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# Evaluation the Effect of Aqueous Grape Extract in Nano – Chitosan – TPP on Chemical Properties of Surimi (*Clupeonella cultriventris*) in refrigerator

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## ABSTRACT

In this study, the effect of microencapsulated grape extract in chitosan nanoparticles (0.5:1 w/v ratio) on surimi prepared from Kilka fish was investigated. Physical properties of nanoparticles (particle size, zeta potential, and PDI) were 177.3 nm, +32.94 mV and 0.405 respectively. Also, morphological characteristics (SEM photo) were studied and the data showed that the produced nanoparticles were in a favorable condition. After preparation of surimi from Kilka fish, treatments included: Surimi (control) Surimi containing chitosan-surimi nanoparticles containing extract and surimi containing chitosan nanoparticles and extract were prepared and chemical factors of deterioration in surimi (pH, TVB-N, TBA and peroxide) were evaluated on different treatments days (0, 1, 3, 6 and 9) at refrigerator temperature. Also, sensory evaluation of treatments was done after cooking by hedonic method. The results showed that the changes in the chemical factors mentioned in the treatments containing chitosan nanoparticles and extract were reduced ($p<0.05$). Sensory evaluation of the use of chitosan nanoparticles along with extract showed a decrease in taste, odor and overall acceptance factors by the consumer. According to the results, The use of chitosan nanoparticles containing extract in surimi of Kilka fish can delay the deterioration of surimi and increase the shelf life of the product and improve organoleptic properties during refrigerated storage.

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## 1- Introduction

Grape is one of the native fruits of West Asia and is one of the oldest plant species that is widely cultivated all over the world. In addition to grape fruit, grape roots, leaves and seeds are widely used in the pharmaceutical industry. [1] Grapes are a perishable fruit with very low stability, which is very vulnerable during the storage period, drying, growth of microorganisms, loss of color, crispness and hydration of the texture are among the damages of grapes during storage. . Microbiologically, the presence of microorganisms on the surface of grapes can damage the safety and quality of the product. Therefore, the use of new methods that increase the shelf life and maintain the quality of grapes has been given much attention in the food industry.[2]

One of the most important advantages of herbal products, including extracts, is their biodegradability.Grape extract is one of the compounds rich in polyphenols such as: catechin, epicatechin, gallic acid and proanticyanidin, dimer, trimer, tetramer compounds such as: procyanidins, which show antioxidant and antimicrobial properties. It is known as an anti-virus and anti-cancer agent. [3]

Chitosan is a natural cationic biopolymer compound (carbohydrate) which is the second natural biopolymer in the world after cellulose and is composed of N-acetyl-D-glucosamine and D-glucosamine units, which are connected by β-1,4-glycosidic bonds with They are related to each other. Chitosan is a linear polysaccharide that is created from the removal of minerals and proteins from chitin and is widely used in the pharmaceutical and agricultural industries. [4]

The cationic properties of chitosan are due to the presence of amine groups, which react with the substances in the solution along with the hydroxyl group of chitosan. In terms of surface absorption ability, amine groups are more important than hydroxyl groups, and it is this amine group that improves the quality of the biopolymer. determines

(poly-b-(1-4)-D-glucosamine) and its derivatives alone in connection with biological or non-biological materials, a suitable material for use in coatings or nano or micro compounds, in order to achieve the goal of high durability in materials It is food. When antioxidants, enzymes and functional and antimicrobial agents such as plant extracts, minerals, probiotics and vitamins and natural and chemical substances are covered by chitosan (and its forms), a higher performance of them is seen. [5] Nowadays, due to the increase in the level of awareness of food consumers, the desire of people to use functional foods and foods containing bioactives has increased a lot. One of the most important advantages of herbal products, including extracts, is their biodegradability. One of the challenges ahead in the use of these beneficial substances is their instability, which are vulnerable to environmental factors such as light, oxygen and temperature, and the other is the persistence of their aroma and flavor in the food product, which affects the properties Its organoleptic has a negative effect. Micro-coating is one of the new and effective methods in maintaining the properties, performance and acceptability of these compounds. [6 and 7]

Nanotechnology, in fact, creates a variety of transfer systems for micro-encapsulated target substances inside capsules, protecting and controlling the release of various bioactives and micronutrients. These transport systems include micronutrients and bioactive compounds that are trapped inside nanoparticles with a size of less than 500 nm. Microcoating technique for nanoparticles loaded with bioactive compounds includes:evaporation,emulsification , coacervation inclusion complexation emulsification-solvent, nanoprecipitation ,supercritical fluid technique، and the range of particle size for each capsule is between 10-1000 nm in these methods.8]

Among the mentioned methods, the ionic gelation method is more effective and accessible due to its simplicity and lack of use of aqueous solvents and high heat. Bonding occurs through creating an electrostatic force between the positive charge of chitosan and the negative charge of tripolyphosphate (TPP). By changing the ratio of chitosan to TPP, the size and surface of chitosan nanoparticles changes. It is necessary to explain that no chemical reaction occurs in this method that causes the creation of toxic

substances. The basis of ionic gelation is the electrostatic reaction between the positive charge of the primary amino group of chitosan and the negative charge of the groups of polyanions such as TPP tripolyphosphate. The ionic gelation method is suitable for hydrophilic polymers, while in hydrophobic polymers, the technique of replacing the solvent by settling the preformed polymer is used with organic solvents: such as ethanol or acetone. [9]

Because seafood is a very rich source of nutrients, it has a special place in human nutrition and is highly popular. In many countries, the use of freshly caught fish is preferable to flavored and processed fish. [10]

Fish oil is an important source of unsaturated fatty acids with double bonds and omega-3, especially EPA and HDA. Because fish is rich in selenium, it has a significant effect in preventing heart attacks. [4 and 11]

The possibility of spoilage of fresh fish and its products is higher than other foods. Fish meat is affected by biological reactions that cause spoilage, such as: oxidation of fats, activity of enzymes and activities of microorganisms that are effective during a short period of storage. [12]

Today, in order to increase shelf life and prevent fish spoilage, different techniques are used, such as cooling the product immediately after catching and storing it in ice, packaging in vacuum and modified atmosphere, irradiation with gamma and UV rays, using preservatives such as organic acids and Salt of organic acids and artificial and natural preservatives (such as natural extracts and essences) are used. [3]

Kilka fish are among the valuable species of the Caspian Sea, which are important due to their high nutritional value, especially omega-3 unsaturated fatty acids. Studies show that the highest consumption of these fishes is for the production of fishmeal and their share of human consumption is low. Therefore, it is possible to increase the use of these fish by adopting different methods such as: preparation of canned food, marinade, various fermented products, etc. Among the species of this family, which include: common kilka, anchovy kilka and large-eyed kilka, which belong to the Caspian Sea. Common kilka (*Clupeonella knifeventris*) has the highest percentage of catch and can be considered as a candidate for the production of products such as surimi.[13]

Surimi was produced for the first time in Japan, which consists of myofibrillar proteins, which after washing and deboning and combining with cold protection materials, turns into a paste. This product is used in the production of seafood and other new products. Traditionally, surimi from the muscles of white fishهای*Alaska pollock (Theragrachalcograma)* Or*Pacific(Merluccius)* has been produced

Surimi is not consumed directly, but is used as an intermediate product in the production of a wide range of food products such as sausages, fish burgers, finger fish, shami, kebabs. Surimi production in 2014 and 2015 It has been more than 80 thousand tons, for its preparation, low-cost and cheap fish are usually used, because by turning this category of fish into value-added products, on the one hand, marine waste is reduced, and on the other hand, The loss of rich protein sources is prevented. One of the most favorable long-term methods for most foods, such as surimi, is freezing, which slows down the chemical and microbial reactions. After defrosting, the changes in the quality of surimi, especially the orientation of protein myofibrils and the reduction of the functional properties of these proteins, such as gel formation. Studies show that in some cases there is no need to use the freezing method and it can be kept in the refrigerator for a short period of time, in which case the use of various preservatives can be necessary to increase its shelf life. [13 and 10]

Considering the increasing development of the use of nanotechnology in the production of preservatives and improving the properties of bioactive compounds on a nanoscale compared to their original form, it is possible to use plant extracts to increase the shelf life and maintain the quality of marine products as well as the value. Nutritional and economic aspects of Caspian sea kilka fish, the present study was conducted with the aim of determining the effect of chitosan nanoparticles containing whole grape seed extract on the chemical factors indicating

spoilage of kilka surimi during the storage period of surimi in the refrigerator.

## 2- materials and methods

### 1-2- Preparation of aqueous grape extract

First, grape seeds (red grapes of the Qalat variety of Fars province).℃ 50 were dried in the oven for 30 minutes and ground until the stage of powder formation and passed through mesh sieve No. 35. Then the resulting powder was dissolved in deionized water at a ratio of 1 to 8 and placed in an ultrasonic bath device (model UP200 h, made in Germany) and kept at a temperature of℃ 55 and were exposed to 35 kHz power for 15 minutes. Then the extracted extract is passed through Whatman number five filter paper to separate the impurities. The obtained extract powder was stored in a closed container, away from light and humidity, to perform experiments. [14]

### 2-2- Preparation of chitosan nanoparticles (with and without extract)

To prepare chitosan nanoparticles, chitosan powder was first dissolved in 1% (volume/volume) acetic acid and after being placed in an ultrasonic bath for 60 minutes to make the solution clear, a 2% (weight/volume) chitosan solution was obtained. By NaOh (N4) solution, the pH of the solution was controlled between 3-4. TPP was dissolved in deionized water using a magnetic stirrer and at ambient temperature to obtain a 2% concentration of TPP solution. 4 ml of TPP solution was added to 100 ml of chitosan solution and it was stirred by a Mantaisi mixer at a speed of 700 rpm for 30 minutes to make the solution uniform, and the stirring process continued for another 30 minutes to complete the gelation stage. Found . The final concentration of chitosan was 2 mg/ml and the final concentration of TPP was 0.7 mg/ml.

The method of producing chitosan nanoparticles containing the extract is the same as the production of chitosan nanoparticles. After dissolving in deionized water with a volume/volume concentration of 0.75, the extract was added to the chitosan nanoparticles solution and stirred for 30 minutes by a magnetic stirrer, and the concentration of chitosan in the extract WithA ratio of 1 to 0.5 was obtained. After adding the extract, stirring continued for another 30 minutes to achieve complete gelation. The final concentration of chitosan was 2 mg/ml and the final concentration of TPP was 0.7 mg/ml [5, 15, 16, 17].

### 2-3- Measurement of particle size, zeta potential and relative particle distribution (PDI) of chitosan nanoparticles

First, 1 ml of the solution of chitosan nanoparticles and chitosan nanoparticles containing the extract was placed in an ultrasonic bath for 30 seconds and in order to measure the size of the particles ، PDI index and zeta potential were used from a zeta sizer device (Malvern ZS equipped with a He-Ne laser) at a wavelength of 633 nm, a power of 40 mW and a fixed scattering angle of °90, and the tests were performed in three repetitions.[15]

### 4-2-Evaluation of microcoating efficiency of nanoparticles containing extract

Nanoparticles suspension at a speed of 12,000 rpm, at a temperature of℃ 4, was centrifuged for 60 minutes. The absorbance of the uncoated essential oil in the range of 250-400 nm by ultraviolet-visible photometer and the standard curve of the extract in ethanol at a wavelength of 274 nm was prepared and the amount of free extract was determined using the equation of the standard curve ($R^2 = 0.999$, $y = 0.0067 x 0.0145$) was done. Finally, the microcoating efficiency of the extract was determined using equation 1:

Relationship 1:

(%) Microencapsulation efficiency = total amount of encapsulated extract / total amount of initial extract x 100

### 5-2- Morphological characteristics of nanoparticles

To determine the morphological characteristics of nanoparticles, SEM (JEOL, Japan, model 0 JSM 640) was used. For this purpose, 1 ml of the solution of chitosan nanoparticles and

chitosan nanoparticles containing the extract, with a weight ratio of chitosan to the extract of 0.5:1, was poured into 50 ml of double distilled water and the resulting mixture was placed in an ultrasonic bath for one minute. . 50 microliters of the above solution was poured and spread on a completely clean glass surface and finally dried at 30 degrees Celsius by oven under vacuum. Before observing the nanoparticles with SEM, the particles were covered with gold using a layering device and finally, the imaging of the samples was done by the SEM device with a power of 26 kW.

**6-2- Preparation of surimi**

In this research of common Kilkai fish*(Cultrienter)*The Caspian sea, which is part of the daily catch of Anzali port, was used. The samples were randomly selected from healthy samples without physical damage and of approximately the same size. (Length between 7-10 cm and weight between 7-10 grams) After washing with water, the samples were immediately transported to the laboratory in a Unilite box with ice in a 3:1 ratio of ice and fish. In order to prepare surimi, the head and tail of the samples were first cut, and after splitting the belly of the fish, the meat was separated from the spine bone and skin. Then it was made uniform by a meat grinder, then with water at approx℃ 5 in a ratio of 3:1, meat to water, washed and the resulting mixture slowly in water for 3 minutes℃ It was stirred for 4±1 and filtered by nylon mesh and washing was done in three complete stages and after each stage of washing, the suspended material on the water was thrown away and the resulting dough, after pressing and dewatering, became surimi. [10]

**7-2- Test** pH

To perform the pH test, first, 10 grams of the prepared surimi sample is homogenized with 90 ml of distilled water and measured by a pH meter in three repetitions. Other samples are prepared in the same way and measured in the machine. [18]

**8-2- Peroxide test**

To perform the peroxide test, first, 60 ml of chloroform, 60 ml of methanol and 30 ml of distilled water were added to 50 grams of surimi sample and mixed well. After 2 hours, the mixture could be separated into three phases, and 20 ml of the lower phase (chloroform phase) was transferred to a 250 ml flask. Then 25 ml of acetic acid and chloroform (in a ratio of 1:1) were added to 0.1 g of each sample and 1 ml of saturated solution of potassium iodide was added to the above mixture and left standing in the dark for 10 minutes. given . 20 ml of distilled water and 1 ml of 1% starch solution were added to the above mixture and then titrated with sodium thiosulfate (0.01 normal), the titration was continued until the blue color of the solution disappeared. Then, through equation 1, the amounts of peroxide in different samples were reported based on milliequivalents of peroxide per kilogram of fat. To prepare the control sample, all the above steps were done without adding surimi.

Relationship 1

(meq / Kg sample) peroxide index□ ( a – b )× N × 100 / W

In this regard, a and b are the sodium thiosulfate used for blank and sample titration, N is the concentration of sodium thiosulfate, and W is the weight of the sample. [18 and 19]

**9-2- Volatile nitrogen bases**TVB-N

To determine the volatile nitrogen bases, 10 grams of the sample along with 2 grams of magnesium oxide (MgO) powder were added to a Keldal flask by adding 500 ml of distilled water, and the resulting mixture was distilled into a solution consisting of 2% boric acid and 1-2 drops of methyl Rejection was added as an indicator. The resulting yellow solution was titrated with sulfuric acid until a purple color was obtained. The amount of volatile nitrogen bases was reported according to equation 2 and in terms of milligrams of nitrogen per 100 grams of surimi sample. [20]

Relationship 2:

Volatile nitrogen bases= 14 x the volume of sulfuric acid used

**10-2- Test**TBA

In order to measure the amount of TBA, first, 5 grams of surimi sample was mixed with 100 ml of trichloroacetic acid (10%) with a weight-volume ratio and it was homogenized for 30 seconds and passed through filter paper. 2 ml of the filtered mixture was added to 2 ml of TBA liquid, 0.02 M in a test tube and the resulting mixture was kept for 60 minutes in℃ 100 was placed in a greenhouse and the sample was cooled at ambient temperature and its absorbance at 530 nm was read using a spectrophotometer against a control sample containing distilled water and after drawing the Malon standard curve in aldehyde + 0.2195, $R^2$ = 0.9947) (y X=0.0676 was determined in terms of mg of malonaldehyde per kilogram of sample. In the control sample, 3 ml of cold trichloroacetic acid was used instead of the sample. [18, 21, 5]

**2-11- Sensory evaluation**

For sensory evaluation, first, 200 grams of surimi were cooked in a high power microwave (700 watts) for 10 minutes, and for sensory evaluation, 15 evaluators. It was trained in the age group of 25-27 years. In the sensory evaluation, aroma, smell, taste and general acceptance of the product were evaluated. The hedonic method was used for sensory evaluation, and 1-5 points were considered. A score of 5 is completely acceptable (good smell and taste of the fish and accepted by the judges) and 1, (unfavorable smell and taste and not accepted by the judges). A score of less than 3 points is considered critical. The evaluations were done on day 0 (control) and on the first, third, sixth and ninth days. [22, 19, and 5]

**12-2- Statistical analysis**

In this research, SSPS software (19) and Excel software were used for statistical data analysis and software drawing. The Kolnogorov-Simirnov test was used to normalize the data and then to determine the significance or non-significance of the differences. Tukey's test and one-way analysis of variance (ANOVA) (one level of 5%) were used between the numbers related to each index and the comparison of treatment averages.

## 3- Data discussion and analysis

**1-3 - Physical characteristics of nanoparticles**

The distribution of the particle size profile of chitosan nanoparticles and chitosan nanoparticles containing extract can be seen in graphs 1-a) and 1-b, the zeta potential of chitosan nanoparticles and chitosan nanoparticles containing extract can be seen in graphs 1-c and 1-d to be Chitosan nanoparticles have an average diameter of 208.5 nm and chitosan nanoparticles containing extract are 177.3 nm. Zeta potential of nanochitosan particles is +36.3 mV and chitosan nanoparticles containing extract is +32.94 mV.For the physical stability of the nano-suspension by electrostatic repulsion, a minimum zeta potential of ±30 mV is required. [5] Positive zeta potential increases the stability of nanoparticles and prevents their condensation (aggregation) and prevents adhesion well, and because of that, it indirectly causes reactions with the negative charge of the cell surface. . [16]

PDI of chitosan nanoparticles was 0.363 and chitosan nanoparticles containing extract was 0.405. PDI between 0.1 and 0.25 PDI of nanoparticles and above 0.5 shows their non-uniform distribution. [23]

**2-3- Morphology of nanoparticles and percentage of microcoating efficiency**

The percentage of microcoating efficiency in nanoparticles containing extract at a concentration of 0.5 to 1 extract to chitosan was determined to be 53.15%. In the morphological investigations, the sample of chitosan nanoparticles and chitosan nanoparticles containing the extract was used at a weight ratio of 1:0.5 of the extract to chitosan, which is shown in Figure 2(a) and 2(b), respectively.

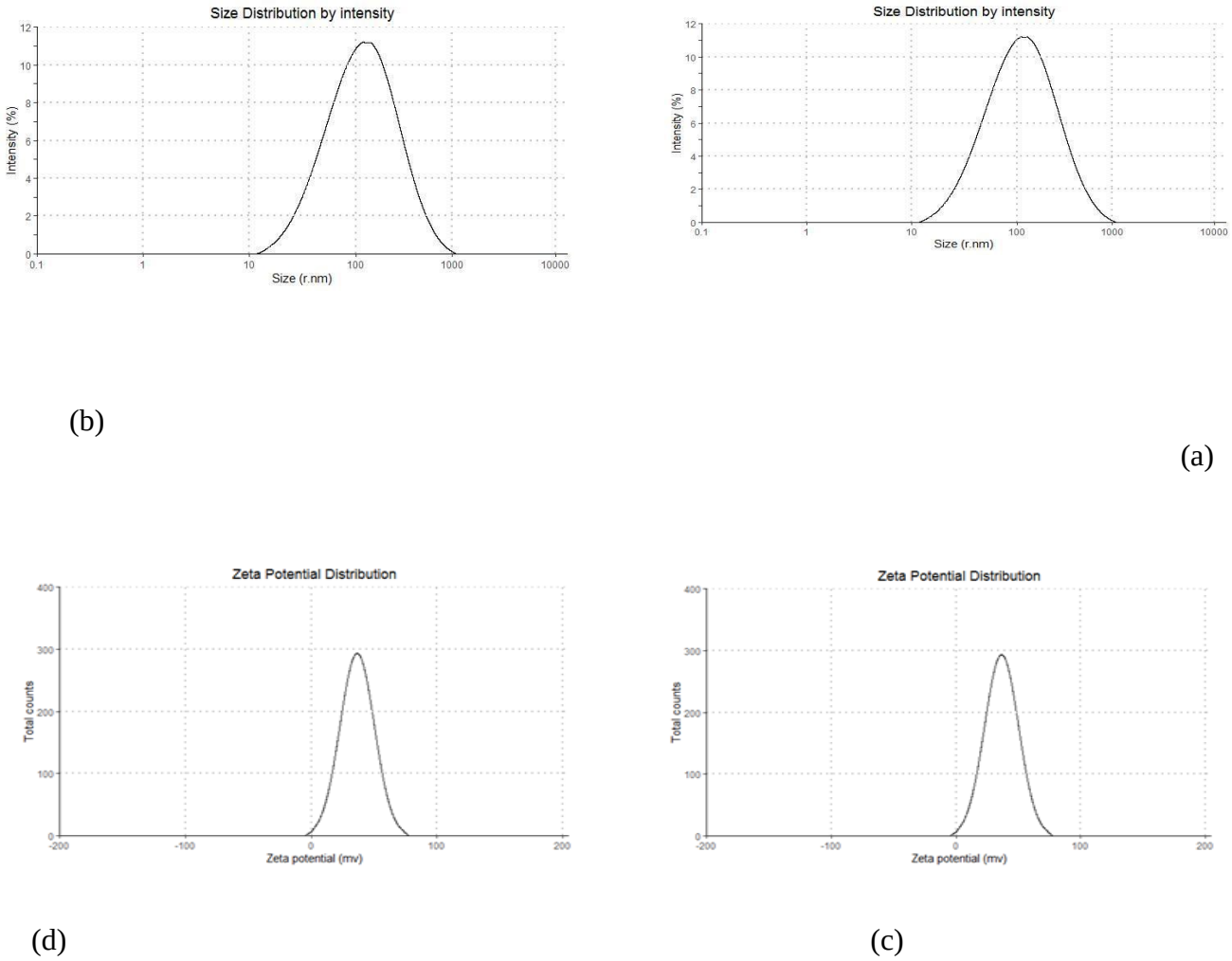


Figure 1 : size distribution intensity graphs of nanochitosan (a) and nanochitosan with extract (b) and zeta potential distribution intensity of nanochitosan (c) and nanochitosan with extract(d)

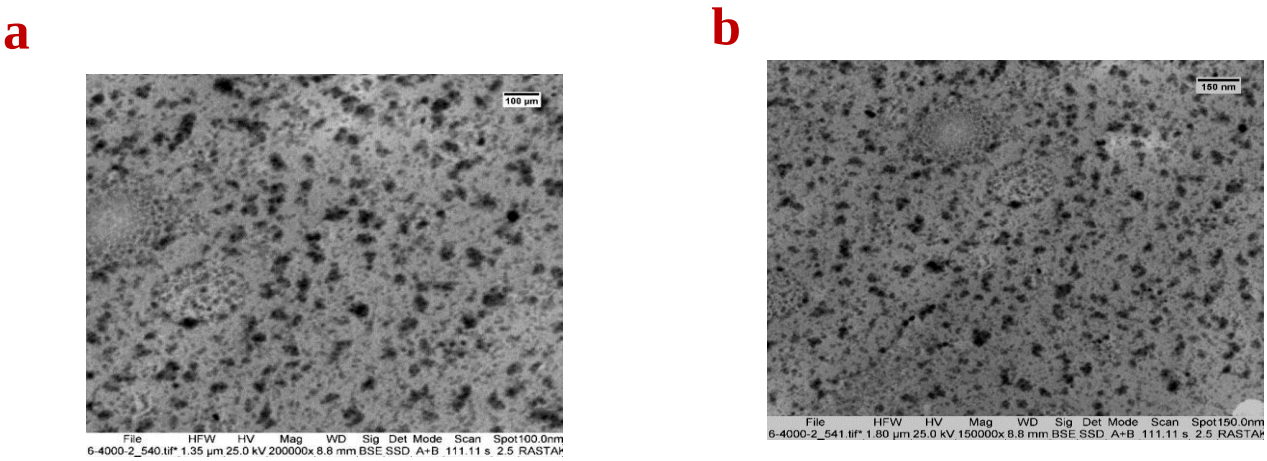


Figure 2 : SEM image of nanochitosan (a) and nanochitosan with extract(b)

As can be seen in Figure 2 (b), the particles are almost single and spherical, there is no high accumulation in the particles, and the size of the particles is about 150 nm. In both images, accumulation can be seen in some points, which may be caused by the softening of nanoparticles and the adhesion of adjacent particles to each other. Also, this adhesion can be attributed to the presence of extract in the surface parts of nanoparticles, which causes a reduction in surface charge. Reduction of interparticle repulsive force and as a result the accumulation of particles.[20]

**3-3-PH**

As shown in graph 3, in the case of the Surimi sample, pH shows a significant increase from day zero to day nine ($p<0.05$), the lowest value on day zero is 6.96 ± 0.03 and the highest value is 6.96 ± 0.03 On the ninth day, 7.23 ± 0.01 was observed. Regarding the surimi sample with chitosan nanoparticles, significant changes in pH were observed from day 0 to day 9. ($p<0.05$), the highest value was 7.45 ± 0.04 on the ninth day and the lowest value was 7.45 ± 0.04 on the sixth day. There was 7/94. In the surimi sample containing the extract, pH showed significant changes from day zero to day nine ($p<0.05$), the lowest value corresponding to day zero was 7.14 ± 0.02 and the first day was 7.13 ± 0.005 and the highest , corresponding to the ninth day, 7.27 ± 0.02 was observed. In surimi samples with chitosan nanoparticles containing extract, pH showed a significant decrease from day zero to day nine ($p<0.05$), the highest pH value on day zero was 7.18 ± 0.005 and the lowest value was on day nine. 6.14 ± 0 was observed.

The increase in pH values during cold storage can be considered due to the activity of Surimi enzymes such as lipase and protease [10] Another reason for the increase in pH is the increase in volatile amine levels in Surimi. [5] In the sample containing chitosan nanoparticles and chitosan nanoparticles containing extract, a decrease in pH values is observed with the passage of time, which can be attributed to the precipitation of alkaline, calcium, magnesium and sodium phosphate during the storage period. It was concluded that chitosan and chitosan nanoparticle containing the extract are effective in maintaining the pH of the product. [24 and 25] Also, due to the antimicrobial activity of nanoparticles, pH increase is prevented. [26] The lowest pH index was seen in chitosan nanoparticles containing extract, which can be due to the properties in grape extract that act on DNA and RNA due to special compounds and prevent the growth and metabolism of microorganisms (warm bacteria) Prevents positive and negative, mold and yeast.... [27]

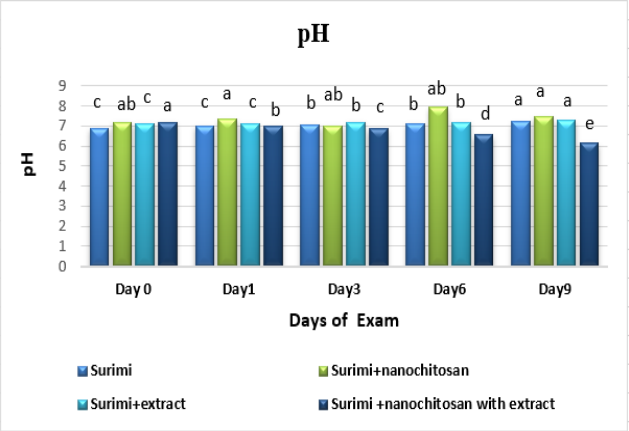


Figure 3 : change in pH value in different treatments during refrigerated storage . means with different small letters in the same day represent significant difference at ($p<0.05$)

**4-3- Peroxide**

As shown in graph 4, with the passage of time, the number of peroxide in surimi increases, which is significant ($p<0.05$). 0.0 and its highest value was observed on the ninth day, 5.56 ± 0.56. In surimi samples containing chitosan nanoparticles, the peroxide number increases significantly with the passage of time from day 0 to day 9 ($p<0.05$). The lowest amount of peroxide value is 0.78 ± 0.02 on the zero day and the highest value is 3.61 ± 0.24 on the ninth day. In surimi samples containing grape extract, the peroxide number goes through a significant increase from day 0 to day 9. ($p<0.05$), the lowest value of peroxide number corresponds to day zero, 1.04 ± 0.18, and the highest value on day 9. It was 3.45 ± 0.18. In surimi samples with chitosan nanoparticles containing extract, the

peroxide value goes through a significant increase from day zero to day nine. ($p<0.05$), the lowest value of peroxide number corresponding to day zero is 0.91 ± 0.12 and the highest It was 3.11 ± 0.05 on the ninth day. Peroxide is one of the primary products resulting from the oxidation of fats, which plays an essential role in the autoxidation of fats and their decomposition into carbonyls and other compounds. The results indicate that in the case of structured degradation products (such as surimi) during Cold storage, the standard of 10 milliequivalents of peroxide per kilogram of fat is acceptable.

Chitosan delays the oxidation of fats by chelating iron ions in the system, thus delaying the peroxidative activity or conversion to iron ions. According to reports, 1% of chitosan independently or in Binding to natural antioxidants is effective in reducing the oxidation of fats in frozen veal. [10] The phenolic compounds of the extract cause the formation of phenolic radicals by trapping free radicals in the first stages of oxidation and inhibit the oxidation of fish oils by delaying the spread of radicals. [19]

In the study of the effect of edible chitosan coating containing grape extract on the shelf life of rainbow trout fillets at refrigerator temperature, a decreasing trend in peroxide levels was reported. [18] also a research on the effect of chitosan on the shelf life of fish surimi*(pangasianodon hypophthalmus)* It was checked during the cold storage period and according to the present research, they confirmed the effect of chitosan on the reduction of peroxide number. [10] in investigating the effect of chitosan nanoparticles containing fennel essential oil on the shelf life of fish fillet*(Spindle spindle)* During the storage period, it was investigated and its reducing effect on the amount of peroxide value was reported. [19] Also, in two separate studies, they investigated the effect of different percentages of mint and oregano extracts on the short-term storage of ordinary Kilkay surimi in the refrigerator, and confirmed the effect of these two extracts on the reduction of peroxide number, which is similar to the present study. It is consistent. [13 and 21]

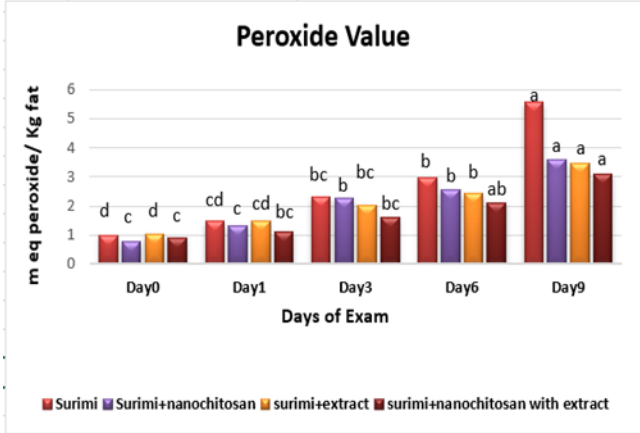


Figure4 : change in peroxide value in different treatments during refrigerated storage . means with different small letters in the same day represent significant difference at ($p<0.05$)

### 3-5- Measurement of thiobarbituric acid (TBA)

As shown in diagram 5, in the case of Surimi sample, TBA changes showed a significant increase from day zero to day nine ($P<0.05$), with the lowest value on day zero being 2.46 ± 0.15 and Its highest value was 4.46 ± 0.1 on the ninth day. In the surimi sample containing chitosan nanoparticles, TBA changes from day zero to day nine had a significant increase ($P<0.05$), its lowest value corresponds to day zero, 2.46 ± 0.41, and the highest value corresponds to day nine, 1/1 0 ± 4 was determined. Regarding surimi sample with extract, the amount of TBA showed significant changes from day 0 to day 9 ($P<0.05$), the lowest amount of TBA corresponding to day zero was 2.63 ± 0.3 and the highest amount was 16.0 on day 9. It is 3.56 ± 0. In the case of surimi sample with chitosan nanoparticles containing extract, TBA showed significant changes ($P<0.05$), the lowest amount of TBA corresponding to the first day was 1.96 ± 0.25 and the highest amount was 47.47 ± 0.26 on the ninth day. 2 showed. TBA is one of the diagnostic indicators

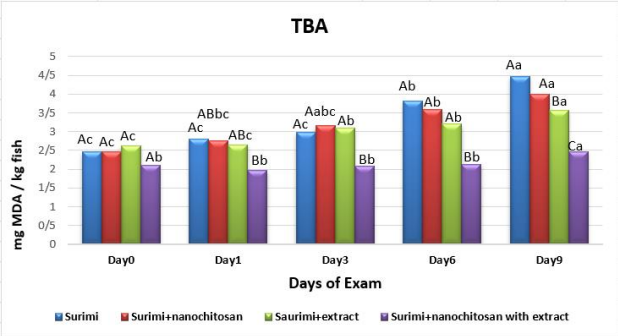


Figure5 : change in TBA value in different treatments during refrigerated storage . means with different small letters in the same day represent significant difference at ($p<0.05$).

The destruction of the product is caused by the oxidation of fats and the formation of hydroperoxide during storage. [10]

TBA is one of the secondary oxidation products that causes adverse changes in the sensory properties of the product. The standard set for TBA in fish is 4-10 mg of malon in aldehyde per kilogram of fish, and its acceptable limit for consumption 0.5 mg of malondialdehyde per kilogram of fish. In high quality fish products, TBA less than 3 mg of malondialdehyde per kilogram of fish, it has been determined that this amount should not exceed 5 mg of malondialdehyde per kilogram of fish. [19] The amount of TBA more than 1-2 mg of malondialdehyde per kilogram of fish indicates the beginning of unpleasant odor and taste changes in the product. [10] The reason for the increase in the amount of TBA during surimi storage in the refrigerator can be attributed to the loss of part of the fish muscle water and the oxidation of unsaturated fatty acids.

In the samples that only contain extracts, the increase in the TBA amount can be due to the increase in the amount of phenol aldehyde compounds that are caused by the decomposition and destruction of phenolic agents.[18] The reason for the decrease in the increasing trend of TBA in the samples containing chitosan gel Compared to the control, it can be considered due to the chelating effect of chitosan on the free ions that are released from the homoproteins during the heat process or during storage, therefore, it inhibits the oxidation of fat in the product. [10] The reason for the decrease in the increasing trend of TBA in surimi samples that contain chitosan nanoparticles in which the extract is loaded can be attributed to the effect of phenolic compounds and catechins in the extract, which have a high inhibitory power of free radicals and also chelate They are metallurgists. [28]

### 6-3- Measurement of volatile nitrogen bases(TVB-N)

As shown in Figure 6, in the case of the Surimi sample, the TVB-N factor increases from zero to the ninth day, and this increase is significant. ($P<0.05$) The lowest TVB-N in On day zero, it was 7.34±0.28 and its highest value was 23.13±0.23 on the ninth day.

In the case of the surimi sample with nanochitosan gel, the TVB-N factor increases from zero to the ninth day, and this increase is significant. ($P<0.05$) The lowest TVB-N on day zero is 0.29. ± 7.35 and its highest value was 23.13 ± 0.23 on the ninth day. In the case of surimi sample with TVB-N factor extract, it goes through an increasing process from zero to the ninth day and this increase is significant. 7.7 and its highest value was 13.23 ± 0.23 on the ninth day.

In the case of the surimi sample with nanoparticles containing TVB-N factor extract, it goes through an increasing trend from zero to the ninth day, and this increase is significant. ($P<0.05$), the lowest TVB-N on day zero is ±0.15 4.93 and its highest value was 19.53 ± 0.45 on the ninth day.

TVB-N is the product of the activity of enzymes in meat as well as bacterial spoilage, the amount of which can be an indicator to determine spoilage in fish.[13]

The presence of fish in cold conditions slows down the production of TVB-N compared to the conditions in which it is placed at ambient temperature. [3]

The lower presence of nitrogenous bases in Surimi can be due to the lower amounts of TVB-N in it. The higher bacterial loads observed in the control samples can be a justification for

increasing the amount of nitrogenous bases in them. The decrease in the amount of TVB-N in the treatments containing the extract loaded in chitosan nanoparticles can be due to the lower number of bacteria that decompose into trimethylamine, peptides, amino acids, etc. [10 and 27]

The decreasing trend observed in the treatments containing the extract loaded in nanoparticles can be due to the antimicrobial activity of chitosan and the extract in the microcapsule, which can cause a decrease in the bacterial population or the oxidative capacity of the bacteria. The TVB-N standard is mentioned in some sources as 35 mg of nitrogen per 100 grams of sample and in some sources as 30 mg of nitrogen per 100 grams of sample. [10] Among other reasons for the increase of TVB-N during storage, we can mention the increase in the activity of spoilage bacteria and the internal enzymes of bacteria. [5] During a research, the effect of chitosan on the durability of fish surimi*(pangasianodon hypophthalmus)* It was checked during cold storage and according to the present research, the effect of chitosan on the reduction process Addition TVB-N confirmed. [10] also the effect of chitosan nanoparticles containing fennel essential oil on the shelf life of fish fillet*(Spindle spindle)* During the maintenance period. They investigated and reported its decreasing effect on the increasing trend of TVB-N. [19] In another study, the effect of pomegranate and orange extract coating in connection with chitosan nanoparticles on increasing the quality of fillet.*(Silver Carp)* It was investigated during storage in the refrigerator and a decreasing effect on the increasing trend of TVB-N was seen, according to the present research. [5] also confirmed the reducing effect on the increasing trend of TVB-N during the coating of salmon with grape seed extract-carvacrol microcapsules in chitosan film. [26]

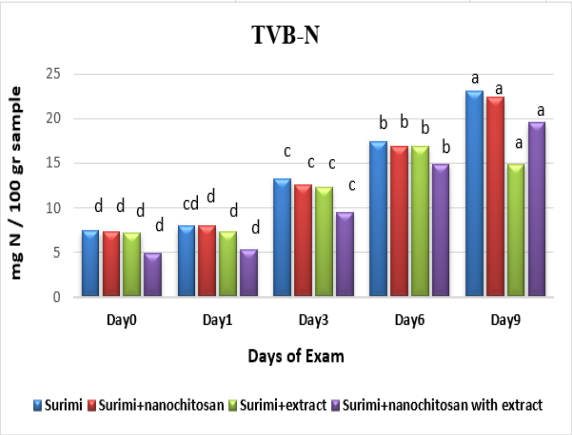


Figure 6 : change in TVB - N value in different treatments during refrigerated storage . means with different small letters in the same day represent significant difference at ($p<0.05$)

7-3 - Sensory evaluation

1-7-3- Determining the quality of sensory index of taste and flavor in different treatments during the storage period

In the surimi sample, the sensory index of taste decreases significantly over time, from day zero to day nine. ($p<0.05$) the highest score was on day 5 and the lowest score was 2.51 ± 0.27 on the ninth day. In surimi samples containing chitosan nanoparticles, with the passage of time from day zero to day nine, the sensory index of taste decreased significantly ($p<0.05$) so that the highest score on day zero was 5 and the lowest score on day nine was ±0.08. 3/59 was obtained. In the case of surimi samples containing extract, from day zero to day nine, there is a significant decreasing trend ($p<0.05$) in the sensory index of taste and taste, to. The highest score on the zero day was 5 and the lowest score was 3.42 ± 0.17 on the ninth day. In the case of surimi samples with chitosan nanoparticles containing extract, the sensory index of taste decreases significantly from day zero to day nine ($p<0.05$), the highest score on day zero is 5 and the lowest score is 13/±0.08. 4 was observed on the ninth day.

As shown in Table 1, the sensory changes of taste and taste in all treatments on the zero, first and third days had non-significant changes ($p>0.05$), but on the sixth day of storage in the refrigerator, there was a significant difference

between all treatments. It was observed ($p<0.05$) on the ninth day of storage between surimi treatments containing chitosan nanoparticles and surimi containing extract, non-significant sensory changes in taste and taste ($p>0.05$) and significant changes between surimi and surimi treatments with chitosan nanoparticles containing extract. ($p<0.05$) was observed.

3-7-2- Determining the quality of odor index in different treatments during the storage period

The sensory index of smell in surimi samples decreases significantly with the passage of time from day zero to day nine ($p<0.05$). The highest value of this index corresponds to day zero and the lowest value corresponds to day nine, 2.55 ± 0.22. In the case of surimi samples containing chitosan nanoparticles, there is a significant decrease in the sensory index from day 0 to day 9 ($p<0.05$). 3 were observed. In the case of surimi samples with extract, the sensory index of smell decreases significantly with the passage of time from day 0 to day 9 ($p<0.05$). The highest value of this index was on day 05 and the lowest value was 3.68 ± 0.15 on day 9. Was seen .

Regarding surimi samples with chitosan nanoparticles containing extract, the sensory index of smell shows a significant decrease from day 0 to day 9 ($p<0.05$). 4.12 ± 0.00 was determined

As shown in Table 2, the sensory changes of smell on the zero day and the first day of the storage period were non-significant ($p<0.05$). On the third day, the changes between surimi treatments and surimi containing chitosan nanoparticles were non-significant ( $p>0.05$) and significant ($p<0.05$) was determined between the treatments of surimi containing extract and surimi along with chitosan nanoparticles containing extract. On the sixth day, surimi and surimi treatments containing nanoparticles showed non-significant changes ($p>0.05$) and in the treatments of surimi containing extract and surimi together with chitosan nanoparticles containing extract, there were no significant changes ($p>0.05$) and compared to the previous two treatments. were determined to be significant ($p<0.05$), also on the ninth day, the changes of this index in all treatments were determined to be significant ($p<0.05$).

3-7-3- Determining the overall acceptance index of different treatments during the maintenance period

The sensory index of smell in surimi samples decreases significantly with the passage of time from day zero to day nine ($p<0.05$). The highest value of this index corresponds to day zero, 5, and the lowest value corresponds to day nine, 2.55 ± 0.22. In the case of surimi samples containing chitosan nanoparticles, the sensory index of smell has a non-significant decrease from day 0 to day 9 ($p<0.05$). 3 was observed. In the case of surimi samples with extract, the sensory index of smell decreases significantly with the passage of time from day 0 to day 9 ($p>0.05$). The highest value of this index is on day 05 and the lowest value is 0.13 on day 9. ± 3.8 was seen. Regarding the samples of surimi with chitosan nanoparticles containing extract, the smell index shows a significant decrease from day zero to day nine ($p<0.05$), the highest value of this index on day zero is 5 and the lowest value is on day nine. 0 ± 1.4 was determined. As shown in Table 3, the changes in the quality of overall acceptance in the treatments showed non-significant changes ($p>0.05$) on the zero day, the first day and the third day. On the sixth day and the ninth day, the changes between the treatments were as significant ($p<0.05$) was observed.

Table1 : change in taste value in different treatments during refrigerated storage . Means with different small letters in the same day represent significant difference at ($p<0.05$)

| Treatments<br>Day of exam | Surimi | Surimi + nanochitosan | Surimi + extract | Surimi + nanochitosan with extract |
|---|---|---|---|---|
| Day 0 | $^{Aa}$ 0 ± 5 | $^{Aa}$ 0 ± 5 | $^{Aa}$ 0 ± 5 | $^{Aa}$ 0 ± 5 |
| Day 1 | $^{Aa}$ 0 ± 5 | $^{Oops}$ 0 ± 5 | $^{Aa}$ 0± 5 | $^{Aa}$ 0± 5 |
| Day 3 | $^{Ab}$ 0.22 ± 4.23 | $^{Aabc}$ 0.05 ± 4.55 | $^{Ab}$ 0.1± 4.38 | $^{Oops}$ 0.53 ± 4.61 |

| | | | | |
|---|---|---|---|---|
| Day 6 | And 0.23 ± 3.42 | Abc 0.10 ± 4.07 | ABc 0.05 ± 4.1 | Oops 0.1 ± 4.46 |
| Day 9 | Cd 0.27 ± 2.51 | Bc 0.08 ± 3.59 | Bd 0.17 ± 3.42 | Ab 0.08 ± 4.13 |

Table2 : change in odor value in different treatments during refrigerated storage . means with different small letters in the same day represent significant difference at ($p<0.05$)

| Treatments<br>Day of exam | Surimi | Surimi + nanochitosan | Surimi + extract | Surimi + nanochitosan with extract |
|---|---|---|---|---|
| Day 0 | Aa 0 ± 5 | And 0 ± 5 | Aa 0 ± 5 | Aa 0 ± 5 |
| Day 1 | Aa 0 ± 5 | Abc 0 ± 5 | Aa 0± 5 | Aa 0± 5 |
| Day 3 | BCb 0.03 ± 4.26 | Bb 0.11 ± 4.49 | Cb 0.12± 4.16 | Ab 0.07 ± 4.78 |
| Day 6 | Bc 0.09 ± 3.26 | Bc 0.15 ± 3.35 | And 0.16 ± 3.78 | And 0.1 ± 4.08 |
| Day 9 | 0.22 Dd ± 2.55 | Cd 0.06 ± 3.05 | Bc 0.15 ± 3.68 | And 0.06 ± 4.12 |

Table3 : change in total acceptance by consumers in different treatments during refrigerated storage . means with different small letters in the same day represent significant difference at ($p<0.05$)

| Treatments<br>Day of exam | Surimi | Surimi + nanochitosan | Surimi + extract | Surimi + nanochitosan with extract |
|---|---|---|---|---|
| Day 0 | Aa 0 ± 5 | And 0 ± 5 | Aa 0 ± 5 | Aa 0 ± 5 |
| Day 1 | Aa 0 ± 5 | Aa 0 ± 5 | Aa 0± 5 | Aa 0± 5 |
| Day 3 | Aa 0.26 ± 4.62 | Ab 0.21 ± 4.6 | Ab 0.29± 4.46 | Aa 0.16 ± 4.76 |
| Day 6 | Bb 0.25 ± 3.22 | And 0.15 ± 4 | And 0.04 ± 4.07 | Ab 0.1 ± 4.23 |
| Day 9 | 0.14 Cb ± 3 | ABc 0.1 ± 3.82 | Bc 0.13 ± 3. 8 | Ab 0.06 ± 4.1 |

a research on the edible coating of chitosan containing grape seed extract on the shelf life of rainbow trout fillets during storage at refrigerator temperature, when the fish fillets were coated with grape seed extract and in terms of color, smell and overall acceptability up to six days did not improve. After six days, signs of spoilage appeared and scores tended to be less than 5. When grape seed extract was added to the samples, the sensory scores improved significantly until day nine, on the last day (day 15 ) the samples containing chitosan and extract got a good score, while the uncoated samples got a poor score, which is consistent with the results

of the present study. This issue can be related to the unique activity in improving texture and sensory characteristics as well as the absence of smell and aroma in meat products. [18] In

another study on the effect of pomegranate and orange peel extract along with nano-chitosan in fish fillets, the data showed that no significant difference was observed between the control samples and other treatments until the sixth day, but from the sixth day to the twelve related points There was a big drop in the overall acceptance of the sample, which was mainly observed from the eighth day onwards, which could be due to the change in the chemical and microbial characteristics of the samples. that this process of changes in scores is consistent with the current research.[5]

## 4- General conclusion

Based on the results of this research, the use of chitosan nanoparticles containing whole grape seed extract in Kilka fish surimi reduces the rate of its chemical spoilage during storage at refrigerator temperature. In the surimi samples containing nanoparticles, spoilage agents at the nd of the storage period (9th day) were significantly less than other control samples, and the taste, aroma and smell factors and the overall acceptance of the product by the customer were improved. Therefore, according to the mentioned findings, chitosan nanoparticles containing grape extract can be used as a natural preservative in Kilka surimi.

## 5- Resources

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**مجله علوم و صنایع غذایی ایران**

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مقاله علمی_پژوهشی

# بررسی اثر عصاره آبی دانه انگور در نانوذرات کیتوزان – تری پلی فسفات بر روی خصوصیات شیمیایی سوریمی تهیه شده از ماهی کیلکا در طی دوره نگهداری در یخچال

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**چکیده**

در این پژوهش تاثیر عصاره دانه کامل انگور ریزپوشانی شده در نانوذرات کیتوزان (به ترتیب به نسبت ۰,۵ به ۱ وزنی / حجمی ) بر روی سوریمی تهیه شده از ماهی کیلکا مورد بررسی قرار گرفت . خصوصیات فیزیکی نانوذرات ( اندازه ذرات ، پتانسیل زتا و PDI به ترتیب : ۱۷۷/۳ nm ، ۳۲/۹۴+ mv و ۰/۴۰۵ تعیین گردید . همچنین خصوصیات مورفولوژی ( عکس SEM ) مورد بررسی قرار گرفت و داده ها نشانگر این بودند که نانوذرات تولیدی در وضعیت مطلوبی قرار دارند . پس از تهیه سوریمی از ماهی کیلکا ، تیمارها شامل : سوریمی (شاهد ) – سوریمی حاوی نانوذرات کیتوزان – سوریمی حاوی عصاره و سوریمی حاوی نانوذرات کیتوزان به همراه عصاره آماده گردید و فاکتورهای شیمیایی نشانگر فساد در سوریمی (pH ، TVB-N ، TBA و پر اکسید ) بر روی تیمارهای مختلف در روز های آزمایش ( روز صفر ، اول ، سوم ، ششم و نهم ) در دمای یخچال مورد بررسی قرار گرفت . همچنین ارزیابی حسی تیمارها پس از پخت با روش هدونیک انجام پذیرفت . نتایج نشانگر این بودند که تغییرات فاکتورهای شیمیایی ذکر شده در تیمارهای حاوی نانوذرات کیتوزان به همراه عصاره در مقایسه با سایر تیمارها کاهش ($p<0.05$) یافته است . در خصوص ارزیابی حسی استفاده از نانوذرات کیتوزان به همراه عصاره افت کمتری را در فاکتورهای طعم و مزه ، بو و پذیرش کلی محصول توسط مصرف کننده نشان داد . با توجه به نتایج بدست آمده ، استفاده از نانوذرات کیتوزان حاوی عصاره در سوریمی ماهی کیلکا ، موجب به تاخیر انداختن فساد سوریمی و افزایش ماندگاری محصول و بهبود خواص ارگانولپتیکی در طی دوره نگهداری در یخچال می شود .