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Investigation of Antimicrobial and Antioxidant properties of Green Tea Extract Encapsulated in Chitosan-Citrate Nanogel on shelf life of Rainbow Trout (*Oncorhynchus mykiss*) Surimi

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ABSTRACT

In the interest of increasing demand for natural foods free of artificial preservatives, this study aimed to control the antioxidant properties of green tea extract (GT) by encapsulating it in chitosan nanoparticles (CS-NP) and investigate its preservative effects on surimi. The results demonstrated that GT-loaded chitosan nanoparticles (CS-NP-GT) were significantly effective in reducing lipid oxidation in surimi, as determined by Thiobarbituric acid and free fatty acid analysis. Chemical, microbial, and sensory analyses of surimi treated with CS-NP-GT showed a significant difference compared to other treatments ($p < 0.05$). Over the storage period, surimi treated with CS-NP-GT exhibited a reduction of 2.6 log cycles in lactic acid bacteria, 2.55 log cycles in *Enterobacteriaceae*, 4.32 log cycles in aerobic mesophilic bacteria, and 2.61 log cycles in mold and yeast. Additionally, in sensory evaluation, E-0.1-GT received a higher score on the ninth day of storage compared to surimi prepared with other treatments. These findings indicate that encapsulating green tea extract with chitosan nanoparticles is a promising technology for controlling chemical, microbial, and sensory changes in surimi, thereby extending its shelf life.

1- Introduction

Lipid oxidation and microbial spoilage are the primary degradation processes observed in rainbow trout (Zeng *et al.*, 2023). Due to the adverse effects associated with the use of artificial preservatives, such as mutagenesis, carcinogenesis, and poisoning, there has been a growing demand for natural food products. Consequently, research has focused on exploring natural antioxidant and antimicrobial compounds (Safari *et al.*, 2018). The leaves of the tea plant *Camellia sinensis L.* contain polyphenolic components and flavonoids, which exhibit potent antioxidant and antibacterial properties (Phuyal, 2023). Among the polyphenols found in green tea, catechins are the main constituents responsible for their antibacterial activity against both gram-positive and gram-negative bacteria, as well as fungi (Desiriani *et al.*, 2023).

Catechins can also prevent lipid oxidation in food by neutralizing peroxyl radicals and inhibiting the lipid oxidation process (Vuong *et al.*, 2011). The presence of orthodihydroxyl groups and hydroxyl moieties at positions 3', 4', and 5' on the β-ring within the catechin molecules contributes to their antioxidant and antimicrobial activities (Sabaghi *et al.*, 2020; Sabaghi *et al.*, 2015). However, catechins in their free form suffer from chemical instability, low bioavailability, poor absorption, and loss of antibacterial and antioxidant capabilities, rendering them unsuitable as natural preservatives in food (Bora *et al.*, 2018).

Previous studies have demonstrated that encapsulation can enhance the bioactivity and bioavailability of polyphenols (Bagheri *et al.*, 2016). Several nanoencapsulation techniques for green tea extract have been proposed in the literature, and the use of catechins has proven effective in extending the shelf life of stored products. Nanogel carriers, particularly those made from natural polysaccharides and proteins like chitosan, have emerged as a prominent delivery strategy in the fields of food, biology, and drug delivery. Chitosan, known for its biocompatibility, biodegradability, low toxicity, and minimal immunogenicity, has also shown strong antibacterial effectiveness against various foodborne pathogens (Darmadji and Izumimoto, 1994; Kamil *et al.*, 2002; Shahidi *et al.*, 1999). Catechins have been successfully encapsulated with chitosan polymer in various nano-systems, including tubes (Wang *et al.*, 2021), hydrogels (Dahiya *et al.*, 2017), particles (Gómez-Mascaraque *et al.*, 2019; Kaur *et al.*, 2017; Li *et al.*, 2018), and others. However, to the best of our knowledge, no study has investigated the nanoencapsulation of green tea (GT) using citrate polyanion and chitosan polymer. Therefore, this study aimed to synthesize chitosan-citrate nanogel and evaluate the effects of GT, GT-loaded CS nanoparticles (CS-NP-GT), and CS nanoparticles (CS-NP) on the shelf life of rainbow trout surimi.

2- Materials and methods

Chitosan (75–85% deacetylation, low molecular weight), N-hydroxysuccinimide (NHS), and 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide (EDC) were provided by Sigma-Aldrich Chemical Co. Other chemicals and culture media used in this research were provided by Merck, Germany.

Preparation of chitosan nanoparticles containing green tea extract

The Darras et al. method was utilized to make modified chitosan nanoparticles (Darras *et al.*,

2010). The chitosan powder was dissolved in distilled water containing hydrochloric acid and then sonicated for 20 minutes at room temperature with a 60 percent maximum power ultrasonic homogenizer (Hielscher ultrasonic, Germany). Further, the solution containing 0.1 gr of citric acid was dissolved in distilled water, 0.1 gr of EDC, and 0.1 gr of NHS were added to the chitosan solution at room temperature stirring at 250 rpm for 2 h to form a covalent bond between the $NH3^{+}$ groups of chitosan and the COO^{-} groups of citrate, the pH of the CS-citrate solution was set at around 7 by precipitating chitosan-citrate with diluted soda. To eliminate impurities, the precipitate was centrifuged at 9000 rpm for 18 minutes and washed three times with ethanol and once with distilled water. To load the green tea extract in the chitosan nanoparticles, the nanogel was dissolved again by setting pH at 4 and the extract was added dropwise to the chitosan-citrate nanogel while sonicating (Piran *et al.*, 2020).

Properties of nanoparticles

The size, zeta potential, and scattering coefficient of nanoparticles were determined using chitosan nanoparticles suspension containing green tea extract (CS-NP-GT) and chitosan nanoparticles (CS-NP). To eliminate cohesive particles, each of these suspensions was first put in an ultrasonic bath for one minute. The particle size, PDI index, and zeta potential of CS-NP and CS-NP-GT were then examined using a Nano Malvern ZS (Malvern Instruments, United Kingdom) equipped with a He-Ne laser at 633 nm wavelength and a 90° fixed scattering angle (Ghahfarokhi *et al.*, 2016).

Rafiei et al.'s method was used to determine encapsulation efficiency (Rafiee *et al.*, 2017). To do this, CS-NP-GT was centrifuged to separate the phenolic components of green tea extract loaded from free phenolic compounds using a centrifuge (Sigma, Germany). The volume of primary phenolic compounds and the volume of unloaded polyphenolic compounds were then measured using the Folin – Ciocalteau technique . The following formula was used to calculate the percentage of encapsulation efficiency:

$$(1)\quad \%\ \text{Encapsulation Efficiency} = \left(\frac{C_{intial} - C_{sup}}{C_{intial}}\right) \times 100$$

C_{sup} is the volume of unloaded polyphenolic compounds in the liquid after centrifugation, and $C_{initial}$ is the initial volume of GT phenolic compounds utilized to make CS-NP-GT.

To investigate the In vitro release of the extract from nanoparticles, chitosan nanoparticles containing green tea extract were first centrifuged. In the next step, the tubes containing the sample were kept in a shaker for 120 hours at 200 rpm. During this period, a specified volume of samples was taken and replaced at predefined intervals with an equal amount of fresh buffer media. The Folin-Siocalto technique was used to determine the quantity of extract, and the cumulative release percent of the extract from nanoparticles was determined using the equation below.

$$CR\ (\%) = \sum_{t=0}^{t} \frac{M_t}{M_0} \times 100 \qquad (2)$$

CR is the cumulative release rate in percentage, M_t is the amount of extract (phenolic compounds released) at time t, t is the time of sampling, and M_0 is the amount of extract loaded in the prototype (M Ghaderi-Ghahfarokhi *et al.*, 2017).

Surimi preparation and treatments

Rainbow trout (*Oncorhynchus mykiss*) were purchased from a fishmonger and transported to the

lab on ice. The fish were then processed into fillets by removing the offal, shells, and bones. After rinsing the fillets with cold water, they were ground using a Meat Mincer (Panasonic MK-G1800, Japan) equipped with a 3 mm pore diameter disk. The ground meat was washed twice with cold water, with each wash using an amount of water four times the weight of the minced fish meat. In the second wash, 0.1 percent sodium chloride was added to enhance water drainage. Following the washing process, the fillets underwent a final drying stage to eliminate any remaining moisture (Park, 2005).

The experimental treatments included a Control (no additive treatment), F-0.1-GT (treatment containing 0.1% free green tea extract), 0.1-CS-NP (treatment containing 0.1% chitosan nanoparticles), and E-0.1-GT (treatment containing 0.1% encapsulated green tea). All analyses were conducted over a period of 9 days during refrigerated storage at 4 °C.

chemical Analysis

pH Analysis

10 g of rainbow trout surimi was completely homogenized and filtered using 100 ml of distilled water while stirring at 1000 rpm. The pH was measured at room temperature using a pH meter (Istek, South Korea) as described by Goulas and Kontominas (2005).

Determination of Peroxide Value (PV)

To measure the peroxide number, we must first extract the oil from the rainbow trout surimi samples. Surimi oil was extracted using chloroform, methanol, and water according to Bligh and Dyer's (1959) method (Bligh and Dyer, 1959). In a shaker, 20 g of rainbow trout surimi was mixed well with 100 ml methanol and 40 ml chloroform. After that, another 40 ml of chloroform was added and mixed for another two minutes. Then 80 mL of distilled water was added, and the mixture was agitated for another 2 minutes. The oil was separated by centrifugation, with the lower phase comprising oil and chloroform. The solvent was separated using a rotating device. The surimi oil was extracted, then 30 ml of chloroformic acetic acid (3: 2), 0.5 ml of saturated potassium iodide solution, 30 ml of distilled water, and 0.5 ml starch solution were added to the Erlenmeyer. The amount of iodine emitted was then determined by titration with 0.01 N sodium thiosulfate solution. The amount of peroxide is calculated using the equation below (Egan and Sawyer, 1997).

PV = (1000 × normality × thiosulfate consumption volume) / (weight oil) (3)

Evaluation of total volatile basic nitrogen (TVB-N)

Goulas and Kontominas (2005) methods were used to determine total volatile nitrogen (Goulas and Kontominas, 2005). For rainbow trout surimi, 10 g of the product was blended with 50 ml of distilled water using a mixer. The solution was then transferred into a 500 ml balloon with 200 ml of distilled water, 2 g of magnesium oxide, and a drop of silicone oil. The titration was carried out using a 250 ml Erlenmeyer flask with 25 ml of 2% boric acid solution and a few drops of methyl red and methylene blue reagents at the end. The titration was completed when the distillation volume reached 125 ml.

% TVB-N (mg) = (V × C × 14 × 100) / 10 (4)

V is the volume of hydrochloric acid consumed and C is its concentration.

Determination of thiobarbituric acid reactive substances (TBARS)

The malondialdehyde-thiobarbituric acid (MDA-TBA) reaction is commonly used to detect lipid oxidation in meat tissue. The pink complex formed is measured with spectrophotometric analysis when one molecule of malondialdehyde combines with two molecules of thiobarbituric acid. First, 200 mg of rainbow trout surimi was placed in a 25 mL volumetric flask. To dissolve and mix the material, 1 ml of butanol was added. Then, the mixture was pipetted into a dry test tube containing 5 mL of TBA reagent (200 mg of 2-TBA in 100 ml of butanol, filtered, and kept at 4°C for less than 7 days). The contents of the test tube were thoroughly mixed using a vortex mixer. Afterward, the test tube was immersed in a 95°C water bath for two hours. Finally, after the fluid inside the test tube had cooled, a spectrophotometer set to 530 nm was used to evaluate and record the absorbance of the fluid.

The amount of TBARS (mg malondialdehyde/kg tissue) was obtained by the following formula (Ojagh *et al.*, 2010). As is the absorbance rate of a test tube solution, was measured at 530 nm against a water blank. A reagent blank was run and absorbance (Ab) recorded. TBA value (mg of malonaldehyde equivalents/kg of tissue) was obtained by the formula.

$$\text{TBARS} = \frac{50\times(\text{As}-\text{Ab})}{200} \quad (5)$$

Determination of Free Volatile Fatty Acids (FFA)

For FFA analysis, the lipid was extracted from rainbow trout surimi samples using chloroform: methanol (2:1 v/v) as detailed by Bligh and Dyer (Bligh and Dyer, 1959). The lipid extract was deposited in Pyrex tubes and all solvent was evaporated with nitrogen. Then 3 mL of cyclohexane was added, followed by 1.0 mL of cupric acetate-pyridine reagent with agitation of the biphasic system for 30 s. After centrifugation at 2000 g for 10 min, the upper layer was read at 710 nm using a spectrophotometer. The FFA concentration in the sample was calculated according to micromolar oleic acid based on a standard curve spanning a 2-14 micromoles range. (Bernárdez *et al.*, 2005).

Microbial Analysis

On experimental days, a 10 g sample of rainbow trout surimi was combined and homogenized with 90 ml of a 0.85% NaCl solution. The necessary dilutions were then prepared and used for microbial counting. After incubation, the results were converted to log10 colony-forming units (CFU) per gram of sample.

lactic acid bacteria (LAB)

The pour plate method was used to count lactic acid bacteria. The culture medium was De Man, Rogosa, and Sharpe agar (MRS), which was prepared according to the instructions and was cooled to 45 °C. The culture medium was added to sterilized plates containing 1 ml of sample dilutions and cultured for 48 hours after solidification at 35 °C (Maryam Ghaderi-Ghahfarokhi *et al.*, 2016).

Enterobacteriaceae

1 ml of sample dilutions was added to the Violet Red Baile glucose agar (VRBGA) medium. The

pour plate method was utilized for this purpose. After cooling to 45 °C according to the preparation instructions, the culture medium was applied to sterile plates containing 1 ml of sample. after solidification, the plates were incubated at 30°C for 24 hours. Then big colonies with purple halos were counted (Chytiri *et al.*, 2004).

mesophilic bacteria

Mesophilic counts were determined using Plate Count Agar (PCA). 0.1 ml of various dilutions were spread on the surface of dry media using an L-shaped bent rod across the surface of the culture medium. The plates were then incubated at 30° C for 48 hours (Chytiri *et al.*, 2004).

mold and yeast

Mold and yeast counts were performed on Yeast Extract Glucose Chloramphenicol (YGC) medium. Incubation was performed at 25 °C for 5 days (Maryam Ghaderi-Ghahfarokhi *et al.*, 2016).

Sensory evaluation

Sensory evaluation of rainbow trout surimi samples was conducted on days 0, 3, 6, and 9 by 20 people (10 females and 10 males, ages 35-25). The evaluation of raw samples was based on color, odor, and overall acceptability. The samples were sealed in nylon bags and evaluated immediately after opening. Cooked rainbow trout surimi samples were also evaluated for taste. The 5-point hedonic method described by Pesavento et al. (2015) was used (Pesavento *et al.*, 2015).

Statistical analysis

All measurements were replicated three times.The treatments include formulations (control, F-0.1-GT, 0.1-CS-NP and E-0.1-GT), the storage time and the intractions of these treatments in rainbow trout surimi samples were analyzed using analysis of variance (ANOVA). Duncan test at confidence level 95% was used to compare the means using IBM® SPSS® software version 23. Microsoft Excel software was used to draw graphs.

3- Results and discussion

Characteristics and release profile of nanoparticles

The first goal of this study was to fabricate chitosan nanoparticles and then use chitosan-citrate nanogel to encapsulate green tea extract. Nanoparticle size, zeta potential, and polydispersity index (PDI) are among the factors that are important in controlled release systems of bioactive compounds (Bulmer *et al.*, 2012). Theoretically, the ideal nanoparticle has the smallest size, highest zeta potential, and lowest PDI (Bulmer *et al.*, 2012). Table 1 shows the important properties of chitosan nanoparticle loaded with green tea extract.

Table 1 Some physicochemical characteristics of chitosan nanoparticle loaded with green tea extract (CS-NP-GT), (CS:GT raito 1:0.5)

Particle size (nm)	Zeta potential (mV)	Polydispersity index (PDI)	Encapsulation efficiency (%)
135.43 ± 2.52	40.40 ± 0.2	0.296 ± 0.00	68.37 ± 1.17

Green tea extract demonstrated a fast rate of release of nanoparticles in the early hours. This stage, so

called 'the burst effect'.The release mechanism at this stage can be attributed to the instantaneous release of the extract attached to the particle surface as a result of the slope of the extract concentration (Yoksan *et al.*, 2010). The burst release phase of chitosan nanoparticles was reported for tea polyphenols within 6 hours (Liang *et al.*, 2011) and ellagic acid within the initial 3 hours (Arulmozhi *et al.*, 2013) of release. After this stage, the phase of gradual release of the extract begins. Following this stage, the gradual release phase of the extract commences. Specifically, during this phase, the extract molecules permeate the surroundings from within the nanoparticles, which corresponds to the breakdown of the Polymer network of nanoparticles. (Agnihotri *et al.*, 2004). During this phase, the glass-polymer network undergoes a transformation into a rubber network, leading to the liberation of trapped molecules from the expanded rubber network into the surrounding environment. (Agnihotri *et al.*, 2004). In the final stage of release, the release rate is stable and the release curve becomes flat. Actually, the amount of extract being released is significantly diminished and can be considered insignificant at this point. The results show that not all of the encapsulated extracts in the nanoparticles are released at the end of release due to the inability of the buffer media to destroy or break the structure of the nanoparticles and release the extract (Garg *et al.*, 2011). Encapsulation of the extract into chitosan nanoparticles was able to achieve a sustained release within 120 hours. Previous studies have also reported the sustained release of tea catechins in water within a timeframe of 100 hours. (Hu *et al.*, 2008).

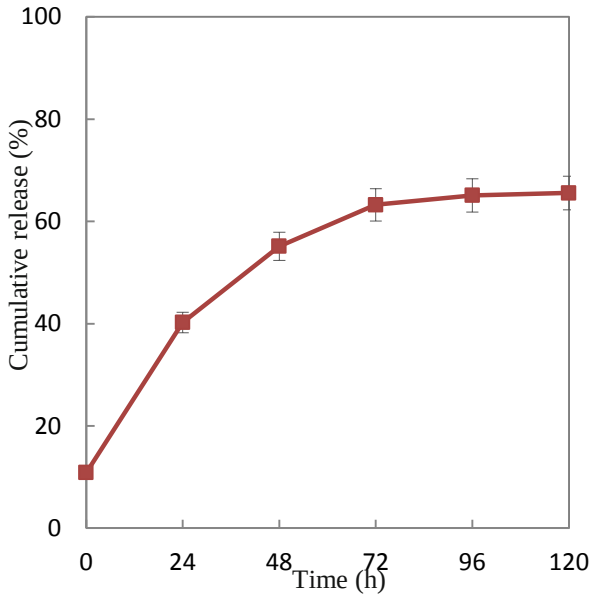

Fig 1 Release behavior of green tea extract from chitosan nanoparticles.

Chemical Changes During Storage

pH

As shown in Figure 2, there is no noticeable difference in the pH changes of the samples over time. The trend of increasing pH is quite obvious in the case of control samples of this trend. This increase in pH may be due to the production of alkaline compounds such as ammonia and trimethylamine by the activity of spoilage-causing bacteria (Fan *et al.*, 2008). From the third day onwards, 0.1-CS-NP and E-0.1-GT showed a decrease in pH, which is probably due to the release of the extract from the nanoparticles. The pH level of meat plays a crucial role in determining its quality and has a significant impact on the breakdown of connective tissue after death. The initial decline in pH is attributed to the activity of lactic acid bacteria. Conversely, as the meat ages, there is a natural increase in pH caused by microbial activity, which leads to spoilage. Phenolic compounds present in the extract elevate the protection of surimi against high levels of internal proteases. As a result, they inhibit protein breakdown and the production of amines (Shahhoseini *et al.*, 2019).

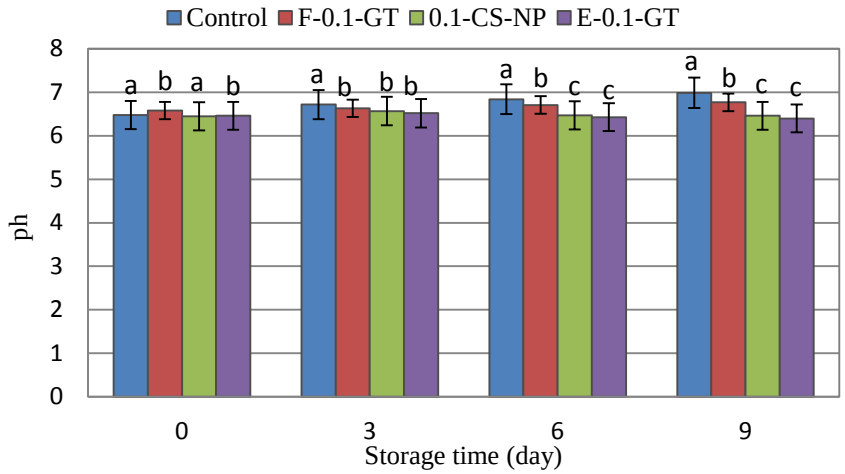

Fig 2 Changes in the pH of rainbow trout surimi samples with different formulation during 9 days of refrigerated storage in 4°C. Different letters in the same column indicate significant differences between treatments ($p < 0.05$).

Peroxide Value (PV)

The changes in peroxide content in mE/kg on days 0 to 9 in the rainbow trout surimi samples are shown in Figure 3, Peroxide values for the control sample increased significantly ($p < 0.05$) over time. At the end of storage time on the ninth day, the amounts of peroxide in each of the control, F-0.1-GT, 0.1-CS-NP, and E-0.1-GT samples showed a significant difference. Samples containing encapsulated green tea, free green tea, and chitosan nanoparticles had lower values, respectively. The results showed that the nanoencapsulated extract is effective in inhibiting peroxide production in the rainbow trout surimi product. Over time, the oxidation level rises as a result of the gradual release of greater quantities of free iron and other pro-oxidants. This release is caused by the increased breakdown of tissue during storage (Chaijan *et al.*, 2006).

The addition of free and encapsulated green tea has a beneficial impact on slowing down the rising trend of peroxide value. poly phenolic extracts possess the ability to neutralize free radicals by providing a hydrogen atom. This ability effectively delay oxidative spoilage in fillets (Hadidi *et al.*, 2022).

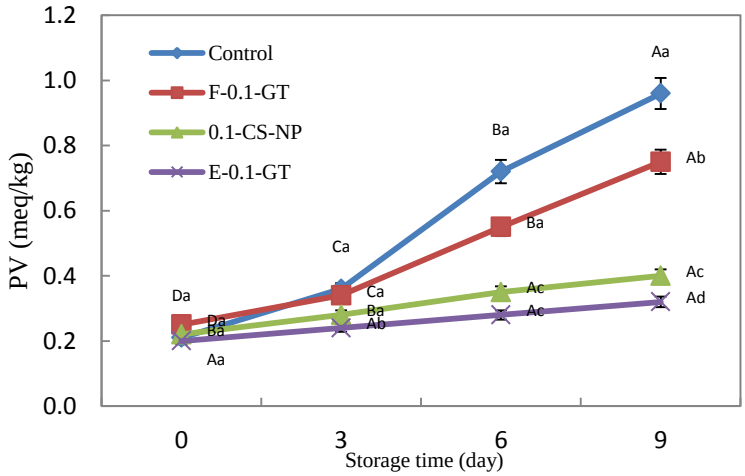

Fig 3 Changes in the PV value of rainbow trout surimi samples with different formulation during 9 days of refrigerated storage in 4°C. Different lower case letters have significant differences in different treatments and different uppercase letters have significant differences at different times ($p<0.05$).

Total volatile basic nitrogen (TVB-N)

Figure 4 depicts the changes in TVB-N for various days. The analysis of the variance of TVB-N and the interaction effects of green tea extract, chitosan nanoparticles, and encapsulated extract were significant ($p<0.05$) from the third day onwards. In general, the findings of comparing the means revealed that the amount of TVB-N in all treatments grew over time, with the control sample having the largest level at the end of the storage period. When the amounts of TVB-N in the E-0.1-GT treatment were compared to the other treatments at the end of the time, was considerably lower ($p<0.05$).

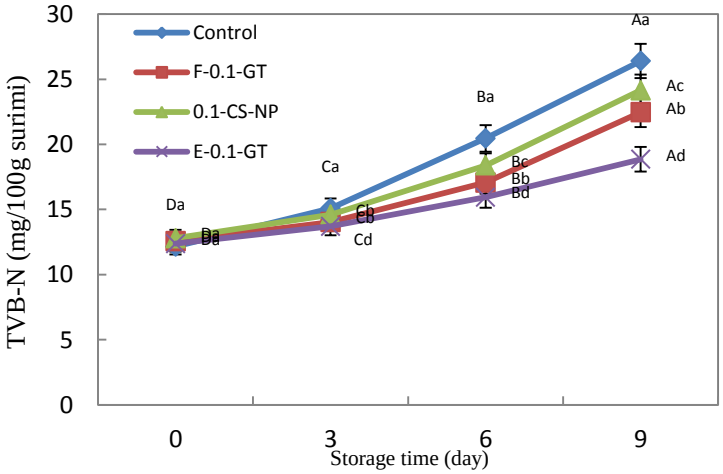

Fig 4 Changes in the TVB-N levels of rainbow trout surimi samples with different formulation during 9 days of refrigerated storage in 4°C. Different lower case letters have significant differences in different treatments and different uppercase letters have significant differences at different times ($p<0.05$).

TVB-N in fish is mainly composed of ammonia and primary, secondary and tertiary amines composed of degradation of proteins and non-protein nitrogen compounds due to microbial activity. Levels of 25-30 mg TVB-N per 100 g of fish muscle are usually concerned as spoiled (Fan *et al.*, 2008; Lopez-Caballero *et al.*, 2000). As shown in Figure 4, TVB-N values increased over time in all samples. The treatments F-0.1-GT, 0.1-CS-NP, and E-0.1-GT were within the acceptable range on the ninth day, while the control treatment exceeded the acceptable range by the end of the period. These findings are in line with the research conducted by Fan et al. as their results demonstrated consistency with regards to the treatment of silver carp using tea polyphenols. According to their findings, samples treated with tea polyphenols exhibited a reduction in TVB-N production. This reduction can be attributed to either the rapid decline in bacterial population triggered by the activity of tea polyphenols, or a decrease in the ability of bacteria to oxidize and deaminate non-protein nitrified compounds due to the presence of tea polyphenols. It is possible that both processes contribute to this effect. (Fan *et al.*, 2008).

Thiobarbituric acid reactive substances (TBARS)

Fish meat is more prone to oxidative degradation due to its high content of unsaturated fatty acids. TBARS is usually measured to measure oxidative degradation of lipids. Produced Malondialdehyde as a result of oxidative spoilage of fats reacts with thiobarbituric acid. Measured malondialdehyde levels determine the rate of lipid peroxidation in biological systems (Khayat and Schwall, 1983). The amount of thiobarbituric acid for different

treatments over 9 days is shown in Figure 5. The amount of TBARS has increased significantly over time in all treatments. The effects of formulation on the amount of TBARS are also statistically significant. The E-0.1-GT and then the F-0.1-GT showed the lowest TBARS values. Other studies have reported similar outcomes. During storing frozen bonito fish by glazing with tea extract, the amount of TBARS was lower than the samples which were not treated with this method. Catechins, which are abundant in green tea, were responsible for inhibiting lipid oxidation (Lin and Lin, 2005). In another study, Song et al. (2020) utilized two antioxidant packages containing rosemary oleoresin and green tea extract to improve the shelf life of minced pork. From the 7th day onwards, the control group showed a rapid increase in TBARS values. However, the meat packaged with antioxidant films demonstrated lower values compared to the control group. Specifically, the meat packaged with films coated with green tea extract exhibited negligible increase in malondialdehyde levels during storage (Song *et al.*, 2020).

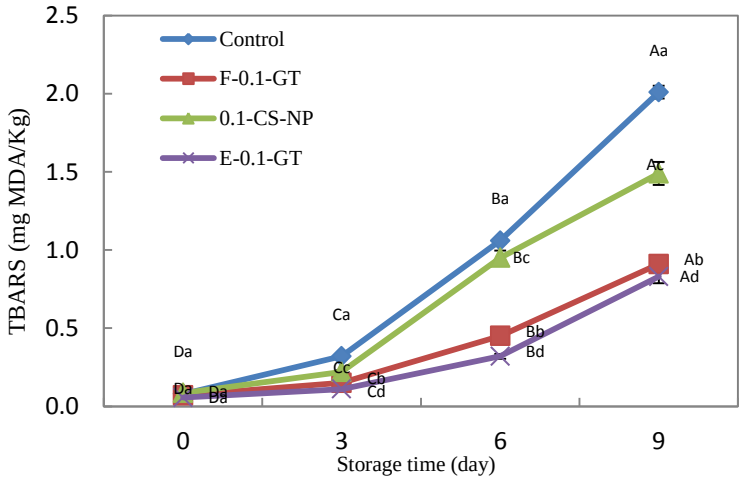

Fig 5 Changes in the TBARS value of rainbow trout surimi samples with different formulation during 9 days of refrigerated storage in 4°C. Different lower case letters have significant differences in different treatments and different uppercase letters have significant differences at different times ($p<0.05$).

Free Volatile Fatty Acids (FFA)

FFA is formed through the hydrolysis of lipids. Therefore, the quantity of FFA can serve as an indicator of the degree of lipolysis, which, in turn, indicates the freshness of the fish (Andevari and Rezaei, 2011).

FFA values in terms of the percentage of oleic acid in rainbow trout surimi under different treatments are shown in Figure 6. The amount of FFA in the samples has increased statistically over time ($p <0.05$). The rainbow trout surimi sample treated with E-0.1-GT has the lowest FFA values indicating that the chitosan nanoparticles containing green tea extract were effective in inhibiting lipid hydrolysis. In Fact the phenolic compounds present in the extract not only directly inhibit free radicals but also have the ability to prevent the accumulation of superoxide and hydroxy free radicals. This is achieved through the inhibition of the xanthine oxidase enzyme's activity (Safari *et al.*, 2018).

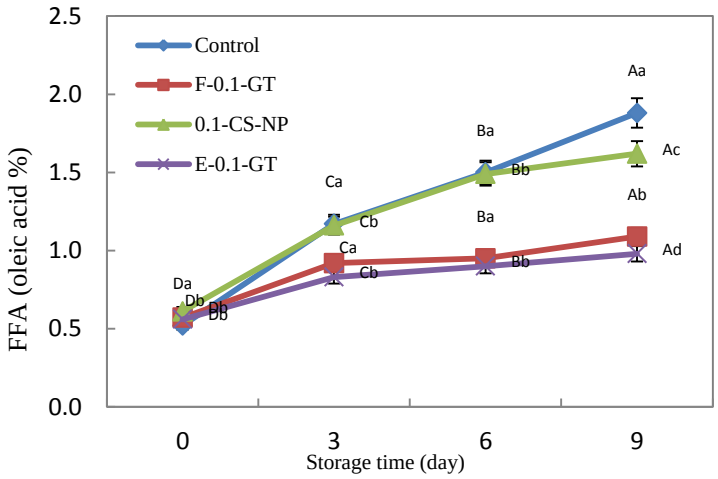

Fig 6 Changes in the FFA value of rainbow trout surimi samples with different formulation during 9 days of refrigerated storage in 4°C. Different lower case letters have significant differences in different treatments and different uppercase letters have significant differences at different times ($p<0.05$).

Microbiological Changes During Storage

The microbial quality of the fish used to create the product strongly influences the quality of seafood, such as surimi. Factors like habitat, geographical location, and water temperature exert a significant influence on the microbial flora and marine organisms. In cold-water fish, gram-negative bacteria are the predominant microbial flora (Park, 2005), while gram-positive mesophilic bacteria are commonly found in hot-water fish.

Lactic acid bacteria (LAB), have the ability to thrive in both aerobic and anaerobic conditions. LAB is a natural component of the microflora present in fresh rainbow trout fillets (Behnam *et al.*, 2015). On day 0, there were 2.12 ± 0.01 log CFU/g of LAB in the control sample, which increased to 5.4 ± 0.04 log CFU/g after 9 days of storage. On the ninth day, 0.1-CS-NP exhibited a reduced LAB count (2.8 ± 0.03 log CFU/g) compared to the other treatments. There was a significant difference in the growth rate of LAB between the control sample and the other treatments on the third, sixth, and ninth days. Additionally, the growth rate of LAB in the control sample was higher than that of the other treatments. The LAB counts in F-0.1-GT samples remained relatively stable from day 0 to 6, but showed a significant increase on the ninth day. On the other hand, Figure 7 illustrates that the LAB counts in E-0.1-GT samples did not exhibit a statistically significant difference between day 0 and day 9. This suggests that the LAB counts in these samples did not experience a significant increase, indicating that the nanoencapsulated green tea effectively inhibited the growth of lactic acid bacteria in surimi.

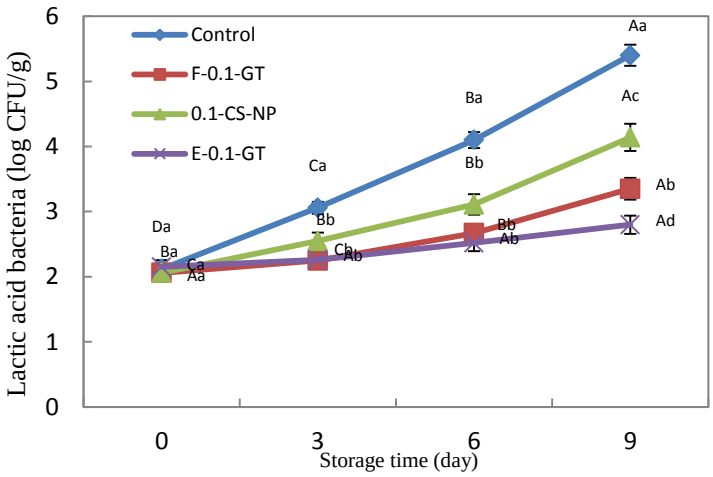

Fig 7 Changes in Lactic acid bacteria counts of rainbow trout surimi samples with different formulation during 9 days of refrigerated storage in 4°C. Different lower case letters have significant differences in different treatments and different uppercase letters have significant differences at different times ($p<0.05$).

The changes in the *Enterobacteriaceae* population during the nine-day storage period are depicted in Figure 8. The control sample, F-0.1-GT, and 0.1.1-CS-NP treatment exhibited a statistically significant increase in the *Enterobacteriaceae* population from days 0 to 9. Among the treatments, the E-0.1-GT treatment displayed the lowest growth rate. While the free extract demonstrated a reasonable inhibitory effect, its impact was significantly enhanced upon nanoencapsulation. Gram-negative bacteria are generally considered resistant to natural antimicrobial compounds due to the unique properties of their outer membrane. However, the addition of green tea extract, particularly in its nanoencapsulated form, has been shown to have significant beneficial effects in reducing the population of these microorganisms. In rainbow trout, *Enterobacteriaceae* is one of the most common microbial groups (Huber *et al.*, 2004). *Salmonella*, *Shigella*, *Enterotoxigenic Escherichia coli*, *Yersinia pestis*, and *Klebsiella* are all members of the *Enterobacteriaceae* family. Trimethylamines, ketones, esters, aldehydes, NH3, and acids are all produced by them, and they all induce food spoiling. *Enterobacteriaceae* dominated the cultured flora in a study conducted by Virta (2009) on the isolation and identification of the most important spoilage microorganisms in rainbow trout (Virta, 2009). Since *Enterobacteriaceae* is dominated flora in rainbow trout, this bacteria is present in rainbow trout fillets. Thus is more likely to be present in products made from rainbow trout such as surimi too (Chytiri *et al.*, 2004). The presence of Enterobacteriaceae in surimi is reasonable due to the acidic environment, low NaCl levels and refrigeration in vacuum containers. usualy The predominant microbial population in surimi consists of psychrotrophic Enterobacteriaceae, lactic acid bacteria, and gram-negative fermenting bacteria. (Gram and Dalgaard, 2002).

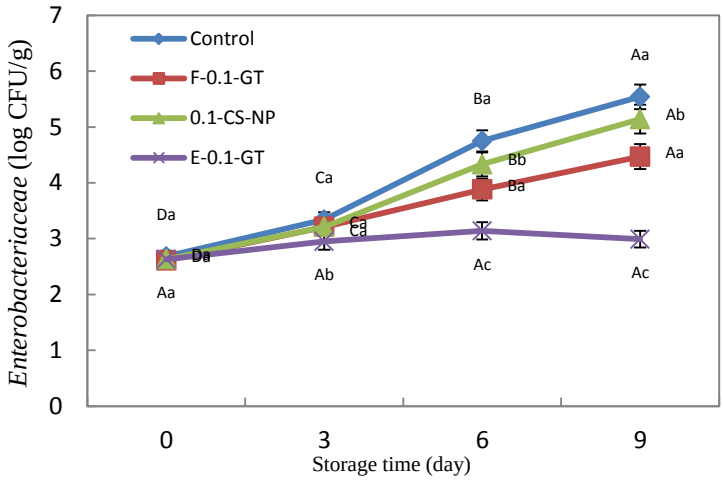

Fig 8 Changes in *Enterobacteriaceae* counts of rainbow trout surimi samples with different formulation during 9 days of refrigerated storage in 4°C. Different lower case letters have significant differences in different treatments and different uppercase letters have significant differences at different times ($p<0.05$).

Figure 9 depicts the logarithm of counting aerobic mesophilic microorganisms. The primary impacts of The results of the analysis of variance indicate that treatments, storage duration, and their combinations had a significant influence ($p<0.05$) on the quantity of aerobic mesophilic bacteria. The presence of primary aerobic mesophilic bacteria on day 0 within the control samples of rainbow trout surimi, as well as the treated samples, displayed a range of 4.08 to 4.12 log CFU/g. This range serves as an indication of the excellent initial quality of the fish utilized in the production of surimi. According to the International Institute on Microbiological Specifications for Foods (ICMSF., 1998), a microbiological maximum limit of 7 log CFU/g has been set for aerobic mesophilic bacteria. This limit serves as a microbiological criterion for assessing the shelf life of a product throughout its storage period (Rodrigues *et al.*, 2016). The population of mesophilic bacteria in rainbow trout surimi samples treated with E-0.1-GT showed significantly lower counts on day 9 compared to other samples, indicating a notable impact of the nanoencapsulated green tea extract on the mesophilic bacteria population. The antibacterial properties of green tea extract can be attributed to its polyphenoles and catechins. These catechins are responsible for inhibiting the growth of microorganisms by causing leakage of cellular material from the cells and interfering with DNA and RNA metabolism. (Ultee *et al.*, 1999). According to Chitiri et al. (2004), rainbow trout fillets were found to have a higher presence of mesophilic bacteria compared to fresh fish. This is likely attributed to secondary contamination that occurs during the filleting process. Secondary contamination can occur through various means such as storage containers, cleaning water, blades and cutting boards, employees, and other sources (Chytiri *et al.*, 2004). Therefore, it is plausible that some of the mesophilic bacteria detected in surimi were introduced through secondary contamination at different stages of the manufacturing process.

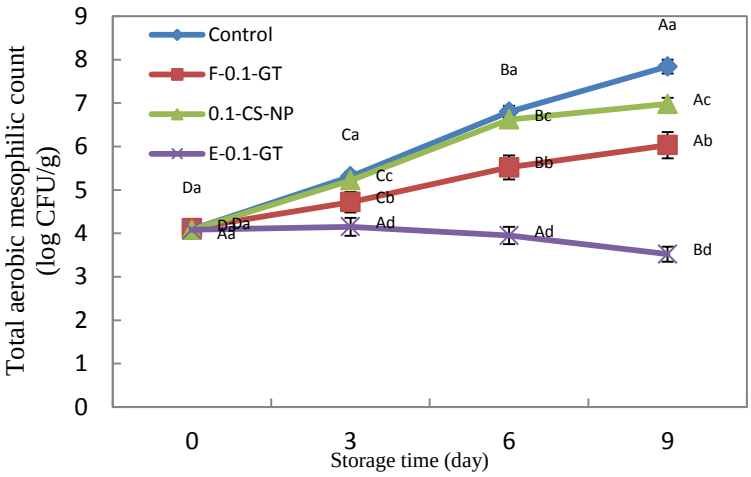

Fig 9 Changes in total aerobic mesophilic counts of rainbow trout surimi samples with different formulation during 9 days of refrigerated storage in 4°C. Different lower case letters have significant differences in different treatments and different uppercase letters have significant differences at different times ($p<0.05$).

The count of mold and yeast increased from approximately 3 log CFU/g at the beginning of the storage period to around 5 log CFU/g for control treatments, F-0.1-GT, and 0.1-CS-NP. The growth of mold and yeast in E-0.1-GT was slower compared to other treatments, indicating that treating nanoparticles with green tea reduced the development of mold and yeast. It is uncommon to find mold and yeast contamination in fresh fish. Mold and yeast have the ability to thrive in conditions with low water activity (a_w). Specifically, molds can grow at a_w levels of 0.80 or lower, whereas yeasts require a_w levels of 0.88 or higher (Park, 2005). Yeast tends to flourish in moist environments, such as fish and salted fish products. On the other hand, molds are more prone to causing spoilage in salted seafood (Abbas *et al.*, 2009). Given the inclusion of salt in surimi products and the potential for secondary contamination, it is possible for mold and yeast to be present in this particular product.

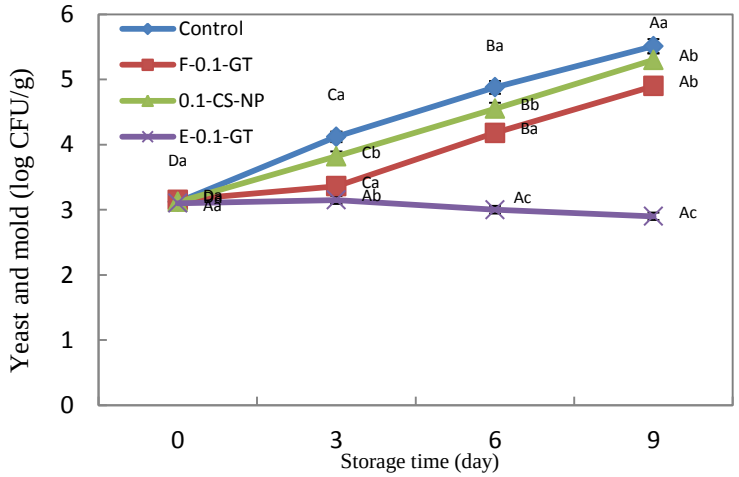

Fig 10 Changes in yeast and mold counts of rainbow trout surimi samples with different formulation during 9 days of refrigerated storage in 4°C. Different lower case letters have significant differences in different treatments and different uppercase letters have significant differences at different times ($p<0.05$).

Sensorial Changes During Storage

The color of surimi treatments was assessed during storage. The results are illustrated in Figure 11, where the E-0.1-GT treatment consistently obtained the highest scores throughout all testing days. However, by the end of the storage period, all sample scores fell below the acceptable range. Another study conducted on rainbow trout fillets demonstrated significant improvement in color preservation when 1.5% cinnamon essential oil was added to the chitosan film used for maintaining the fillets at 4°C. On the 16th day of storage, the color scores of the control samples, as well as the samples coated with chitosan film and chitosan film containing essential oil, were recorded as 2, 3.8, and 4.5, respectively. This suggests that incorporating cinnamon essential oil into the chitosan film has beneficial effects in maintaining the color quality of rainbow trout fillets (Ojagh *et al.*, 2010).

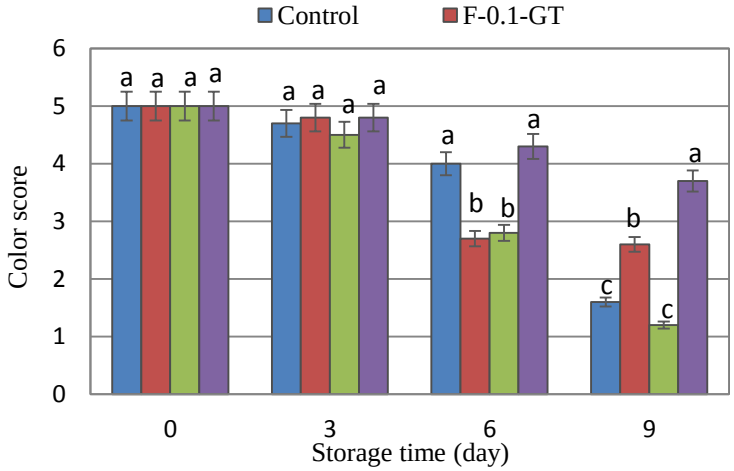

Fig 11 Changes in color of rainbow trout surimi samples with different formulation during 9 days of refrigerated storage in 4°C. Different letters in the same column indicate significant differences between treatments ($p < 0.05$)

In the next step, we evaluated the odor of raw rainbow trout surimi samples to determine spoilage caused by microbial and oxidative factors during different storage days. Evaluators were instructed to assign a lower score to samples with unpleasant smells resulting from microbiological and oxidative decomposition. A score of 5 indicated the natural smell of rainbow trout surimi, while a score of 1 indicated an unpleasant smell associated with spoilage. The changes in smell of the rainbow trout surimi samples over a period of 9 days of storage are presented in Figure 12.

From the figure, it is evident that there was no statistically significant difference between the different treatments on the production day and the third day of storage. The scores recorded on these two days were higher than 4, indicating acceptable odor. However, on the sixth day, a significant decrease in scores occurred, with all samples scoring below 4, which is considered unacceptable. This decline in scores can be attributed to microbial growth and lipid oxidation, despite the E-0.1-GT treatment on days 6 and 9 yielding the highest score compared to other samples.

According to Ojagh et al. (2010), starting from the 8th day of refrigerated storage, microbial spoilage and significant lipid oxidation resulted in undesirable sensory characteristics in rainbow trout fillets, including off-odour, sliminess, and

discoloration (Ojagh *et al.*, 2010). In a separate study, the use of clove essential oil and thyme essential oil encapsulated with chitosan helped maintain the sensory quality of minced mutton for an extended period. The addition of clove and thyme essential oils to minced mutton increased the shelf life of the samples in terms of color and odor (Aliakbarlu and Khalili Sadaghiani, 2015).

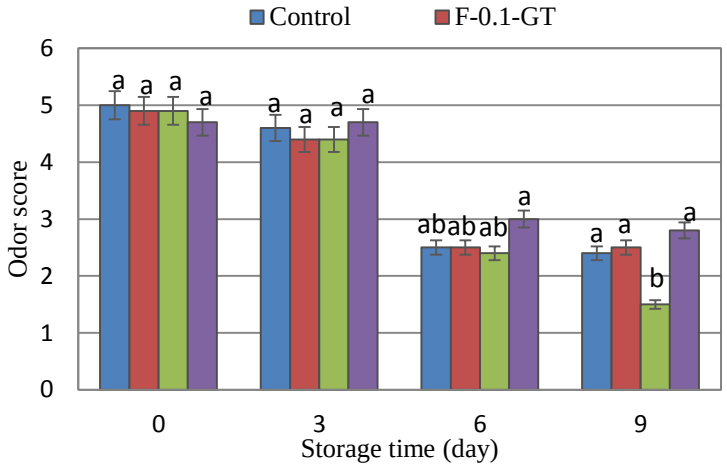

Fig 12 Changes in odor of rainbow trout surimi samples with different formulation during 9 days of refrigerated storage in 4°C. Different letters in the same column indicate significant differences between treatments ($p < 0.05$).

Due to the possibility of microbiological spoilage, the taste of cooked (fried) rainbow trout surimi was evaluated on manufacturing day and the third day. The findings of the analysis of variance revealed no significant differences between the samples, however, the 0.1-CS-NP treatment scored higher than the other treatments on both the production day and the third day. The taste of fried rainbow trout surimi with encapsulated green tea extract was accepted, indicating that nanoencapsulation was successful in covering the taste of the green tea extract in the product On both the production day and the third day, samples with free extract had lower taste scores due to the distinct flavor of green tea in the surimi product. The encapsulation of essential oils and plant extracts serves to minimize the negative impact on sensory characteristics of food products. The taste of essential oils and plant extracts, similar to any other condiment, may be unappealing to certain customers. Consequently, samples incorporating these natural preservatives may receive lower ratings during sensory evaluation. However, for a specific group of consumers who do not appreciate the authentic taste of the product, the addition of essential oils or extracts in appropriate quantities can enhance the product's appeal (Michalczyk *et al.*, 2012).

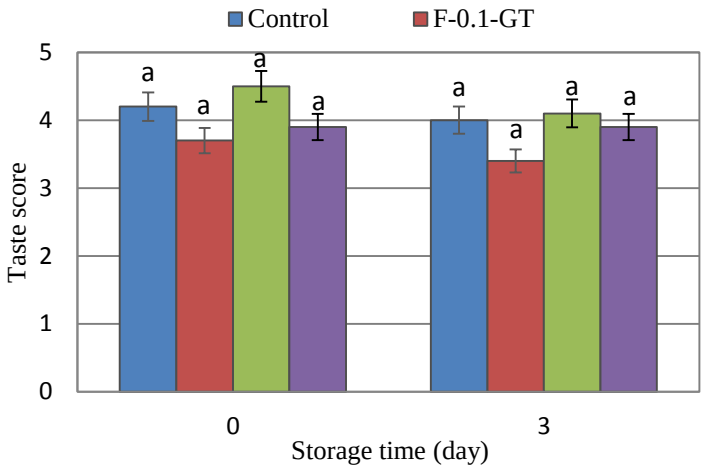

Fig 13 Changes in taste of rainbow trout surimi samples with different formulation during 9 days of refrigerated storage in 4°C. Different letters in the same column indicate significant differences between treatments ($p < 0.05$).

Figure 14 depicts the findings of the overall acceptability examination of the rainbow trout surimi samples. The analysis of variance reveals a significant impact of formulation and time on the overall acceptability of the samples subjected to different treatments. Furthermore, the total acceptance rating score of the samples decreased as time progressed. The overall acceptability score for all samples dropped on the sixth day of sensory evaluation. The control treatments, F-0.1-GT and 0.1-CS-NP, scored below the acceptable range, whereas the treatment E-0.1-GT received the highest score. When rainbow trout fillets were covered with chitosan and cinnamon essential oil to extend their shelf life at 4 °C, the study yielded similar results. The fillets treated with chitosan and cinnamon essential oil exhibited the highest overall acceptance compared to the other samples (Ojagh *et al.*, 2010).

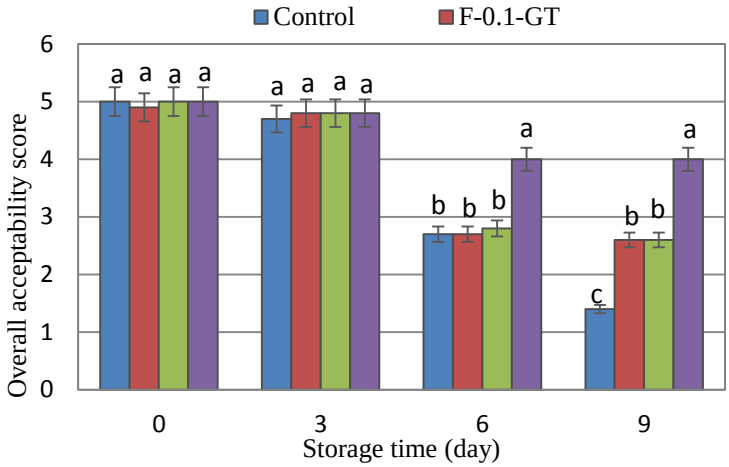

Fig 14 Changes In overall acceptability of rainbow trout surimi samples with different formulation during 9 days of refrigerated storage in 4°C. Different letters in the same column indicate significant differences between treatments ($p < 0.05$).

Fish can be consumed by humans when it achieves a minimum score of 4 in terms of sensory characteristics (Fan *et al.*, 2008; Ojagh *et al.*, 2010). When considering a score of 4 as the threshold for accepting or rejecting surimi, the results indicated that color, odor, and overall acceptability characteristics were unacceptable for most samples on the ninth day. Only rainbow trout surimi with nanoencapsulated green tea extract achieved an acceptable score in terms of color and overall acceptance. Due to the risk of food poisoning, the taste of the samples was not evaluated on days 6 and 9. The sensory evaluation results aligned with the findings from the chemical and microbial analysis Due to the high oxidation of lipids and the growth of microorganisms, the control sample deteriorated and received the lowest scores in sensory evaluation. However, the nanoencapsulated extract with antioxidant and antibacterial properties was able to mitigate the negative effects of lipid oxidation and microbial growth, thereby preserving the original quality of the product and extending its shelf life. These results are supported by other international research findings. For instance, Zhang et al. (2016) conducted a study on the antimicrobial activity of catechin-loaded chitosan nanoparticles, measuring the minimum inhibitory concentration and minimum bacterial concentration. Their study demonstrated that catechin reduced the growth of pathogens, particularly Escherichia coli and Listeria innocua. Moreover, the smaller particle size of the nanoparticles allowed for superior penetration into the bacterial cell wall, interfering with the synthesis of bacterial cell membranes (Zhang *et al.*, 2016). Another study investigated the antimicrobial activity of chitosan nanoparticles containing catechin against gram-negative and gram-positive bacteria. Encapsulated catechin exhibited higher antimicrobial effects compared to free catechin (Li *et al.*, 2018). Additionally, in a study conducted by Dai and co-workers

(2020), the antioxidant activity of chitosan and β-lactoglobulin complex nanoparticles loaded with epigallocatechin gallate was investigated. The measurement of DPPH and Ferric Reducing Antioxidant Power (FRAP) indicated that encapsulation could provide a prolonged-release rate and decreased EC50 values (Dai *et al.*, 2020).

In another study on the encapsulation of catechin in chitosan nanoparticles, it was found that the nanoencapsulated catechin displayed higher and extended antioxidant activity compared to free catechin hydrate (Kaur *et al.*, 2017). Li and co-workers (2018) also claimed that nanoencapsulation of catechin within chitosan demonstrated a higher DPPH free-radical scavenging activity compared to free catechin (Li *et al.*, 2018).

Furthermore, in a relevant study, the measurement of radical scavenging activity by DPPH and $ABTS^{\bullet+}$ showed that encapsulation of the bioactive compound had a pronounced impact on the release rate of catechin (Tang *et al.*, 2013).

Conclusion

The antioxidant and antibacterial properties of green tea extract were preserved through encapsulation with chitosan nanoparticles. The preservation effects of the green tea extract were sustained until the end of the storage period due to the controlled release from the chitosan nanoparticles. When nanoencapsulated extract was added to rainbow trout surimi samples, it had beneficial effects in reducing the progression of lipid oxidation compared to the control sample. Additionally, the amount of primary and secondary oxidation products was significantly reduced. The study on the effect of free and encapsulated extract on the microbial properties of rainbow trout surimi revealed that encapsulating the extract in chitosan nanoparticles further enhanced its ability to reduce the population of aerobic mesophilic bacteria. Rainbow trout surimi containing encapsulated extract was authorized for sensory assessments, specifically in terms of color and overall acceptability, until the final day of storage. It received the highest score compared to other treatments at the end of the storage period. Based on the findings of this study, it can be concluded that green tea extract encapsulated with chitosan nanoparticles can serve as a natural preservative, replacing synthetic preservatives, to effectively maintain the microbiological, chemical, and sensory quality of surimi.

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ریزپوشانی عصاره چای سبز با استفاده از نانوژل کیتوزان-سیترات و بررسی خواص آنتی اکسیدانی و ضد میکروبی آن بر ماندگاری سوریمی قزل آلای رنگین کمان

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چکیده

به دلیل افزایش تقاضای مصرف کنندگان برای استفاده از مواد غذایی طبیعی و عاری از نگهدارنده‌های مصنوعی، این مطالعه به منظور حفظ خاصیت آنتی‌اکسیدانی عصاره چای سبز(GT) از طریق ریز پوشانی آن در نانو ذرات کیتوزان (CS-NP) و بررسی اثرات نگهدارندگی آن بر روی سوریمی انجام گرفت. نانوانکپسولاسیون عصاره چای سبز با استفاده از نانوژل کیتوزان-سیترات انجام شد. نتایج نشان داد که عصاره ریز پوشانی شده (CS-NP-GT) بر روی کاهش اکسیداسیون چربی سوریمی از طریق تعیین تیوباربیتوریک اسید و اسیدهای چرب آزاد به طور قابل ملاحظه‌ای موثر بوده است. آنالیزهای شیمیایی، میکروبی و حسی سوریمی با تیمار CS-NP-GT تفاوت معنی داری در مقایسه با سایر تیمارها نشان داد ($p<۰/۰۵$). در پایان دوره نگهداری سوریمی با تیمار CS-NP-GT سبب ۲/۶ سیکل لگاریتمی کاهش در جمعیت باکتری-های اسید لاکتیک، ۲/۵۵ سیکل لگاریتمی کاهش در جمعیت *انتروباکتریاسه*، ۴/۳۲ سیکل لگاریتمی کاهش در جمعیت باکتری‌های مزوفیل هوازی و ۲/۶۱ سیکل لگاریتمی کاهش در جمعیت کپک و مخمر شد. نتایج این پژوهش نشان داد که ریز پوشانی عصاره چای سبز با نانوذرات کیتوزان یک فناوری نویدبخش در جهت کنترل تغییرات شیمیایی، میکروبی و حسی نامطلوب سوریمی و افزایش ماندگاری این محصول است.