to prolong the shelf life of items while its antioxidant properties help combat oxidative processes that can affect food quality. Additionally SO is being studied as a substitute for preservatives reflecting the growing preference among consumers for natural and transparent ingredients, in food products [8].

The biopolymer chitosan which is obtained from crustaceans has a lot of favorable criteria including biocompatibility, biodegradability and the antimicrobial properties. It has gained immense prominence in all of the areas such as food preservation [9]. Nanochitosan (CTn), the ultrafine variety of chitosan, has added advantages because of its large total surface area and reactivity. The new dimension of CTn provides more effective interactions with microbial cell membranes and thus resulting in enhanced antimicrobial activity. On the other hand, CTn shows improved solubility and bioavailability which makes it perfect for the application of food preservation and packaging fields [10].

The employment of CTn as a carrier for phytocompounds and plant extracts used for green Nano synthesis of SeNPs in a mixture (e.g. CTn-SO/SeNPs) presents an intriguing avenue in food preservation, synergizing the unique properties of both natural ingredients together with the Nano element [11]. CTn is a polysaccharides, offers thickening and adhesive qualities, contributing to improved texture and making up a protective hindrance contra to humidity leakage with antimicrobial along with free radical scavenger properties [12]. SO, known for its antimicrobial and antioxidant properties, complements this by inhibiting the growth of spoilage microorganisms and mitigating oxidative reactions that can compromise food quality. The mixture of CTn, SO and SeNPs not only holds promise in extending consumption period before expiry attributed to perishable products but also aligns beside rising customer request backing sustainable, simple and natural food preservation methods [11].

In the food sector, the use of green nanotechnology is becoming increasingly important as it provides sustainable answers for enhancing food safety, quality and preservation. Consequently, scientists are working tirelessly to explore how eco-friendly conscious nanoparticles (NPs) and processes can be used to address various problems in food production and preservation. Also, green nanotechnology has been employed in ensuring food safety through the development of biosensors that rapidly detect contaminants guaranteeing a safe supply chain. As such, with the demand for sustainability measures arising in the industry of food production, green nanotechnology presents itself as a hopeful way out in resolving these difficulties which further leads to improvements in many components of food production, packaging and safety [13].

During this investigation, the suitability of SO as a food-grade additive for the preservation of refrigerated African catfish fillet was assessed. Additionally, the synthesis of SeNPs using SO was conducted (SO/SeNPs), and their Nano conjugation with CTn (CTn-SO/SeNPs), aiming to collectively evaluate their effectiveness as robust additives for preservation and grade boosters for fish cuts.

Material and Methods

Preparation of Salvia officinalis extract (SO)

Petals stalks of *Salvia officinalis* plants were gotten from the ARC "Agricultural Research Centre, Giza, Egypt". The flowers were cleaned using soft filter cloth to eliminate any traces of dust and insects. Flowers were naturally air-dried for one week in the shades at 25-28ºC temperatures, turning them from time to time to prevent molding. Samples of the dried materials were weighed and ground with a mortar and pestle so that we could store them in a desiccator. In addition to this 50 g of air-dried roots were soaked in lukewarm distilled water (35^{o}C) with amount of 250 mL along with ongoing shaking using an incubating shaker. The mixtures after the steps were 1st filtered with filter paper, then centrifuged for 10 minutes at 2320 xg, moved to evaporate to dryness at 40ºC rotary evaporator [14].

SO Phenolic and Flavonoid Contents Determination

HPLC "High Performance Liquid Chromatography" evaluation employed 1260 series Agilent, Germany. The separation process utilized A Hypersil GOLD C18 column (4.6 mm × 250 mm internal diameter, 5 μm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B), with a flow rate of 1 mL/min. The mobile phase followed a linear gradient program, beginning with 82% A at 0 minutes, shifting to 80% A from 0 to 5 minutes, moving to 60% A from 5 to 8 minutes, holding at 60% A from 8 to 12 minutes,

reverting to 82% A from 12 to 15 minutes, maintaining 82% A from 15 to 16 minutes, and finishing with 82% A from 16 to 20 minutes. The multi-wavelength detector was prepared to read at 280 nm, and an injection volume of 5 µL was used for each experiment sample solution. In this analysis, the column temperature was maintained at 40°C throughout the experiment.

Preparation of SO/SeNPs

The fabrication of selenium nanoparticles (SeNPs) involved sodium selenite transition through ascorbic acid, in presence of SO solution. To initiate the process, an aqueous solution (10 mM) of $Na_2SeO_3.5\ H_2O$ has been set, then merged with SO solution (0.1%, w/v) both of similar amount (90 mL), undergoing vigorous stirring within a duration of 90 minutes. Following this, while maintaining the agitation, ascorbic acid solution was added (56.7 mM) to previously composed mix (10 mL), and the agitation persisted for an additional 60 minutes in darkness. The formed selenium nanoparticles were evidenced by a deep, orange-colored solution. The resulting SeNPs were then collected through a centrifuge (10500 xg, 25 min, Sigma 6-12K, Germany), reset up in water Milli-Q purification system, subjected to lyophilization, and subsequently stored at (25 ± 2°C) [3].

Preparation of CTn loaded with SO/SeNPs

Chitosan (CAS 9012-76-4; Sigma-Aldrich, Germany), with ≥80% deacetylation and molecular weight of ~100 kDa, was dissolved in 1.5 % acetic acid to give the pH of 4.7 ± 0.2 and carry out the pH adjustment using sodium hydroxide. The strained solution was then filtered using 0. 45 µm millipore syringe filter, then TPP solution "sodium tripolyphosphate; 1.0 %" was inserted drip by drip (0.3 ml/min) to chitosan solution under magnetic agitation that was continued for 1 hour at 25°C, which lead to the chitosan nanoparticles genesis. The nano-particles were subjected to refinement using a centrifuge (9000 x*g* for half an hour) to eradicate redundant unreacted chitosan. The sample was bathed in purified water to completely eliminate all sodium hydroxide. For fabricating CTn–SO/SeNPs nanocomposites, SO/SeNPs were added and mixed with TPP solution just before nanoparticle formation. The previous mixtures were added dropwise relative to the quantity of chitosan, and 1% Tween 80 was incorporated while being magnetically stirred for 2 hours, before the next step, centrifugation [10,15].

Optical Analysis of SeNPs

To validate the composition of SeNPs and detect their Surface Plasmon Resonance (SPR), indicative of free electrons at the exterior of the NPs, the spectrum of SO/SeNPs were examined using an ultraviolet-visible spectrophotometry (model UV-2450, Shimadzu, Japan) in UV-visible region of 200-600 nm for the optical characterization.

NPs Size and Charge Assessment

Measuring the scale and electrokinetic potential of SO/SeNPs and CTn particles was carried out using Dynamic (DLS) Light Scattering procedure, utilizing a zeta-sizer apparatus (Zeta plus, Brookhaven, USA). The DLS was used to determine the particle size and zeta potential in the dispersed phase of nanoparticles. This technique is very important as it determines the extent to which the nanoparticles are stable and uniformly distributed in the solution, thus its effectiveness in biopreservation.

Fine structure Analysis of NPs

The structural characteristics such as size, shape, morphology, and distribution of SO/SeNPs and CTn-SO/SeNPs particles were measured using TEM "transmission electron microscopy; Leica, Leo 0430; Cambridge, UK). The selection of TEM for imaging of the selenium nanoparticles (Se NPs) was because of high resolution offered by the technique. This technique also enables us to well observe its size, shape, and dispersion situation of the nanoparticles needs for the further production, in order to understand the structure and uniformity of the nanoparticles.

Morphology of the synthesized nanocomposite (CTn-SO/SeNPs) was observed under SEM "JEOL, JSM IT100, Scanning electron microscope, Japan" at 15 kV accelerating voltage. SEM is employed for determining the molecular form of the sample to monitor whether the mixtures are well mixed during the polymerization step of the process. The SEM technique is useful in producing images of the topography and surface features and surface profile, hence, gives an initial understanding on how the nanoparticles interact with chitosan matrix.

FTIR analysis

To discern the distinctive biochemical bonding and interactions within the generated mixtures, the spectra of sage extract (SO), synthesized selenium nanoparticles (SO/SeNPs) and synthesized Nano-chitosan loaded with sage extract with Nano selenium (CTn-SO/SeNPs) were subject to spectrophotometric analysis. This involved utilizing Fourier-transform infrared spectroscopy (FTIR) on a JASCO FT-IR-360 instrument, Tokyo, Japan, covering a wavenumber range of 450–4000 cm^{-1}.

Fish preparation

Fillets of *Clarias gariepinus* fish that weighing approximately 500 g/fish were gotten from Seafood processing plant, Kafrelsheikh University, Egypt. The sliced fillets (each with ~ 6 × 8 × 2 cm dimensions and 40 g weight) were categorized to four groups: Initial group was denoted as the control group (C), was dipped in sterilized purified water for

2 minutes, drained off within an additional 2 minutes, packaged in green pouches, and refrigerated for 25 days. Next in order, the SO-treated group (S) was immersed in a 0.5% sage extract (SO) solution for 2 minutes, drained off within 2 minutes, then packaged and stored in the refrigerator for the same duration. The SO/SeNPs treated group (SS) was immersed in a 0.5% SO/SeNPs solution for 2 minutes, drained off within 2 minutes, packaged, and stored in the fridge for the same period as well. The last group was soaked in 0.5% CTn-SO/SeNPs mixture (M group) for 2 minutes, with another 2 minutes to dry, then packed, and stores in the refrigeration temperature for 25 days like the previous treatments [3,5,16].

The selection of fish with specific sizes and weights was intentional and based on several factors, e.g. uniformity in experimental conditions, commercial relevance, ease of handling and processing and representative sampling.

Examination and sampling of the fillets were conducted for each group at eight-day intervals (e.g. at 0, 8, 16 and 25th day), involving 12 slices from each group in each session, through 25 days.

Fish Samples' Storage Conditions:

Temperature: The fish samples were kept at cool at temperature of 4 °C all the time during the analyses. *Relative Humidity*: The storage conditions relative humidity varied slightly with an average of about 85%, which is important for preventing fish fillets dryness. *Packaging*: After filleting the fish each fillet was put in a separate sterile polyethylene bag to reduce the exposure of external pathogens and not allow cross contamination between different samples. Airflow and Ventilation: There was also a provision of a controlled airflow regulation to maintain the temperature and humidity level in the store unit. *Monitoring*: A digital thermometer and hygrometer were used for monitoring temperature and humidity respectively with data loggers.

Sensory evaluation

A group of seven experienced panelists, chosen from faculty of Aquatic and Fisheries Science, Kafrelsheikh University (Department of Fish Processing and Biotechnology) undertook the sensory assessment. The fillet samples, diced into cubes, underwent a 5-minute oven cooking process to evaluate color, aroma, taste, consistency, and overall liking. Utilizing a rating scale spanning from 1 to 10, the evaluators provided scores for each sample, with any receiving a score below 4 considered unsatisfactory and subsequently discarded [17].

Estimation of pH

Sample homogeneity was achieved by soaking it in ten times volume of potassium chloride solution and homogenized. After that, the pH of the solution was read using a pH meter once the values had normalized to a stable number [18].

Estimation of Total Volatile Basic Nitrogen and Thiobarbituric Acid Reactive Substances

The assessment of Total Volatile Basic Nitrogen (TVB-N) estimate performed according to the officially described technique of hydrodistillation [19]. This process involves extracting TVB-N by using an alkaline solution and subsequently titrating the ammonia yield. Initially, 10 g of homogenized flesh was mixed with 7.5% trichloroacetic acid (20 mL) and sieved by Whatman no. 1 membrane. The seived yield underwent hydrodistillation using a VELP UDK-6 apparatus (Milan, Italy), beside mixing with NaOH 10% (3 mL). Vapor concentrate was assembled in a beaker that has boric acid (4%) supplemented with 1 mL of a blended index (methylene blue/methyl red in a 1: 2 ratio). The resulting alkaline mixture was then titrated with 0.025 N H_2SO_4.

To determine TBARS (Thiobarbituric Acid Reactive Substances), spectrophotometry was utilized to estimate optical density at 532 nm wavelength [20]. 5 g fillet sample was blended alongside 11% trichloroacetic acid for 1 minute at 11100 x*g* using an IKA Homogenizer (Wilmington, USA). Subsequently, the homogenized sample was ice bathed for 1 minute and blended for an additional 1 minute. The resulting mixtures were filtrated using Whatman No 1, 20 mM thiobarbituric acid solution (1 mL) was mixed with 1 mL of the filtrated yield, followed by putting in a dark incubator condition for 20 h at 25°C. The density of absorbance was then measured at 532 nm using an Ultraviolet-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The output was expressed in µg of MDA (Malondialdehyde) per gram.

Psychrophilic Bacterial Count

A sterile buffered peptone water volume of approximately 225 ml was mixed with 25 g of fish flesh sample for 1 minute. Subsequently, a 10-folds serial dilution was made up as outlined in the method [21].

Aseptically, composed dilution (0.1 mL) was introduced to two sterilized culture plates, each containing approximately 15 mL of sterilized-dissolved Agar counting plates. The mixture was allowed to harden before being placed at a temperature of 7 °C for duration of 10 days following completion. Subsequently, the count was conducted [22].

Statistical analysis

The numerical datum, with a sample size (n) of 3, underwent a two-way ANOVA, and the outputs were stated in the form mean and standard error of the mean (SOM). Then, Tukey's test was chosen as post

hoc test and implemented by GraphPad Prism 8.0.2 software. Statistical significance was determined with p-value <0.01.

Results and Discussion

HPLC

The concentrations of phenolic acids and flavonoids (μg/g) in sage herbal extract (SO) were Gallic acid 1859.05, Chlorogenic acid 2274.27, Methyl gallate 143.88, Caffeic acid 608.00, Syringic acid 1090.09, Ellagic acid 210.52, Vanillin 2995.28, Ferulic acid 2329.55, Naringenin 521.42, Quercetin 2388.44, Cinnamic acid 157.57, Kaempferol 3801.84 and Hesperidin 161.71 μg/g (Table 1).

Sage extract (SO) is famous for its abundance of phytochemicals, e.g. phenolic compounds and flavonoids; which also exhibit antioxidant properties by neutralizing free radicals. Also, SO has shown antimicrobial activity in the previous related studies. That is why this herb has a dual role, one being health promotion while the other is food preservative [23].

Specifically, table (1) provides a detailed account on various phenolic acids in SO which are capable of triggering oxidation reactions as well as showing antibacterial action through numerous pathways – both incidents are commonly attributed to their high concentration [24].

In addition to this, seven phenolic acids and flavonoids showed very high values among others that were considerably significant. It is therefore necessary to conduct an extensive discussion on these findings.

Two phytochemicals from the flavonoid class namely quercetin and kaempferol have been widely recognized in food industry for their potential health benefits. Acting like natural antioxidants, they are key players in eliminating free radicals leading to food protection against oxidative processes that cause spoilage. Moreover, kaempferol contributes nutritional value and functionality with its possible anti-cancerous effect as well as cardiovascular benefits [25].

The diverse applications of vanillin, gallic acid and syringic acid in the food industry are due to their distinct flavors and beneficial effects. Vanillin is a popular flavoring agent with a sweet taste that resembles vanilla in many food products. It has also been found to exhibit antimicrobial activity as well as serve as an antioxidant which helps in preserving food [26]. Gallic and syringic acids, belonging to phenolic compounds, are antioxidants that eliminate free radicals in the body hence avoiding oxidative damage. They act as natural preservatives used in food processing since they inhibit spoilage organisms [27,28]. Syringic Acid combined with Gallic Acid and Vanillin has a dual purpose of improving taste while providing vital antioxidative and antimicrobial activities making it ideal for contemporary functional foods that demand for naturalness with functionality [29].

Ferulic and chlorogenic acids are among the most common bioactive compounds used as functional ingredients in health-promoting foods and beverages. Besides being antioxidants, these compounds have potential cardiovascular health benefits along with anti-inflammatory, weight management capabilities and anti-cancer properties [30].

The concentrated, aromatic and herby flavor of SO is characterized by its strong taste [31]. The strong taste is enjoyed as it enhances different kinds of dishes but sage extract should be used sparingly so that other ingredients are not overpowered and to allow them to stand out. So, the minimum amount of dosage should be used in catfish fillet since its culinary value can still be realized while maintaining safety standards [32]. Therefore, a concentration of 0.5% was used for this study.

Optical analysis

The occurrence of SO/SeNPs was obviously discovered through observing a change in the solution to orange-red or slightly brownish color (Fig 1, Upper box). The cause of this color transformation is assigned to the SPR (Surface plasmon resonance) featured of SeNPs which often happens in the wavelength range between 260 and 400 nm, as reported in some publications [33].

The reduction mechanism of the SeNPs was said to be in association with SPR excitation, which implies that this phenomenon could be responsible for the absorption peak at the desired wavelength (277 λmax) and the color variations of NP solutions (shown in Fig.1, curve). These observations indicate the formation of SeNPs [34].

Size and charge

DLS analysis results indicate that the average charge of SeNPs is -26 mV. This confirms the success of SeNPs synthesis with stabilization of the formed nanoparticles by the capping ability of sage solution [3].

DLS analysis states the size of the synthesized particles where mean diameter is 17.9 nm and median diameter is 22.9 nm. Previous research stated that SeNPs size can range from 5 to 100 nm which is further proof to the success of SeNPs formation [13,35].

The random distribution of SeNPs in sage solution was investigated by TEM scanning as in Figure 2/A. The results of size and diameter match those of DLS analysis as size range 2.12 - 42.36 nm and mean size 13.48 nm.

The particles' shape appears to be quite uneven in Figure 2/B and this is usual in nano composites

chitosan and in this research is due to the nano combination (CTn-SO/SeNPs). A few particles seem clusterized by condensing together to form a new and larger mass. This happens possibly due to high surface energy in at least one of the nanoparticle containing component, which results in attraction of the particles, causing coagulation.

From SOM analysis, it could be noted that the surface of the particles seems to be relatively rough, as is characteristic of chitosan because it is biopolymeric in nature [12,36].

Biochemical bonding of FTIR analysis

The FTIR spectra of SO, SO/SeNPs and CTn-SO/SeNPs mixtures were presented to highlight the primary organic bonding and groups within Sage with their possible combination with SeNPs and NC preparation (Fig.3).

The prominent oscillation groups identified in FTIR spectra of SO have peaks at 1464 cm^{-1}, indicating the stretching oscillation of N–H bonds in proteins, at 1393 cm^{-1}, indicative of C–N bonds in aromatic amino group, at 1094 cm^{-1}, associated with carbonyl C–O–C or C–O extension of amide bonding, at 1048 cm^{-1}, is a carbohydrate indication, and at 879 cm^{-1}, representing the extension oscillation of CH_2 bonds in ethyl alcohol. Other significant peaks included those at 3406 cm^{-1}, caused by to extension oscillation of hydroxyl group (–OH) present in phenolic compounds. Additional peaks were observed at 2976 cm^{-1}, corresponding to the uneven elongation of methyl group ($–CH_3$), and at 2931 cm^{-1}, related to C-H bonds elongation in alkanes or secondary amines. Noteworthy peaks were also observed at 1653 cm^{-1}, indicate extension oscillation of C–C linkages in flavonoids and terpenoids, as well as carbonyl group (C–O) in amides either proteins that refer to extension oscillation [37,38].

The FTIR inspection of the formed SeNPs presented in Fig.3 shows peaking at 3434 cm^{-1} correlates to the stretching of O–H bonds in phenolic compounds or ethanol. Peaks at 2938 and 2977 cm^{-1} represent elongation of C–H linkages in alkynes. There is a band at 1571 cm-1 indicates the asymmetric stretching of N–O bonds in nitrogen containing complexes compounds. Another group at 1426 cm^{-1} is attributed to the stretching of C–C bonds (in rings) of aromatics. The peaked linkage at 1375 cm^{-1} is associated with the bending of CH bonds in alkanes, while peaks at 1077, 1044, together with 1012 cm^{-1} corresponding the stretching of C–N bonds in amines. A peak at 923 cm^{-1} signifies the bending of O–H bonds in carboxylic acids. Peaks at 817 and 649 cm^{-1} indicate elongation of C–C bonds in alkyl halides, while weaker bands at 510 together with 461 cm^{-1} result from the bending of C–N–C bonds in aminated groups. These findings suggest the involvement of several functional groups that could play roles in the assembly and balancing of the SeNPs [39,40].

It was stated in literature that substituent group (–COO) of SO can react with the aminated group ($–NH_3$) of CTn through ionic bonding, into which an anionic complex between the two compounds forms. The typical vibrational absorption bands highlighted in Fig. 3 consists of significant bands in the range of 1800 and 700 cm^{-1}. The bands found in 1605, 1520 and 1445 (cm^{-1}) are the indications of the aromatic ring stretching in the molecule. Strong support for the phenolic groups is the band at 1360 cm^{-1} which originates from the OH stretch, and the one at 1180 cm^{-1} (C–O stretch). The peak at 1684 cm^{-1} would be caused by the shifted linkages that exist because the extended carboxylic acid group and ester groups are present, which usually appear from 1725-1750 cm^{-1}. Also, In Fig. 3, a few peaks were observed to have lost some of their intensity following the preparation of the mixture. The relatively narrowing of the distinctive bands measured by FTIR observation may be ascribed to the partial retention of antioxidants that have not yet been released from the nanoparticles [35,37].

Sensory testing

During visualizing the transformations in different characteristics like those depicted in the Fig. 4 given below and by adhering to the statistical analysis of the data, it was discovered that the scores for the treatments differ drastically based upon the storage time, the treatment, or both the two factors depending upon the experiment.

The Tukey multiple comparison tests have given a statistic result which shows that day zero was the day with the highest scores for all attributes. The trends gradually changed throughout all treatments, including the control group, over the 25-day period, with variations in scores observed on the days when the test was conducted.

From the beginning of the study, the scores were always above 9 on the first day. Later they began to decline progressively. On the 8^{th} day, the score for the C group declined significantly while the drop in the S, SS and M groups was only negligible, with the SS group experiencing the least decrease. Out of these, on the 16th-day, the decrease in the C group was very substantial, while the decreases in the other groups were comparatively minor with slightly more decrease in the S group but not much in the M group and SS group. By reaching day 25 of the investigation, the C group developed the first signs of spoilage, scoring less than 4. But the other groups were within the boundaries, the SS group getting the highest score and the M group second and the S group third.

The results shown in Fig. 5 showed that the texture and the color also followed similar traits to

that of the general acceptance among all the treated groups. Significantly, the color was superior to the SS group in the whole process, probably due to the formation of orange SeNPs that gives a significant appearance to SS group throughout the experiment. Also the newly formed composite of CTn-SO/SeNPs and SO/SeNPs of M and SS groups respectively had the best texture results and this may be due to the effect of nanoparticles on this attribute as stated previously in researches that nanoparticles decrease adhesiveness and gumminess of fillet, and this may return to the antimicrobial effect of the nanoparticles that produce substances lead to changes in textural properties. This is the cause of the better textural properties in nanoparticles treated groups [11,12,41].

In this instance, C group was refused due to odor and the flavor on the 25th day while the other groups continued to be acceptable during the whole experiment and were scored quite high. Furthermore, this might be related to the distinct aromatic property of Sage.

From the above results it can be wrapped up for the odor, flavor, altogether with overall suitability of C group is rejected by the panelist at the end of the experiment whereas that of S, SS, and M groups is favorable and satisfactory for an extended period and has a superior degree of sensorial properties when analogized with C group. This finding suggests that the fillet could maintain its freshness for whatsoever extend has been subjected to experimentation beyond the 25 days.

pH

In fish postmortem state, the general pH normally appears between 6.0 and 6.8, as cited in various studies. Some literature declares that the pH value around 7.0 is own to fresh fish [3,42].

The pH level increased markedly in C group throughout the experiment. At the 8th day, the values remained the same in both S & SS groups, with a slight increase in M group. At the 16th day, SS group had insignificant increase while S group had slight increase, whereas M group had insignificant decrease in value. At the 25th day, the increase in S and M group was clear, while in SS group was way less than in the previously mentioned groups.

The rise of pH in fish fillet might have its roots in several effects: microorganisms, catalytic enzymes activities, and chemical processes become active after the death of the fish.

However, an increase in pH can be predominately ascribed to the microbial activity, in which certain bacteria were able to degrade muscle glycogen into lactic acid during the earliest stage of spoilage, consequently accelerating a decrease in pH (acidification). Nevertheless the microbes that decompose the organic waste may produce ammonia and some alkaline substances as the decomposition goes on, hence increasing the pH value of the wastes. Apart from that, the hydrolysis of protein, represented by the group of proteolytic enzymes, can also partake in the changes of pH value by the conversion of proteins into basic amino acids. Along with that, chemical reactions take place and produce alkaline compounds that also increase this pH value [43].

The cause of dropping of pH in the M group at 16th day may be attributed to SO/SeNPs release from the NC which is capable of reducing pH of fish fillet. On top of that, the mixture has substances that affect pH like phenolic compounds, which are known as acids. Nevertheless, the magnitude of pH decrease will depend on the concentration of the extract employed as well as the period of application [44].

Chemical testing

Tolerable threshold for TVB-N in fish fillet is noted as 35 mg/100 g, stemming from proteinous compounds breakdown caused by bacterial activity or autolytic enzymes [3,45].

As presented in Table 2, TVB-N concentration in C group was significantly raised when analogized with that of the other treatments; the elevation was observed beginning from the 8th day onward until the terminal of investigation period. The values continued to fluctuate staying just below the unfit limit up to the 16th day. The investigation persisted till the 25th day and by this time it crossed the admissible standard and the product for consumption was unsatisfactory in C group. The change in M group was observable starting from the 16th day onwards till the terminal of investigation with complete convenience regarding product consumption; all estimates in this group remained well within the admissible standard. In case of SS group, the estimate rose on the 25th day, but it never came close to the admissible standard. As such, those outcomes can suggest that CTn-SO/SeNPs mixture played a role in slowing down TVB-N rates of increase while on the other hand SO/SeNPs seemed to considerably slow down TVB-N rates of increase which were kept close to fresh estimates.

Upon closer examination of the treated groups over the course of the experiment, it becomes evident that the S group exhibits the lowest increase. This outcome is surprising, as it was initially anticipated that the SS or M group would demonstrate the lowest values due to the presence of SeNPs or CTn loaded with SO/SeNPs, that are admitted to have free radical scavenging altogether with germicidal properties, potentially inhibiting the production of TVB-N [5,12,46]. A previous study mentioned that the optimal concentration of CTn for the maximum effect on freshness is 2% which is more than the used concentration in this study, thus the raise in M group maybe due to the low concentration of CTn used in the treatment [47]. Also, chitosan is rich in amino group

in its chemical composition, this maybe an additional cause for the slightly higher level of TVB-N throughout the experiment [48]. The cause for using NC in combination with SO/SeNPs is that NC is suggested to limit the trace possibility of toxicity by SeNPs and this may be related to its adsorption and chelation ability and thus being the safest option with maximum profitability [49–51]. Nevertheless, more investigation is required for comprehensively explicating any potential indirect effects of SeNPs and CTn-SO/SeNPs mixture on TVB-N levels, especially in distinct food matrices and under varying storage conditions. With more reinforcement of the role of nanotechnology in causing this effect, as previous results demonstrated that different nanoparticles constrict the raise in TVB-N levels [3,45].

Fish lipids, containing PUFA, are prone to deterioration, leading to TBARs release. The acceptable threshold for TBARS is ranging from 1-2 mg malondialdehyde/kg and may extend to 4.5 mg malondialdehyde/kg [3].

The TBARS levels increased in all groups throughout the experiment, with the most significant increase observed in the C group. The SS group exhibited the lowest increase, followed by the S group then M group. This could come from the free radical scavenging merits of SeNPs, SO, and CTn, which reduce the likelihood of oxidative rancidity in the treated fillet [9,12,52–55].

The variance in the permissible threshold of TBARS observed in previous studies impacts the level of acceptability. Hence, the results presented in Table 3 are cross-referenced with the findings from the organoleptic examination and those in Table 2. It is notable that while samples from C group were rejected on day 25, those from the remaining treated groups remained acceptable until the terminal of investigation. This suggests that, concerning this research, the admissible limit for TBARS is set at 1 malondialdehyde/kg.

Psychrophiles Counting

Psychrophiles grow well in cold environments especially in the required temperature range for fish storage used in refrigerators, which in turn decreases the grade altogether with the period till expiration of chilled fillet. These bacteria grow and release enzymatic catalysts which disintegrate proteinous compounds and fats which produce undesirable characteristics in the taste, appearance, and smell, and subsequently, the flesh become unfit for consumption. Moreover, they generate amines as their volatile compounds, and that negatively affects the taste and odors of fish. Moreover, psychrophilic bacteria may generate biogenic amines provoking health issues if consumed in large quantities [34,56]. It was concluded that psychrophilic bacteria, respectively, should have a 4 log CFU/g upper threshold [56].

In terms of acceptability, the level of psychrophilic bacterial count isn't dependable because the limit of permissibility is not set as mentioned in previous studies, but there must be a comparison between the results within Table 4 and these results with those in Table 2 and 3. The refusal was on the 25th day in C group and no rejection in the rest of the groups till the end. With a closer look to Table 4 we find that there's a gradual increase in the log count in C group that is much lesser in S and SS group, and that suggests the bacteriostatic effect of both SO and SeNPs [6,55].

Whereas in M group there's a sudden decrease in the level of Log count that suggests the bactericidal effect resulting from the synergism between SO/SeNPs and NC [9,52]. This result is interesting as by comparing it with the results in table 2 and 3 which show that M group had the highest raise in TVB-N and TBARS estimates in the treated groups in comparison to control group. This may indicate that the increase might be due to the autolytic activity, chemical reactions in addition to the chemicals resultant from the minor microbial activity rather than the action of the microorganisms itself [45].

Biopreservation methods employing green plant extracts, polymers originate from biological sources, and biologically composed NPs were demonstrated to elongate the time till expiration of products and enhancing the overall quality of fisheries products. These findings align with the objectives of this investigation and underscore their relevance in fish processing industry.

Present study therefore holds out a lot of prospects for the industrial application of NP treatments regarding fish fillet preservation, including the employment of SO, SeNPs and their composites. The result of this study shows that those treatments have positive impact on the shelf life and quality of catfish fillets and this has certain potential of commercial application. Outlining the industrial implications and considerations for scalability are well-documented and assured.

The incorporation of natural extracts as well as green synthesis methods supports the increased concern towards environmental and economic friendly ways of food preservation. This can help to improve the marketability NP-treated seafood products to consumers who are aware of the impact of their purchases on environment

Conclusion

Innovative biosynthesis of SeNPs using SO and their nanoconjugation with CTn were achieved in current research. The application of SO, SO/SeNPs and CTn-SO/SeNPs mixture significantly prolonged catfish fillets' shelf life and enhanced their quality

compared to untreated samples. Regarding general acceptance, TVB-N, TBARS estimates and psychrophiles counting the preserved products remained fit for consumption for more than 25 days even under different treatments. Additionally, this demonstrates the validity of SO, SO/SeNPs and CTn-SO/SeNPs usage as applicable in maintaining seafood products particularly catfish fillets. The biological origin of screened materials (e.g. SO and CTn), with their elevated biosafety and minimal toxicity, could provide effectual nano-formulations with high applicability, scalability and industrial implications. Based on the attained findings, it is suggested to examine more biosynthesized nanomaterials and further biopolymers as edible coating for seafood products. The biosafety assessments are also suggested for determination in future work.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The design of the study and execution received formal validation and approval from the "Aquatic Animal Care and Use in Research Committee, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt, "approval number IAACUC-KSU-30-2019".

TABLE 1. The concentrations of phenolic and flavonoids (*μ*g/g) in sage herbal extract (SO).

Phenolic and flavonoid compounds	Concentration in SO (*μ*g/g)
Gallic acid	1859.05
Chlorogenic acid	2274.27
Methyl gallate	143.88
Caffeic acid	608.00
Syringic acid	1090.09
Ellagic acid	210.52
Vanillin	2995.28
Ferulic acid	2329.55
Naringenin	521.42
Quercetin	2388.44
Cinnamic acid	157.57
Kaempferol	3801.84
Hesperidin	161.71

TABLE 2. The TVB-N values of fillets treated groups (S, SS and M groups), compared with the control (C) group, over the refrigerated storage period*

Experimental days	TVB-N (mg/100 g)			
	C	S	SS	M
Zero day	15.690 ± 0.067^{Aa}	ND	ND	ND
8th day	16.667 ± 0.88^{Ba}	15.690 ± 0.067^{Ab}	15.690 ± 0.067^{Ab}	15.833 ± 0.033^{Ab}
16th day	18.747 ± 0.048^{Ca}	14.057 ± 0.088^{Bb}	14.967 ± 0.371^{Ac}	16.667 ± 0.273^{Bd}
25th day	38.990 ± 0.107^{Da}	12.667 ± 0.203^{Cb}	16.400 ± 0.265^{Bc}	18.233 ± 0.145^{Cd}

*Distinct uppercase letter within each column signifies a key difference, whereas changeable lowercase letter within each row means the presence of a key difference, when the p-value is < 0.01.

TABLE 3. The TBARS values of catfish fillet treated groups (S, SS and M groups), compared with the control (C) group, over the storage period at 2°C.

Experimental days	TBARS (malondialdehyde/kg)			
	C	S	SS	M
Zero day	0.430 ± 0.010^{Aa}	ND	ND	ND
8th day	1.353 ± 0.009^{Ba}	0.480 ± 0.017^{Ab}	0.430 ± 0.021^{Ab}	0.657 ± 0.049^{Bc}
16th day	1.663 ± 0.030^{Ca}	0.690 ± 0.017^{Bb}	0.570 ± 0.017^{Ab}	0.810 ± 0.035^{Bc}
25th day	1.757 ± 0.078^{Ca}	0.810 ± 0.035^{Bb}	0.677 ± 0.009^{Ab}	0.937 ± 0.030^{Bc}

*Distinct uppercase letter within each column signifies a key difference, whereas changeable lowercase letter within each row means the presence of a key difference, when the p-value is < 0.01.

TABLE 4. The psychrophiles counting (log CFU/g) of catfish fillet in S, SS and M groups, compared with the control (C) group, over the storage period at 2°C.

Experimental days	Log CFU/g			
	C	**S**	**SS**	**M**
Zero day	3.200 ± 0.000^{Aa}	ND	ND	ND
8th day	4.800 ± 0.058^{Ba}	3.367 ± 0.088^{Ab}	3.267 ± 0.033^{Ab}	1.200 ± 0.058^{Ac}
16th day	5.367 ± 0.088^{Ca}	4.000 ± 0.100^{Bb}	3.600 ± 0.058^{Ac}	2.633 ± 0.033^{Bd}
25th day	6.500 ± 0.115^{Da}	5.133 ± 0.120^{Cb}	4.833 ± 0.088^{Bb}	3.967 ± 0.120^{Cc}

*Distinct uppercase letter within each column signifies a key difference, whereas changeable lowercase letter within each row means the presence of a key difference, when the p-value is < 0.01.

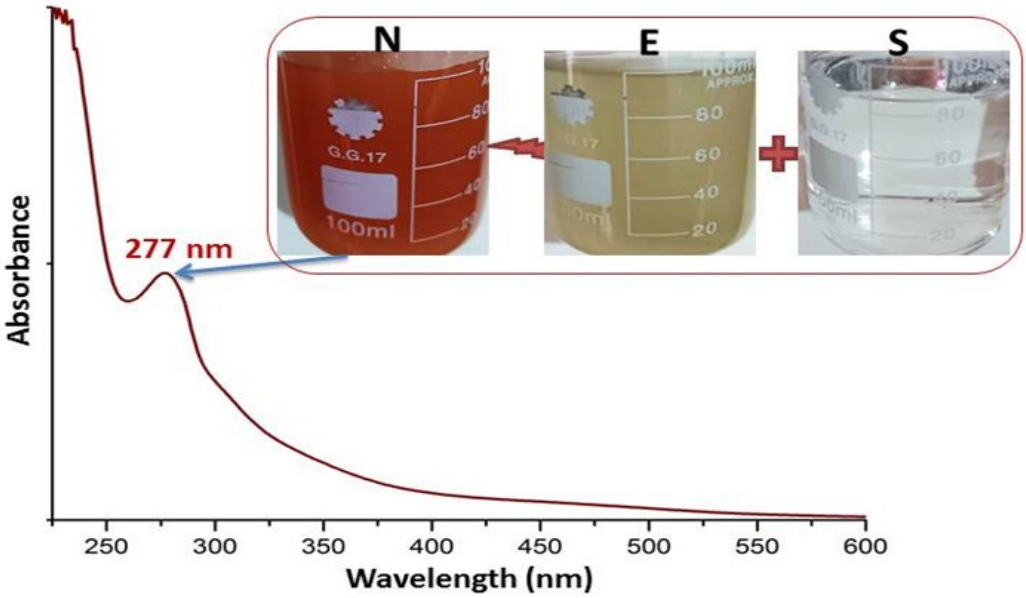

Fig. 1. Color indicator and UV visual spectrum of sage extract-mediated SeNPs, indicated the optical change of selenite solution (S) into SeNPs (N) after reduction with sage extract (E), where the curve appoints the maximum absorbance of Sage-SeNPs solution.

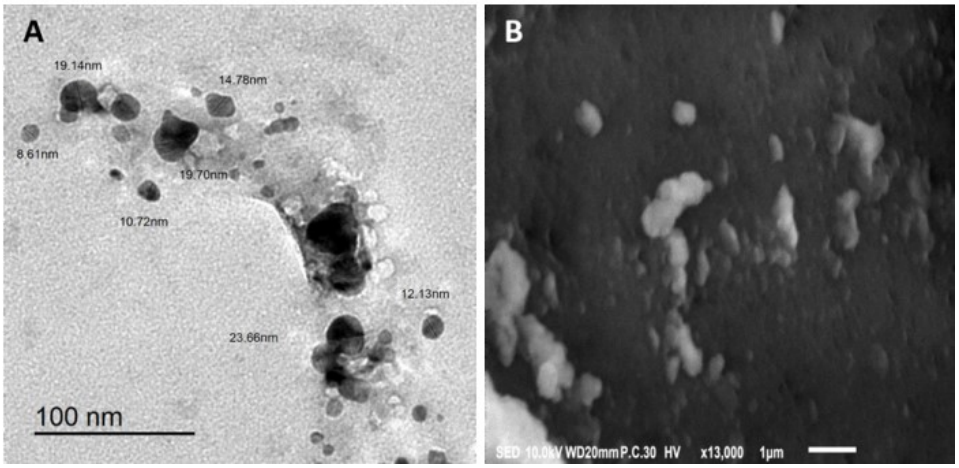

Fig. 2. TEM imaging of SeNPs prepared in SO (A) & SOM imaging of CTn-SO/SeNPs (B)

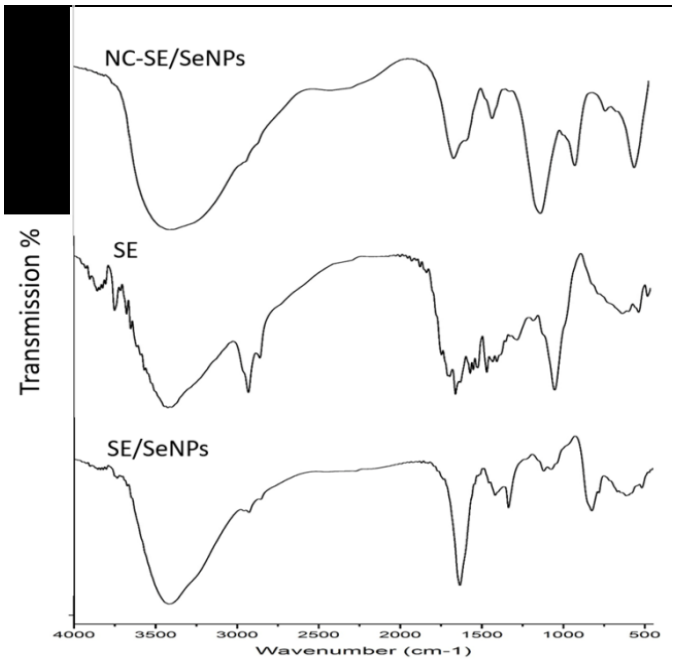

Fig. 3. FTIR spectra for Sage, Sage SeNPs & Sage with SeNPs loaded o Nanochitosan preparations

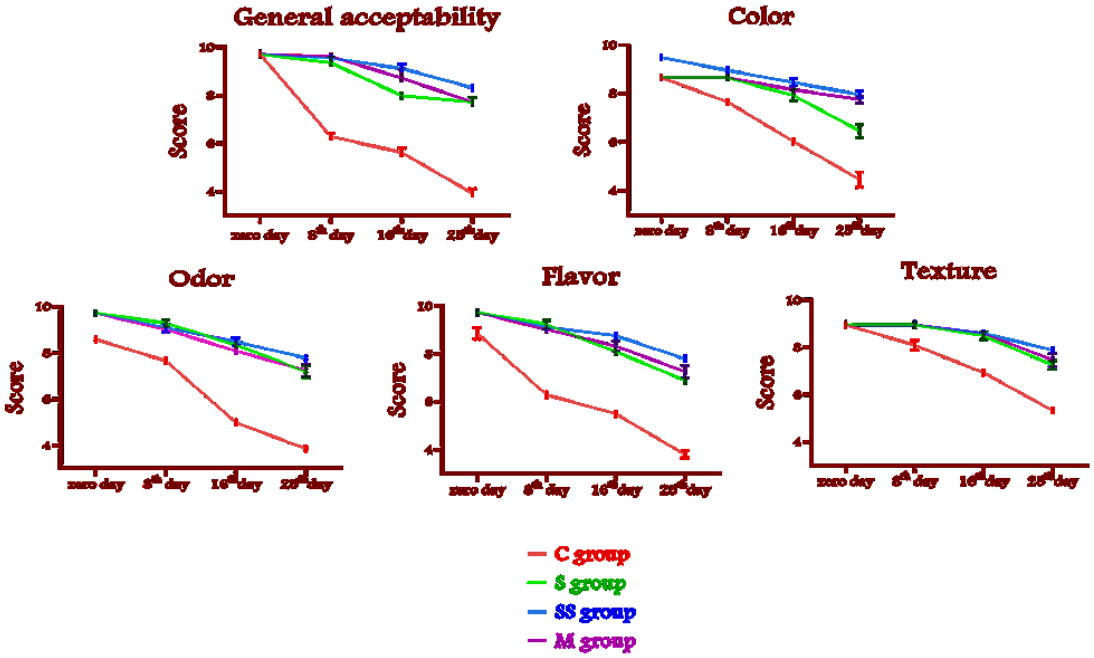

Fig. 4. Mean of sensory attributes of catfish fillets groups, including (C): untreated group, (S): treated with sage extract, (SS): Sage extract with Selenium Nanoparticles and (M): treated with Sage extract/ SeNPs loaded on nano-chitosan mixture

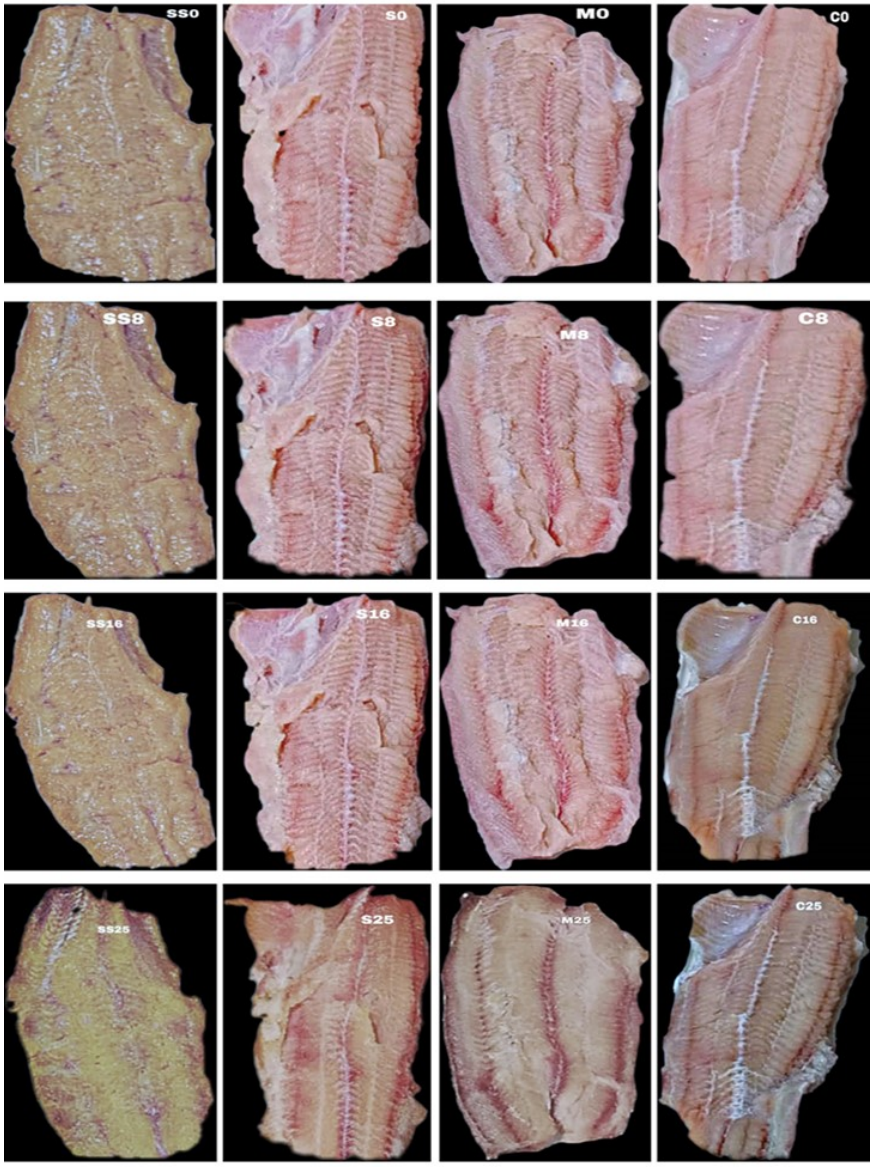

Fig. 5. The variation of appearance and color in catfish fillets groups, including (C): untreated group, (S): treated with sage extract, (SS): Sage extract with Selenium Nanoparticles and (M): treated with Sage extract/ SeNPs loaded on nano-chitosan mixture, during 25 days of storage at 4 °C.

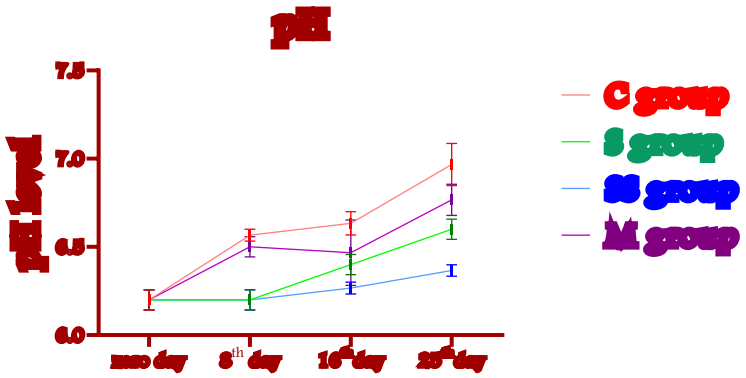

Fig. 6. Means and SD of pH values of catfish fillets groups, including (C): untreated group, (S): treated with sage extract, (SS): Sage extract with Selenium Nanoparticles and (M): treated with Sage extract/ SeNPs loaded on nano-chitosan mixture, during 25 days of storage at 4 °C

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تحسين حفظ شرائح سمك القرموط الأفريقي باستخدام النانو-سيلينيوم المعزز مع المريمية والنانوكيتوزان: التآزر لتعزيز الطزاجة

منار مجدي شهاب ، منى عبد الصمد عسس ، ابراهيم ابراهيم الهواري و أحمد عبد الفتاح طايل

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الملخص

تكتسب أسماك القرامبط الأفريقية اهتماماً متزايداً نظراً لأهميتها الغذائية وسهولة تكاثرها، إلا أن الحفاظ على جودة منتجاتها يُعد تحدياً. تم استخدام مستخلص بتلات نبات المريمية في تحضير جزيئات السيلينيوم النانوية باستخدام إجراء مباشر وسهل. تم دمج جزيئات الكيتوزان مع خليط من مستخلص المريمية وجزيئات السيلينيوم النانوية، وتم توصيف المركبات النانوية الناتجة باستخدام التحليل الطيفي والمجهر الإلكتروني. أظهر مستخلص المريمية، بمحتواه من المركبات الفينولية، فعالية في تحضير جزيئات السيلينيوم النانوية التي بلغ متوسط حجمها 13.48 نانومتر. تم تحقيق وتأكيد الترابط النانوي للمركب باستخدام التحليل بالأشعة تحت الحمراء والمجهر الإلكتروني الماسح. أظهرت عملية الحفظ الحيوي لشرائح سمك القرامبط باستخدام المواد النانوية المصنعة نتائج واعدة للغاية أثناء تخزين العينات المحفوظة في المعاملات لمدة خمسة وعشرين يوماً. كانت أكثر المعالجات الواعدة هي المغمورة بمحلول بنسبة 0.5% من مستخلص المريمية مع جزيئات السيلينيوم النانوية ومدمجاتهم مع النانوكيتوزان للحفاظ على الخصائص الحسية للشرائح (القبول، اللون، الرائحة، النكهة والملمس) طوال فترة التخزين. كان غمر الشرائح بالمركبات النانوية قادراً على تقليل تقدم معايير التلف مثل الأس الهيدروجيني أو pH، النتروجين الأساسي المتطايرأو TVB-N، حمض الثيوباربيتوريك التفاعلي أو TBARs وأيضا عد البكتيريا المسببة للفساد؛ فظهر التأثير بشكل كبير مقارنة بالعينات التي لم يتم معاملتها. قدمت جزيئات السيلينيوم النانوية المحضرة بمستخلص المريمية ومركباتها النانوية مع الكيتوزان أسس فعالة كمواد حفظ قابلة للأكل لحفظ شرائح سمك القرامبط والحفاظ على جودتها.

الكلمات الدالة: التخليق الحيوي، القرامبط، المواد النانوية، المنتجات الطبيعية، حفظ المأكولات البحرية.