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Effects of nanochitosan with and without sodium acetate coating on *Pseudomonas fluorescens* and the quality of refrigerated rainbow trout filets

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Abstract

The present study was conducted to evaluate the effects of nanochitosan with sodium acetate and none-sodium acetate coating on *Pseudomonas fluorescens* and the shelf life of refrigerated rainbow trout (*Onchorhynchus mykiss*). 45 samples of rainbow trout filet in five positive and negative treatments including a control, 1% NCH(T2), 2% NCH(T3), 1% NCH+5% NaOAC1 (V/V, T4), 2%NCH2%+ 5%NaOAC1(V/V,T5), the negative groups incubated by *P. fluorescens* bacteria (PF+NCH (1 and 2%) +5% NaOACl (V/V) (PF+NCH(1 and 2%)) and a negative control during the 16 days. Comparisons of microbial, chemical and sensory analyses were purely done on safe treatments periodically using one way ANOVA and Duncan tests. Results detected significant differences between the control group and both NCH+5% NaOAC1 (V/V), and NCH free of 5% NaOAC1 (V/V) in all amounts of microbial and chemical indices (p<0.05) and there were no significant differences between the two NCH groups (p>0.05). Sensory indices illustrated that filets treated with each groups of NCH±5% NaOAC1(V/V) received good points and their shelf life was 16 days, but control group settled in this status on sixth day of the test. For the two other treatments, shelf life storage increased and remained on 12 th and 16 th day, respectively. Furthermore, third day of the test showed high amounts of bacteria in the *PF* incubation treatment. High levels of bacteria same as that in the control group were determined on sixth day in desirable samples and in NCH+ *P. fluorescens,* while there was an obvious bacterial reduction in bacterium mixed with NCH+NaOAC and kept for 12 days. Overall, utility of NCH+NaOAC increased the shelf life in refrigerator storage and decreased the amount of *P. fluorescens* in it.

Keywords: Nanochitosan, Sodium acetate, Rainbow trout, *Pseudomonas fluorescens*, Shelf life

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Introduction

The use of fresh fish and fillets as healthy diets is important among the nutritionists and food hygiene scientists, and they are trying to improve the preservation methods to enhance shelf life, safety and make the foods more desirable. Rancidity of fish products changes their odor, flavor, taste, color, texture and appearance. While, delaying lipid oxidation, product enhancements are factors that can contribute to the development of quality and quantity of functional meat and fishery products (Hayes *et al.,* 2011). Some chemical and biological matters like sodium acetate and chitosan delivered from crustacean shell as marine organism base on edible activities would be promote the fish and fisheries industries keeping. Effects of sodium organic salts derived from citric, lactic and acetic acids have already been studied on microbial damage of *Salmon* slices during tray-packaged storage at 1ºC (Sallam, 2007). Using 2% sodium acetate improved the (*Etroplus suratensis*) shelf life of fillets with vacuum packing during the 15 days at 2 ºC (Manju *et al.,* 2007). Among the sodium acetate, sodium citrate and sodium lactate on rainbow trout refrigerator storage during 15 days NaA is the best chemical treatment to prolong the shelf-life of stored rainbow trout sticks in refrigeration at 4°C at least by 9 days compared to the other treatments (Kashiri *et al.,* 2011). Chitosan (poly-b-(1–4)-D-glucosamine) and its derivatives, alone or in combination with biological or nonbiological materials had nontoxic, biodegradable, bio-functional and biocompatible characters which provide excellent oxygen barrier properties along with their antimicrobial activity (Kong *et al*., 2010; Ojagh *et al*., 2010 Domard, 2011). Using nano -chitosan coated with pomegranate peel extract was significantly stronger than orange peel extract to shield the refrigerated silver carp fillet and significantly inhibited the lipid oxidation at this time compared with nanochiosan treatment (Zarei *et al*., 2015). Comparing using the chitosan and nanochitosan coating on silver carp fillet during the refrigerator storage exhibited higher antimicrobial activity than chitosan during the storage period. Furthermore, nanochitosan showed a stronger ability to inhibit the total volatile basic nitrogen (TVB-N) content than chitosan and fresh fish samples were treated with a solution of 2% (w/v) medium molecular weight chitosan dissolved in glacial acetic acid randomly and stored in refrigerator for 15 days. They concluded that chitosan coating can retard the microbial growth of treated samples and also extend the shelf life of fresh rainbow trout in the refrigerator at (5±1 ºC) significantly (p<0.05) (Chamanara *et al.*, 2013; Ramezani *et al*., 2015). Chitosan coatings reduced the total viable count (TVC) and extended the shelf life by 3–5 days for chilled salmon (*Salmo salar*) and sardine (*Sardinella longiceps*) fillets (Mohan *et al.,* 2012). Moreover, enzyme activities, lipid oxidation, and microbial activity are often the main causes of spoilage through the accumulation of deleterious substances

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and unpleasant off-odors (López de Lacey *et al.*, 2014). Psychrophilic bacteria such as *Pseudomonas*, *Shewanella* and *Aeromonas* are the dominant micro-biota in chilled fishery products and are involved in spoilage (Parlapani *et al.*, 2014). Whey protein coatings enriched with lactoperoxide and α-tocopherol effectively inhibited bacterial growth (including TVC, Psychrotrophs, *P. fluorescens* and *S. putrefaciens*) and extended the shelf life of pike-perch (*S. lucioperca*) fillets as well (Shokri *et al.*, 2017). However, most early studies have only focused on the effects of edible coatings on the quality of aquatic products in terms of observing bacterial enumeration, physicochemical changes and sensory scores. Information about how edible coatings affect microbial composition during storage is limited due to the inability to enumerate>1% of the total bacterial population when using traditional microbial plate count methods (Ward *et al.*, 1990), and this information is meaningful when exploring the reasons of coating preservation. However, information about how the coating treatment affects microbial composition in fillets during refrigerated storage is limited. Thus, the purpose of this study was to evaluate the effects of nanochitosan mixed with sodium acetate, and how chitosan nanoparticles based coating on *Pseudomonas fluorescens* induced quality changes such as sensory, physiochemical, microbiological in the shelf life of rainbow trout fillets during refrigerator storage.

Materials and methods

Materials

In this study 45 live *Onchorhynchus mykiss* (rainbow trout) between 500 and 600 g weight were provided from a trout pond in Tehran, kept in dried ice bags and transferred to the lab. First, heads and viscera were removed immediately and skinless fillets were prepared.

Prepared chitosan and Nanochitosan

Chitosan films were created based on the method of (Günlü and Koyun, 2013). For the production of chitosan films, chitosan (1 and 2%, w/v) was added into glacial acetic acid (1%, v/v) and the mixture was mixed for 1 hour with a magnetic stirrer at 40 °C (Wisestir MSH 20A, Korea). Then, 2% (v/v) glycerol was added slowly to the mixture as a plasticizer and mixed again with heater magnetic stirrer for 10 min and the foam was removed with a vacuum pump (KNF Neuberger, Germany). 100 ml of the resulting homogeneous mixture was poured on the Styrofoam plates (10 cm×20 cm) to cover the surface and air dried at 45 °C in a drying oven (Labart, DHG 9140 A, Korea) for 24 hours to prepare chitosan-based edible films. Chitosan nanoparticles were prepared based on the ionotropic gelation between chitosan and sodium tripoly- phosphate. Briefly, chitosan with the deacetylation degree of 83% and molecular weight of 850 KDA was dissolved in 1% (v/v) acetic acid to obtain a 1and 2% (w/v) chitosan solution. Sodium tripolyphosphate was dissolved in water to a concentration of 1 and 2%. Under

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magnetic stirring at room temperature, 4 ml of tripolyphosphate solution was added to100 mL of chitosan solution. The mixture was stirred for 60 min, then, treated with sonication at 1.5 kW for 10 min before being used for further analysis (Du *et al*., 2009). Particle size and zeta potential were measured using a ZetasizerNano-ZS-90 (Malvern Instruments, Malvern, UK). The analysis was performed at a scattering angle of 90 degrees at 25°C.

For zeta potential measurements, samples were dispersed in water and measured under the automatic mode (Figs. 1 and 2).

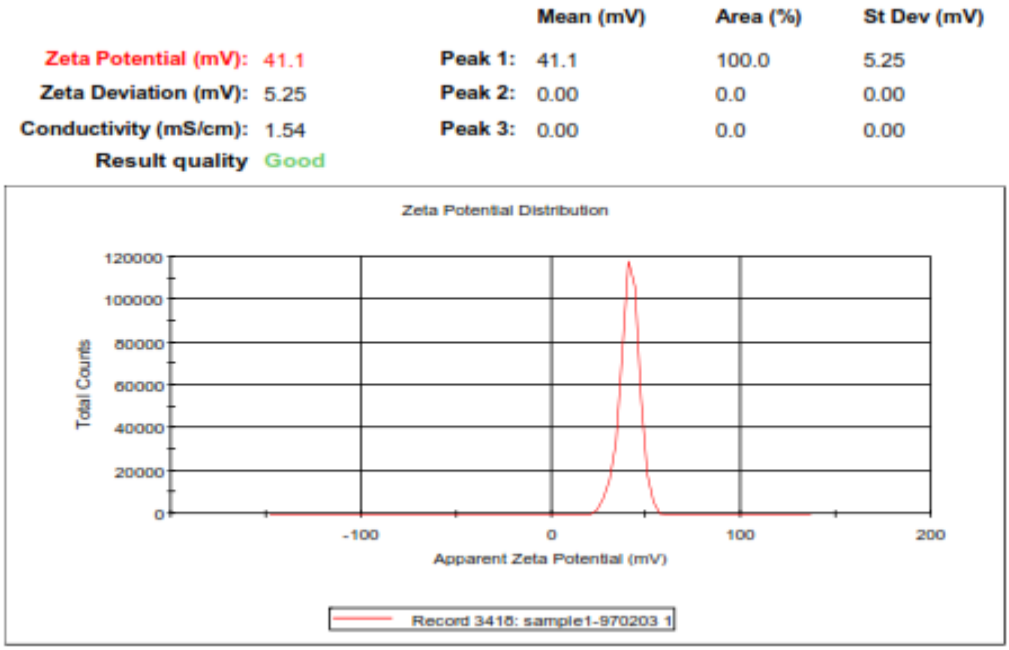


Figure 1: Zeta potential- chitosan nanoparticles 1%.

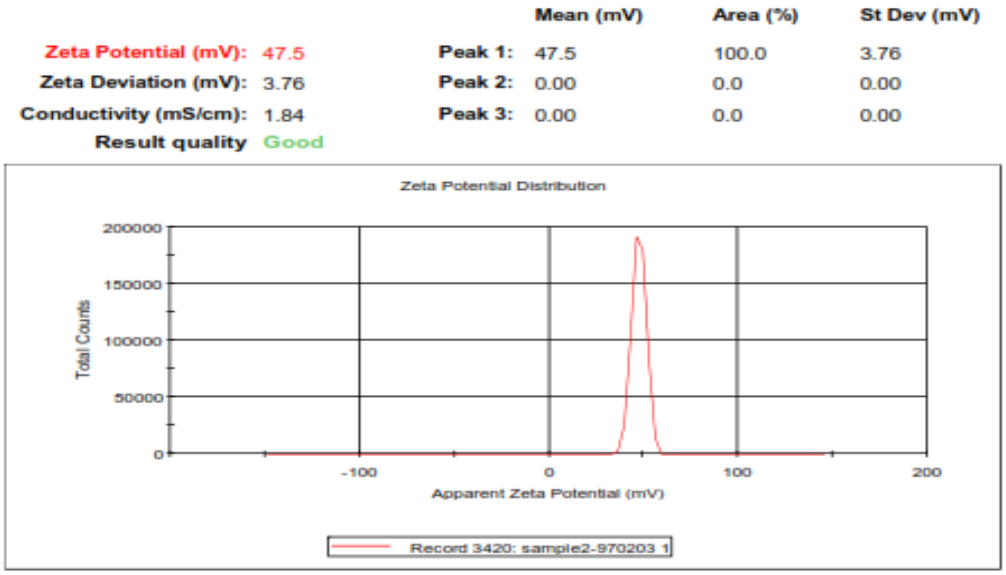


Figure2: Zeta potential- chitosan nanoparticles 2%.

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Evaluation of nanocomposite microstructure

Surface of nanocomposite was evaluated to understand how the nanoparticles were dispersed in the matrix. To prepare the solution for surface analysis, 15 ml of the suspension was poured in 9 cm dia Petri dishes for casting nanocomposite films. Subsequently, the cast petri dishes were dried at ambient conditions for 72 h. Then, surface microstructure of the film samples was examined with a Philips XL 30 scanning electron microscope (Philips, Eindhoven, the Netherlands) under high vacuum condition and at an accelerating voltage of 20.0 kV. The film samples were mounted on the specimen holder with aluminum tape and then sputtered with gold in a BAL-TEC SCD 005 sputter coater (BAL-TEC AG, Balzers, Liechtenstein). The structure of nanoparticle and its nanocomposites was evaluated by XRD measurement. XRD patterns were taken with a Philips X'Pert MPD Diffracto meter, with Cu_ Kα radiation at a wavelength of 1.544 nm, at 40 kV and 30 mA. The films were scanned over the range of diffraction angle 2θ=1–12°, with a scan speed of 1° per min at room temperature.

Assessment and incubation of Pseudomonas fluorescens bacteria

To assess the inhibiting ability of chitosan nano-capsulation on *P. fluorescens* bacteria, fillets were incubated with 1×10^4 cm^2 bacteria using leoflophyltic media (heart-brain broth) at 35 °C for 18h, and BHI media (30 °C for 24h) (Maurice, 1994) and kept in refrigerator temperature for 24 days. Finally, a spectrophotometer was used at 0.1λ wave length.

Bacterial analysis

Total viable count (TVC) and total psychrotrophic count (TPC) were determined using the pour plate method. 10 g of the fish sample was aseptically taken and homogenized in 90 mL of sterile 85% NaCl solution with a blender (HBM-400B, HBM Biomed, Tianjin, China) at room temperature. Appropriate dilutions were serially prepared, and then, 1 ml of each was spread onto plate count agar media (Merck, Darmstadt, Germany). The prepared plates were incubated at 35 °C for 2 days for TVC and at 4 °C for 10 days for TPC (Arashisar *et al*., 2004). Lactic acid bacteria (LAB) were enumerated on de Man Rogosa Sharpe agar (MRS, Oxoid code CM361, Basingstoke, UK) incubated at 30 °C for 24 h under anaerobic conditions (Giatrakou *et al*., 2008). *P. fluorescens* were enumerated on king agar medium at 30°C for 24 h (Mead and Adams, 1977). All counts were expressed as log colony-forming units (CFU) g and performed in triplicate (AOAC, 2002).

Chemical analysis

Chemical analysis of fish fillets were determined to evaluate the changes of fish quality according to chemical spoilage properties such as total volatile base (TVB), thiobarbituric acid (TBA), Peroxide and pH values. Total volatile basic nitrogen (TVB-N) value was estimated by the micro diffusion method (Goulas and Kontominas,

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2005). The micro diffusion method was determined by distillation after the addition of MgO to homogenize fish samples. The distillate was collected in a flask containing a 3% aqueous solution of boric acid and a mixed indicator produced from dissolution of 0.1 g of methyl red and 0.1 g of methylenblue to 100 ml of ethanol. Afterward, the boric acid solution was titrated with a 0.05 M sulfuric acid (H_2SO_4) solution. The TVB-N value (mg N per100 g of fish) was determined according to the consumption of sulphuric acid.

The thiobarbituric acid value was determined colorimetrically as previously described by Erkan and Ozden (2008). The samples (200 mg) were put in a 50 ml centrifuge tube to which 45 ml of a 5 % (w/v) solution of trichloracetic acid (TCA) and 100 µl BHT (Butylated Hydroxy Toluene) were added. Then the solution was blended in a homogenizer at high speed for 2 min. The mixture was filtered through a filter paper. One milliliter of a 0.01 M aqueous solution of 2-thiobarbituric acid and 5 ml of the filtrate were mixed. The mixture was stoppered, vortexed and placed in a boiling water bath (70–80 °C) for 30 min until the pink color appeared. Absorbance (As) was measured at 530 nm against a water blank. A reagent blank was run and the absorbance (Ab) recorded. TBA values were expressed as mg malondialdehyde (MDA) kg^{-1} fish meat. TBA value was determined by the formula.

$$TBA = \frac{50 * (As - Ab)}{200}$$

The peroxide content was determined in the total lipid extracts according to the method of Pearson (Egan *et al*., 1997). Results were expressed in meq oxygen kg^{-1}.

The pH was measured using a pH meter after the sample had been homogenized in distilled water in the same ratio (AOAC, 2002).

Sensory evaluation

The sensory evaluation of the trout fillets during 16 days of preservation was based on a 5-point scale to safe samples, and has not been done for samples incubated with *P. fluorescens* bacteria A 5-member trained panel – 5 men- were asked to judge the texture (5, firm; 1, very soft), color discoloration (5, no discoloration; 1, extreme discoloration), odor (5, extremely desirable; 1, extremely unacceptable/off-odors) and overall acceptability (5, extremely desirable; 1, extremely unacceptable) of the samples. The fish samples were defined as unacceptable when the sensory attributes declined to below 3.0 (Ojagh *et al.*, 2010).

Statistical analysis

The differences between all measurements were evaluated by one-way analysis of variance (ANOVA). Duncan's multiple range tests were used to compare the means to identify which groups were significantly different from other groups. Significance was defined at (p<0.05). All data are presented as mean±SD.

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Results

Total viable count

TVC tolerance is presented in Fig. 3. The control group had maximum levels (9.85 log cfu g^{-1}) of TVC in the last day of preservation and NCH2%+1.5% NaA (3.5 log cfu g^{-1}) had minimum levels in the initial time of storage. Also, two groups of NCH and NCH1%+1.5% NaA had the same point on these days and there was no difference between them (p>0.05). However, the control group showed significant differences with all experimental groups and whole treatments with each other during the storage (p<0.05).

Psychrotrophic bacteria and Lactic acid bacteria count

Amount of Psychrotrophic and lactic acid bacteria will increase to improve the cold storage time. Hence to this, there were high amounts of TPC from tenth to sixteen day of the test in the control group and the low values of that were demonstrated in the NCH (2) % +NaA group. There were significant differences between prohibited treatments and the control group (p<0.05). NCH1% andNCH1%+NaA at zero time had same levels and showed no change, meanwhile, significant differences were detected with greater time of preservation (Fig. 4).

High level of LAB was seen in the control group on the last days of the experiment and the two last treatments with same levels (2.1 log cfu g^{-1}) were the lowest ones, demonstrating significant difference between the control group with others (p<0.05, Fig. 5).There was no significant difference in LAB levels in the two NCH groups same as the two last groups on day zero of the test (p>0.05). Moreover, NCH groups have no difference in LAB amounts during the storage time (p>0.05). In fact increasing the time of refrigeration had positive effects on bacterial load especially on TVC, Psychrotrophic and lactic acid bacteria. As shown in Table 1 there is positive correlation.

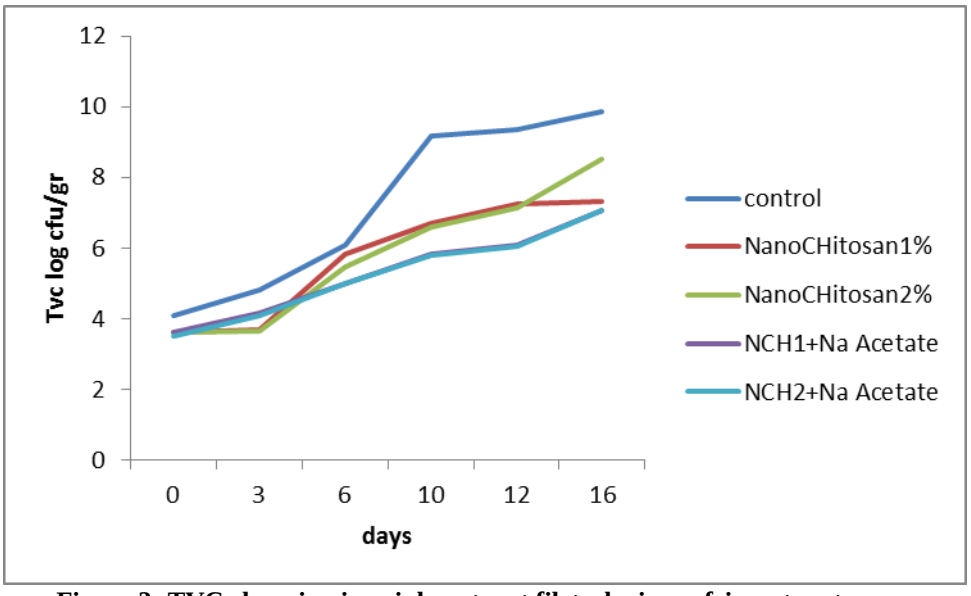


Figure 3: TVC changing in rainbow trout filets during refrigerator storage.

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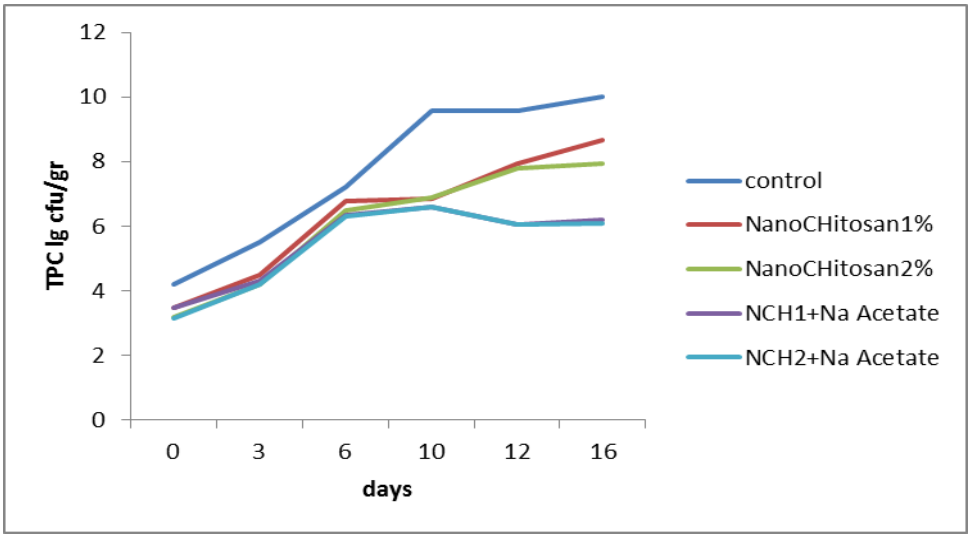


Figure 4: TPC changing in rainbow trout filets during refrigerator storage.

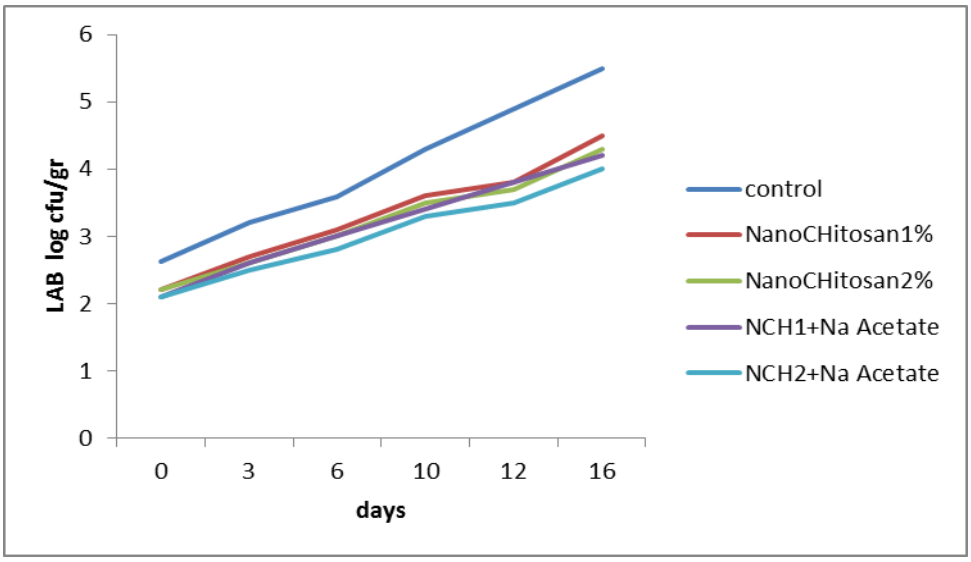


Figure 5: Lactic acid bacteria changing in rainbow trout filets during refrigerator storage.

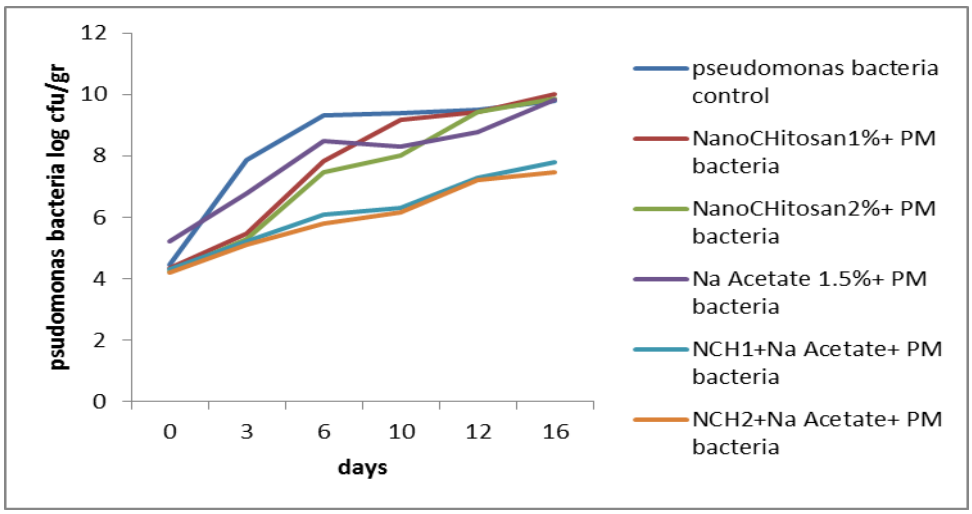


Figure 6: *Pseudomonas fluorescens* changing in rainbow trout filets during refrigerator storage.

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Table 1: Correlation between bacteria during the cold storage.

	TVC	PTC	LAB
Total Pearson correlation	1	0.917**	0.906**
Psychotropic Pearson correlation	0.917**	1	0.879**
Lactic acid Pearson correlation	0.906**	0.879**	1

Pseudomonas fluorescens count

The negative control group (Control+ *P. fluorescens*) had significant difference with others (p<0.05). *P. fluorescens* loading declined on day zero (4.2 log cfu g^{-1}) in NCH (2) % +NaA. (T_5). Fig. 6 illustrated that the negative control group had upper levels of this bacteria (6.47 to 10) at the end of test like other bacterial loadings mentioned above. On the other hand, the combination of NCH plus NaA had better results than NCH groups and showed significant differences (p<0.05).

Total Volatile Basic-Nitrogen

Maximum TVB-N was seen in the negative control group (152.13 mg N $100g^{-1}$ tissue) on 16 days of the test and minimum amount of it was in NCH (2) %+ 1.5% NaA (7.46 mg N $100g^{-1}$ tissue) at zero day of storage. There were significant difference between treatments (p<0.05) But, there was no significant difference between T4 and T5 among the safe samples during the cold storage in the initial time (Fig. 7 a and b).

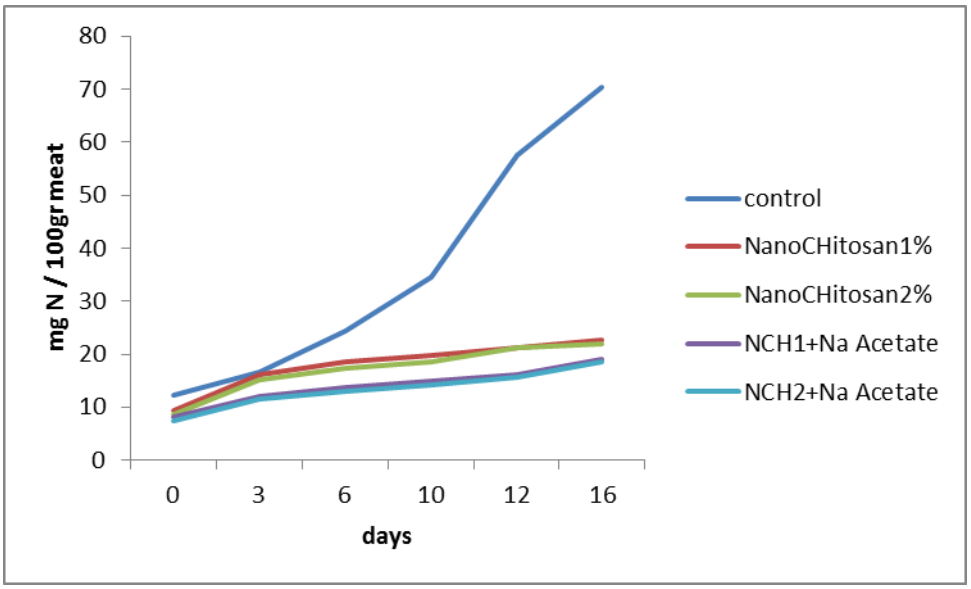


Figure 7(a): TVB-N changing in rainbow trout filets during refrigerator storage.

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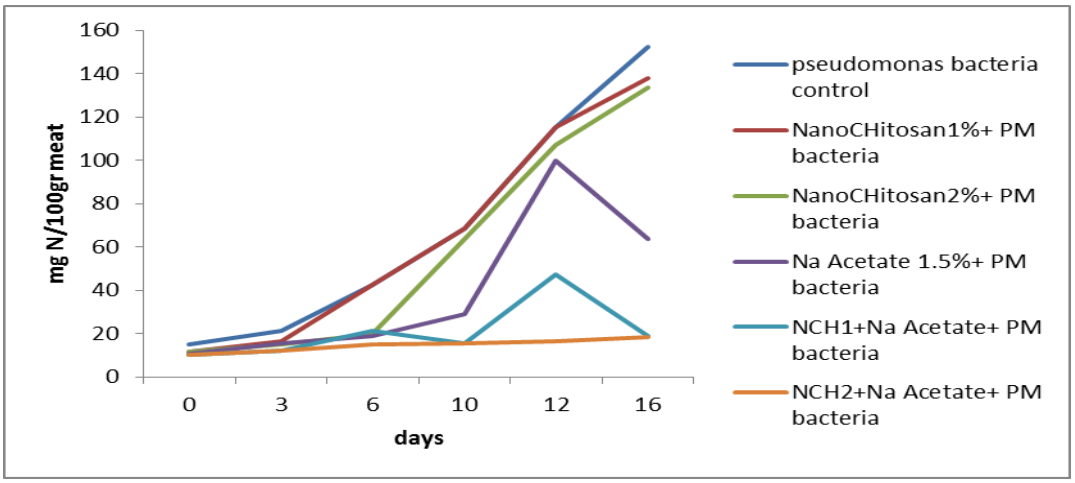


Figure 7(b): TVB-N changing in *Pseudomonas fluorescens* incubated filets during refrigerator storage.

pH

pH was in neutral to alkalis status (6-7.5) in all treatments. As shown in Fig 8a & b there was significant difference between (±control groups) and others in the trial (p<0.05). NCH2%+NaA in both parts of the test had the best results and the lowest point on days 0 and 16 of storage(p>0.05).

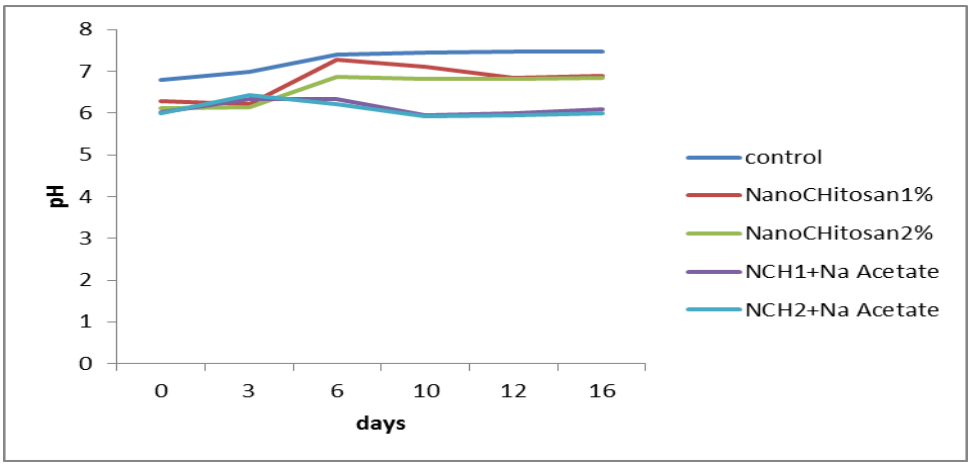


Figure 8(a): pH changing in rainbow trout filets during refrigerator storage.

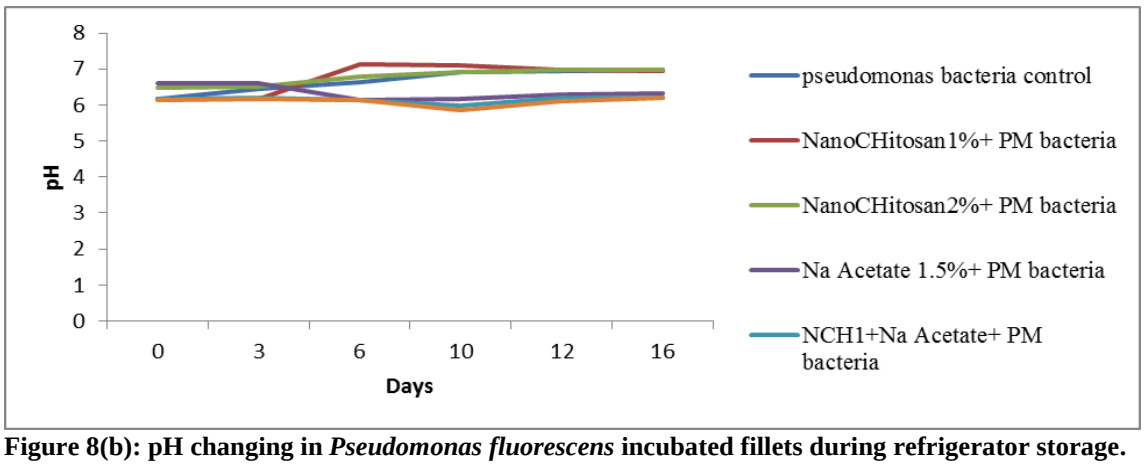


Figure 8(b): pH changing in *Pseudomonas fluorescens* incubated fillets during refrigerator storage.

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Peroxide value

The NCH (2) %+1.5% NaA (1.06 meqo$_2$ kg^{-1} fillet) and NCH (2) %+ 1.5% NaA+*P. fluorescens* (2.03 meqo$_2$ kg^{-1} fillet) have the minimum values of PV higher than the two control groups at first (Fig. 9 a and b). There were significant differences between the test groups during the refrigeration (p<0.05).

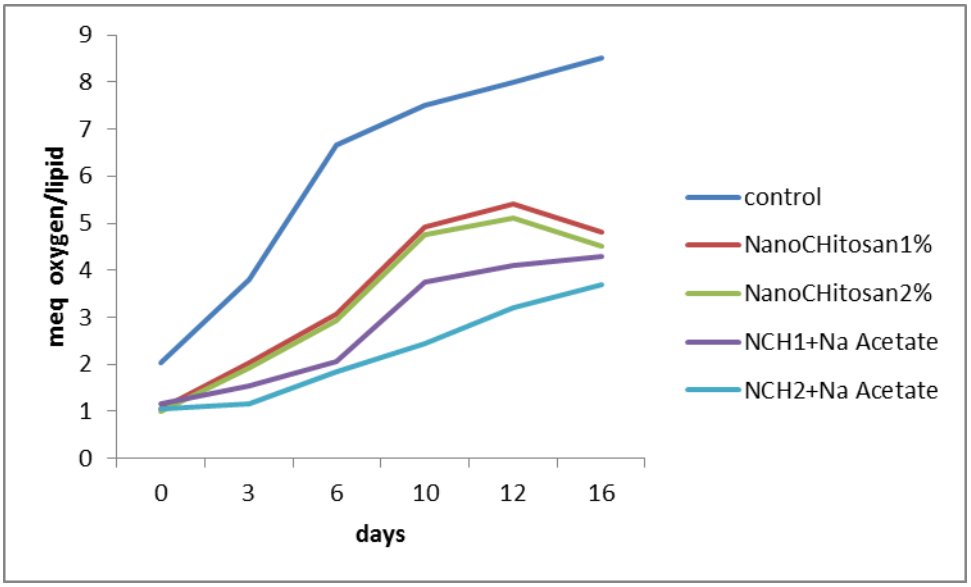


Figure 9(a): PV variations in rainbow trout fillets during refrigerator storage.

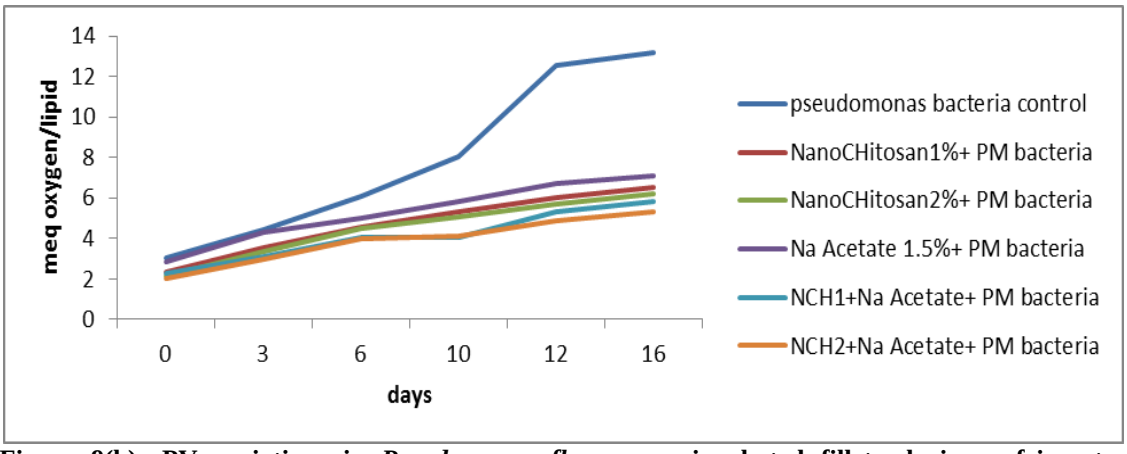


Figure 9(b): PV variations in *Pseudomonas fluorescens* incubated fillets during refrigerator storage.

Thiobarbituric acid value

TBA values of different treatments are presented in Fig. 10 a and b. TBA values increased from 0.7 and 1.05 mg malonaldehyde kg^{-1} of fish to 2.93 and 4.56 mg malonaldehyde kg^{-1} of fish in (±control) groups. The lowest point of TBA was recorded in NCH2%+NaA 1.5% on day 16 of storage. Significant differences (p<0.05) were observed between treatments.

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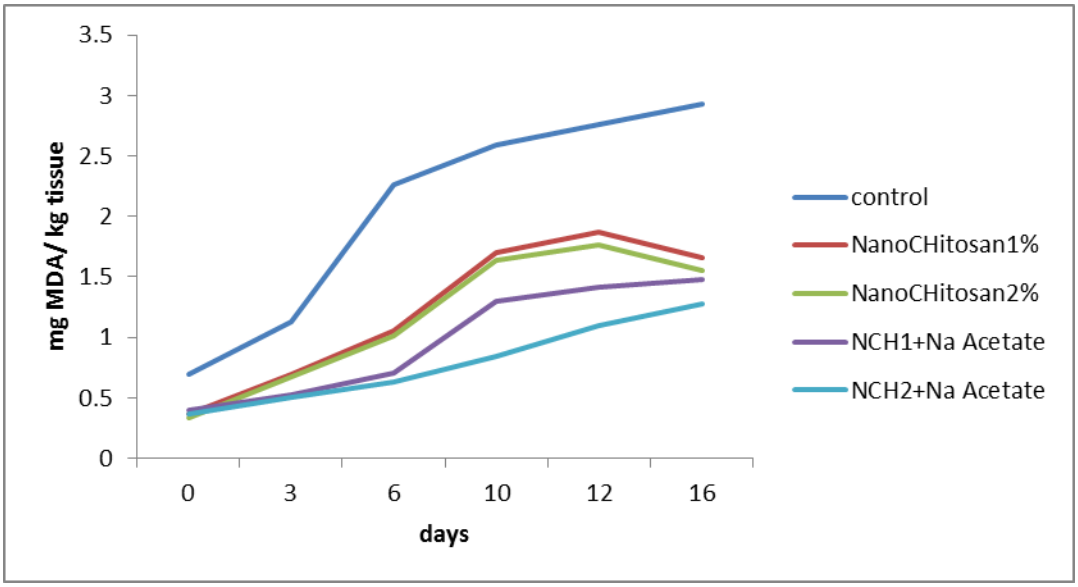


Figure 10(a): TBA variations in rainbow trout fillets during refrigerator storage.

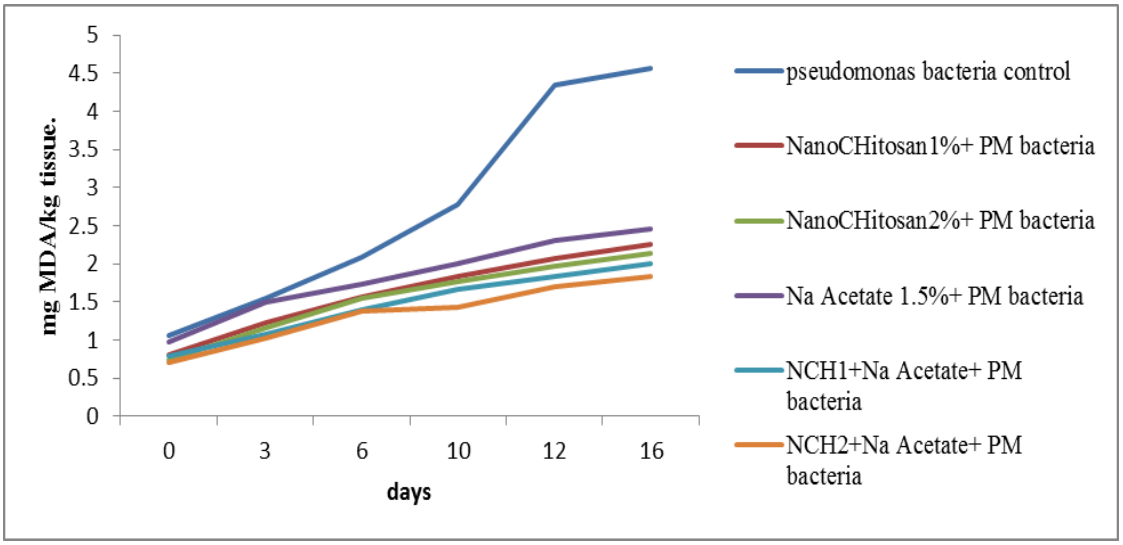


Figure 10(b): TBA variations in *Pseudomonas fluorescens* incubated fillets during refrigerator storage.

Sensory evaluation

Comparison of treatments demonstrated significant differences in texture, odor, color and overall acceptability of trout fillets (p<0.05) (Figs. 11-15). Furthermore, of the treatment with NCH+NaA had the best acceptability on day 16 of preservation. Hence the control group had the least change in all sensory indices on day zero of storage and the greatest changes were seen on day 16 in this group too.

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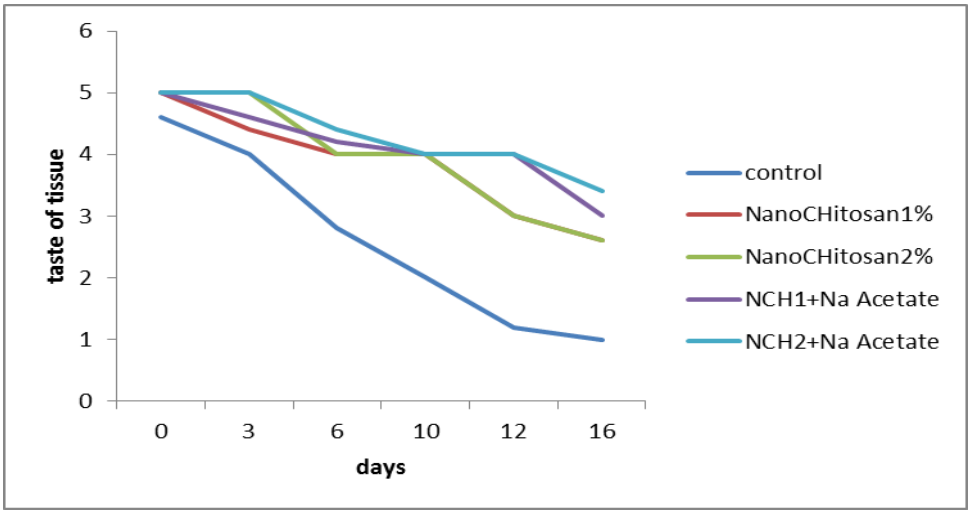


Figure 11: Taste of rainbow trout fillets during refrigerator storage.

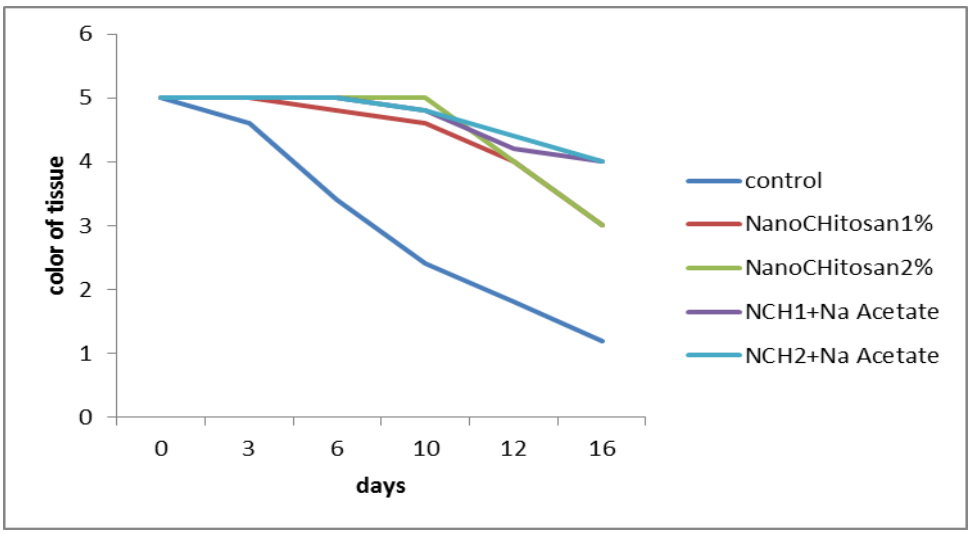


Figure 12: Color of rainbow trout fillets during refrigerator storage.

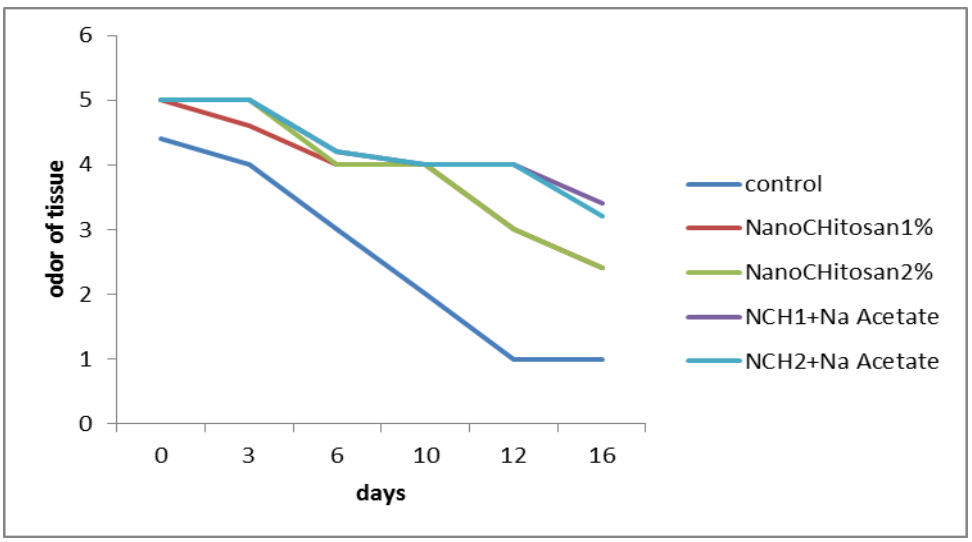


Figure 13: Odor of rainbow trout fillets during refrigerator storage.

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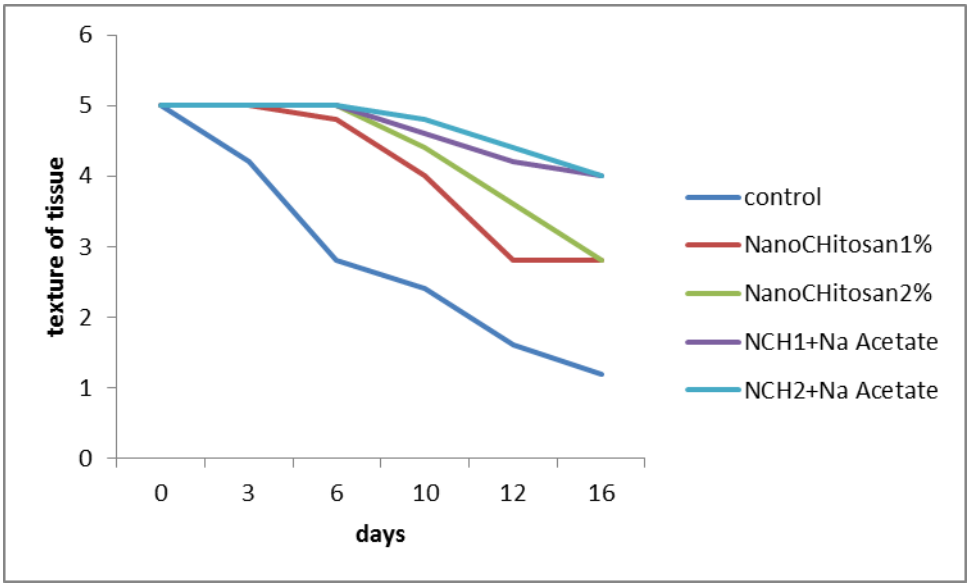


Figure 14: Texture of rainbow trout fillets during refrigerator storage.

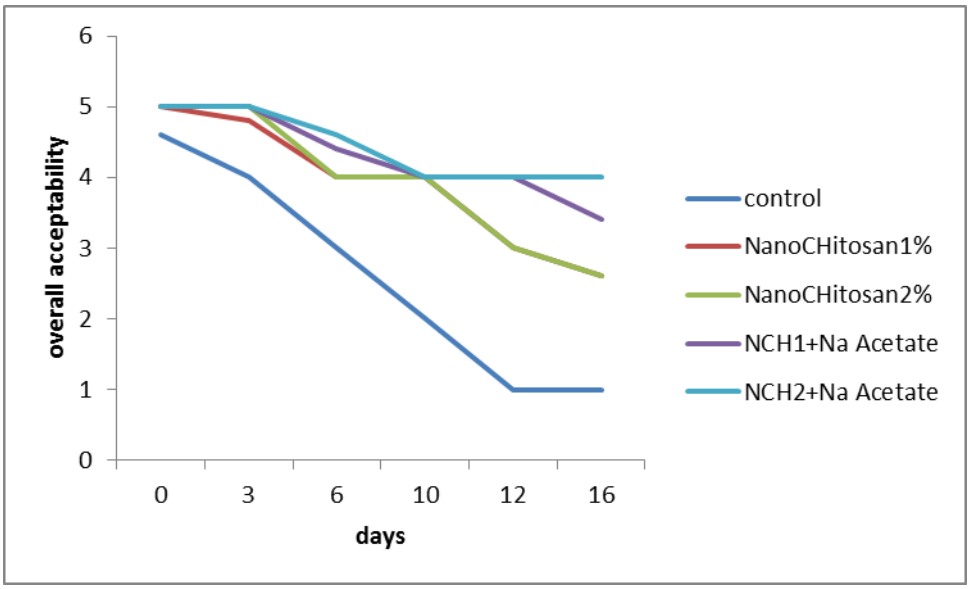


Figure 15: Overall acceptability of rainbow trout fillets during refrigerator storage.

Discussion

Spoilage of fresh and lightly preserved fish is caused by the growth and activity of specific spoilage organisms (SSOs) which produce metabolites causing off-flavors or off-odors and consequently cause consumer food rejection (Gram and Huss, 1996; Gram and Dalgaard, 2002).

However the SSOs are not the same in every case and the microbial flora isolated from seafood differs considerably from one study to another, depending on the species of fish, their environment, the mode of capture, the type of fish product (whole, whole gutted, fillets, slices) as well as the climatic and storage conditions (Gram and Dalgaard, 2002).

Due to the lack of information about nanochitosan and NaA mixture on marine products shelf time during cold storage, comparisons were done with the same studies abut NCH and sodium

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acetate separately and then together. Amount of TVC, TPC and LAB levels in T5 (NCH2%+NaA 1.5%) was 2.1 to 4 (log cfu g^{-1}) 3.5 to 7 (log cfu g^{-1}), and 3.15 to 6.1 (log cfu g^{-1}), respectively during refrigerated storage and did not exceed the maximal permissible limit of 7.0 log10 cfu g^{-1} (ICMSF, 1986) until the end of the storage period (except for TVC, TPC and LAB control group which reached 5.5, 10 and 9.8 (log cfu g^{-1}), respectively on day 16. This gradual increase refers to no use of any prohibiting matter. Due to synergistic effects of nanochitosan and NaA (T5) had the better results than the other groups. Similar findings were reported by Sallam, (2007), Fan *et al.* (2009) and Nowzari *et al.* (2013).

Lopez-Caballero *et al.* (2005) reported that a coating consisting of a blend of chitosan dissolved in acetic acid and gelatin exerted an inhibitory effect on the gram-negative flora of fish patties. According to Ojagh *et al.* (2010), a chitosan coating enriched with cinnamon oil extended the shelf life of fresh rainbow trout during the refrigerated storage by retarding the enhancement of the total viable and psychrotrophic counts because nanoparticle's larger surface area and higher affinity with bacteria cells, which yields a quantum-size effects (Qi *et al*., 2004). According to Tsai *et al.* (2002) pretreatment of fish fillets (*Oncorhynchus nerka*) for 3 h with 1% chitosan solution retarded the increase in the counts for mesophiles, psychrotrophs, coliforms, *Aeromonas* spp. and *Vibrio* spp. This was in agreement with the present study which demonstrated that *P. fluorescens* count in the last treatment was better than in the others and prohibited bacterial load compared to the negative control in the storage time. Khodanazari *et al.* (2018) reported that Chitosan in combination with green tea extract had higher inhibition on microbial growth and the lowest losses in quality of tiger tooth croaker (*Otolithes ruber*) fillets during refrigerated storage. Therefore, NCH coating under acidic environment may result from its polycationic structure due to the protonation of –NH2 on the C-2 position of the D-glucosamine repeat unit. Positively charged chitosan can bind to the bacterial cell surface which is negatively charged and disrupt the normal functions of the membrane, e.g. by promoting the leakage of intracellular components or by inhibiting the transport of nutrients into cells. Chitosan also inhibits the microbial growth by the chelation of essential metals and nutrients, spore components, as well as the penetration of the nuclei of the microorganisms, which leads to the interference with protein synthesis by binding with DNA. Furthermore, chitosan coatings act as an oxygen barrier and thus inhibit the growth of aerobic bacteria (Shahidi *et al.*, 1999; Devlieghere *et al.*, 2004).

Total volatile basic nitrogen (TVB-N), which is mainly composed of ammonia and primary, secondary and tertiary amines, is widely used as an indicator of meat deterioration. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (Kyrana *et al*., 1997; Fan *et al*., 2009). The initial (day 0) value of

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12.36 mg $100g^{-1}$ is higher than the values reported for rainbow trout by Shokri *et al.* (2017) and Kashiri *et al.* (2011). Of course, variation in TVBN values of a particular fish species is related to the fish non-protein nitrogen content, which in turn depends on type of fish feeding, season of catch, fish size as well as other environmental factors. Lastly it is directly related to microbial activity in the fish tissue (Connell, 1995; Goulas and Kontominas., 2005). According to Connell (1995), a level of 35–40 mg TVB-N/100 g of fish flesh is usually regarded as spoiled. In this experiment, the final TVB-N values of both positive and negative treated except controls samples did not exceed the upper acceptability limit after 16 days of cold storage; however, TVB-N increase in negative treated samples was significantly lower than in the control after 16 days of the storage indicating of either a faster reduction of bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds (or both) due to the effect of NcH and NaA.

Generally, the natural pH of live fish is just above 7.0, typically about 7.3, but this value falls markedly after death as the fish goes through rigor mortis and glycogen is converted to lactic-acid. In most species, the post mortem pH is between 6.0 and 6.8. In the present study, a gradual increase in pH values with storage time from 6.8 to 7.48 in control group during storage but in other treatments except incubated bacterial group pH was constant at 6±0.02 in refrigerated time Similar results of pH values on day zero of chilled storage were recorded by Sallam (2007) and Zambuchini *et al.* (2008), while Hernandez *et al.* (2009) reported higher pH values on day zero. Differences among the initial pH values than those reported in other studies may be due to the species, diet, method and time of preservation. pH values reported in the current study were significantly higher ($p<0.05$) in the last two safe treatments than in others. Higher pH values could be attributed to the increase in volatile bases such as ammonia produced by either microbial or muscular enzymes (Lu *et al*., 2010). As regards to the maximum acceptable limit of pH value (6.5) in fish and fish products recommended by EOS (2005), it was evident that a mix of NCH and NaA groups reduced on the 3^{rd}, 6^{th}, 12^{th} and 16^{th} day of storage. However, the samples are still acceptable according to the sensory evaluation results. These results are in accordance with that recorded by Ozyurt *et al.* (2009) that pH values above 7.1 are indicative of decomposition in fish.

Peroxide values and TBARS levels of 5 meq kg^{-1} and 2 mg kg^{-1} of flesh, respectively are regarded as the maximal permissible limit in fish muscle (Connel, 1995). In this study, the initial PV and TBARS values of filets samples were 1.06 meq kg^{-1} and 0.37mg MDA kg^{-1}, respectively. In control samples these parameters reached to the maximal permissible limit after day 6 to day 16 in PV (6.65+ 1.8 meq kg^{-1}) and TBARS (2.26±0.65 mg MDA kg^{-1}) test.; however the final

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PVs and TBARS values of T5 treated samples were within the maximum permissible limit value after 16 and 6 days, respectively from the beginning of the experiment. The data revealed that the combination of 2% NCH+1.5% NaA indicated preservation of fish flesh by inhibiting the oxidation of lipid. This fact was indicative of either a faster reduction of bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds (or both) due to the effect of nano chitosan and sodium acetate in the fish samples. Similar findings were reported by Haghparast *et al*. (2010) that sodium salts, particulary sodium acetate, have antioxidant properties.

The results of the sensory assessment of samples are given in Figs. 5-10. Samples were considered to be acceptable for human consumption until the sensory score reached 3 (Ojagh *et al*., 2010). As shown, until day 6, there was no significant difference in the overall acceptability among all treatment groups ($p<0.05$); however, compared with the fish fillets coated with Nch+ salt overall acceptability of the control samples decreased sharply from days 6 to 16, and they had significantly lower scores on days 10 to 16 ($p<0.05$).

Li *et al*. (2012) observed a significant decrease in the overall acceptability after 8 days of storage of untreated large yellow croaker, which agreed well with a concomitant shift in bacterial counts. Similarly, in this study, sensory evaluation results appeared to be correlated to microbial and chemical value analyses.

The effects of refrigerated storage (+4 °C) on microbiological, chemical, sensory properties of trout filet detected that the control group was safe in this status at 6 days and then all indexes were over the standard value. While the amount of LABs after the 12^{th} day was sharp, amix of chemical and bacteria index showed that day 6 was acceptable for cold storage but in NCH groups day 12 and in the NCH+ NaA group the last day of test was approved. In negative treatments (control) values exceeded standard values on the third day and day 6 and day 12 were affordable for NCHS+ PF and NCH+NaA+PF, respectively. Meanwhile, combinations of NCH+NaA improve fillets acceptability in sensory and biochemical factors during the preservation.

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