



Scientific Research

## Physicochemical characteristics and mineral analysis of white sugar during Operation

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ARTICLE INFO	ABSTRACT
<p><b>Article History:</b> Received:2024/1/24 Accepted:2024/3/16</p>	<p>The purpose of this research was to prepare nanocomposite film based on wheat gluten containing quercetin nanoliposome and zinc oxide nanoparticles and use it to package Rainbow trout; For this purpose, in this research, the effect of quercetin nanoliposome at the levels (0, 5, 10% by volume) and zinc oxide nanoparticles at the levels (0, 3, 6% by weight) using the response surface method in The central composite design template was investigated on the physicochemical properties of the nanocomposite film, and finally, the optimal sample was used in the packaging of Rainbow trout meat to check its properties during the storage period (0, 3 and 6 days). Also, the results of the research on packaged fish meat showed that the use of wheat gluten nanocomposite film containing 10% quercetin nanoliposomes and 6% zinc oxide nanoparticles led to a decrease in peroxide index, volatile nitrogen compounds index, thiobarbituric acid index and total microbial count during the storage period. Became. Also, no significant difference was observed in the fat of the examined samples, and the color of the packaged fish meat was duller than the control sample. Finally, according to the obtained results and investigations, the addition of 10% quercetin nanoliposome and 6% zinc oxide nanoparticles in the wheat gluten nanocomposite film formulation led to the improvement of the properties of the produced film and the properties of the packaged fish during the storage period.</p>
<p><b>Keywords:</b> wheat gluten, zinc oxide nanoparticles, quercetin nanoliposome, Rainbow trout</p>	
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## 1-Introduction

Rainbow trout is a cold water fish and belongs to the open fish family. Its scientific name is *Oncorhynchus mykiss* and its English name is Rainbow trout [1]. Rainbow trout meat is rich in protein, vitamins (K, E, D, A) and minerals (magnesium, calcium, copper, iodine, phosphorus, sulfur, potassium and zinc) and is a rich source of essential amino acids (lysine, leucine, arginine, histidine, valine, tryptophan, etc.) and essential fatty acids (linolenic acid, arachidonic acid and linoleic acid) [2]. Due to the chemical composition of fatty acids with several double bonds and high percentage of protein, fish is one of the fastest perishable foods, and its storage in inappropriate conditions causes the occurrence of enzymatic and microbial activities in fish meat and causes spoilage and reduction in the quality of fish meat. One of the most important types of spoilage in fish meat is autolytic spoilage, bacterial spoilage and chemical spoilage. One of the chemical compounds in the fish body that has a high nutritional value is unsaturated fatty acids [3]. Fatty acids are oxidized in the vicinity of air and lose their properties. Also, fatty acids with adverse changes in the taste and smell of products cause the appearance of signs of corruption in the product and make it unusable. Due to the fact that many fish have large amounts of polyunsaturated fatty acids, therefore, oxidative speed is the most important problem in seafood processing technology [4]; Therefore, according to the explanations provided to prevent adverse changes in fish meat, in recent years, active antimicrobial and smart packaging have been considered as a food safety barrier technology. Edible films and coatings have good mechanical properties, gas and moisture barriers, and when applied to meat and meat products, generally show a positive effect on the sensory properties of the products [5]. Edible films are thin layers of biopolymer materials with a thickness of 250 micrometers. These coatings are placed on the surface or between food components and act as a barrier against the transfer of

materials [6]. One of the most important advantages of edible films is that they can act as a carrier for various additives and compounds such as antimicrobial substances, antioxidants, etc., which in this case are called active packaging. Active packaging is a type of packaging that, in addition to having the main deterrent properties of conventional packaging (such as the deterrent properties against gases and water vapor and mechanical stresses), by changing the packaging conditions, the safety and shelf life or sensory characteristics of the food improve and at the same time maintain the quality of the food [7]. Also, the efficiency and characteristics of this type of packaging depend on the inherent characteristics of the ingredients of the film, i.e. biopolymers (such as proteins, carbohydrates and lipids), softeners and other additives [8]. Therefore, the purpose of this research is to investigate the preparation of quercetin nanoliposome and its use in the preparation of nanocomposite film based on wheat gluten containing quercetin nanoliposome and zinc oxide nanoparticles for the packaging of Rainbow trout meat.

## 2-Materials and methods

### 1-2- Materials

Rainbow trout from Urmia local market (Iran), wheat gluten (75-82% protein) from Golmehr Kermanshah Company (Iran), zinc oxide (20 to 40 nm) from Sepid Oxid Zanjan Company (Iran) and quercetin from FZBIOTECH Company (China) were prepared. Other required chemicals were purchased from Merck (Germany).

### 2-2- Preparation of nanocomposite film

To prepare the studied nanocomposite film, first, 30 grams of wheat gluten powder and 9.9 grams of glycerol were mixed in 135 ml of ethanol using a magnetic stirrer; Then 90 ml of deionized water was slowly added to the resulting homogeneous solution and 0.1 M sodium hydroxide was added to the solution until the solution reached 11 pH. The obtained solution was heated to 75°C for 20 minutes and stirred for 10 minutes under these conditions. Then the solution was

poured into Falcon and centrifuged at 4000 rpm for 20 minutes. The clear supernatant solution was separated from the insoluble part as the basis of the film. Two combinations of quercetin nanoliposome and zinc oxide nanoparticles were added to the solution according to the statistical scheme of Table 1 and stirred for 30 minutes until a uniform solution was obtained. And the D9112 homogenizer (Hidolf, Germany) was used to homogenize the solution for 2 minutes at 13,000 rpm. The final prepared solution was poured onto 9.5 cm plates and dried for 1 day at 25°C and 50% humidity. All the prepared films were kept in special zipped plastic bags in the dark and at a temperature of 4 °C until the tests were performed [9].

### 3- Tests of fish meat

#### 1-3- Measurement of meat fat

The fat content of rainbow trout fillet was measured by the Soxhlet method based on the method of Karimi Sani et al. 2021 [10].

#### 2-3-Measurement of total volatile basic nitrogen (TVB-N)

In order to measure the amount of volatile nitrogen, 10 grams of samples were mixed with 2 grams of magnesium oxide by adding 500 ml of distilled water in a Kjeldahl flask, and the desired extract was added to a solution consisting of 2% boric acid and 1-2 drops of methyl red as an indicator. The resulting yellow solution was titrated with sulfuric acid until a purple color was obtained and expressed as mg of nitrogen per 100 grams of fish meat sample [11].

#### 3-3- Measurement of thiobarbituric acid reactive substance (TBARs)

First, 1 gram of the sample was homogenized in the presence of 5 ml of 5% trichloroacetic acid aqueous solution and 5 ml of BHT<sup>1</sup> solution in hexane with a concentration of 0.8 g/100 ml. Then the present mixture was centrifuged at 3000 rpm for 10 minutes and after discarding the upper layer, 2.5 ml of the lower layer was mixed with 1.5 ml of 2-thiobarbituric acid aqueous

solution with a concentration of 0.8 g/100 ml. It was mixed and the resulting mixture was placed in a hot water bath at 37 °C for 30 minutes. The optical absorbance of the resulting mixture after cooling was calculated at a wavelength of 532 nm and the thiobarbituric acid number was calculated based on micrograms of malondialdehyde per kilogram of the sample using the standard substance 1, 1, 3, 3-tetraethoxypropane [10].

#### 3-4-Measurement of peroxide value

0.3 grams of sample were vortexed with 9.8 ml of chloroform-methanol for 2-4 s. Then 0.05 ml of 10 mM ammonium thiocyanate solution was added to it and vortexed for 2-4 s. After adding 0.05 cc of iron (II) solution, vortexing was done again for 2-4 s. After 5 minutes of incubation at room temperature, light absorption was read at a wavelength of 500 nm [12].

#### 3-5- Identification and counting of total bacteria

In order to determine the microbial load, 5 grams of the sample was transferred to a sterile Stomacher bag with 45 ml of distilled water and homogenized by a Stomacher machine (Stomacher400 manufactured by Seward, England). Then the sample was diluted to a dilution of 10<sup>5</sup> ml. 1 ml of each dilution was placed on the plate and the culture medium of the plate count agar (PCA) was added to it. Each plate was carefully shaken in order to distribute the sample homogeneously. After a few minutes, all the plates were turned upside down and placed in an incubator for 48 hours at a temperature of 37°C. After 48 hours, all colonies were counted [13].

#### 3-6-Measurement the color characteristics of the optimal film in packaging as a color sensor

In this method, during the storage period, the changes to the nanocomposite film for fish meat packaging were measured with a CIE colorimeter (Minolta CR300 Series, Minolta Camera Co. Ltd., Osaka, Japan) and then the data were recorded and

<sup>1</sup> Butylated Hydroxy Toluene

the color changes of the optimal film as a color sensor with the passage of storage time were visually observed.

#### 4-Statistical analysis

The statistical analysis in this research was done in two parts.

First part: the gluten nanocomposite film containing zinc oxide nanoparticles and quercetin nanoliposome is prepared, and then the effect of two variables, the percentage of quercetin nanoliposome and the percentage of zinc oxide nanoparticle, each at three levels in 13 treatments by the statistical software Design expert 11 according to the table below with The response surface method (RSM) of the central composite design was investigated on the properties of the produced film. Also, after analyzing the data, the optimal films were selected based on the factorial plan and the effect of 1) blank film, 2) the interaction effect of both variables with the highest value on the film were investigated.

Second part: the optimal film prepared in the first part was used for the active and intelligent packaging of fish meat, and the effect of storage time and the presence or absence of active film according to Table 1 on the quality characteristics of fish meat were investigated.

**Table 1. Statistical analysis of film samples prepared for packaging Rainbow trout meat**

Sample	Blank	Max (10MI) + Max ZnO (6%)	NL + -
Time (day)	0	3	6

#### 1-4- The results of packaged rainbow trout meat tests

##### 2-4- Thiobarbituric acid index

The results of thiobarbituric acid index of nanocomposite film samples based on wheat gluten in packaged fish meat are presented in graph1-a. As can be seen, according to the results of statistical analysis,

with the passage of time up to 6 days, the index of thiobarbituric acid in coated fish samples increased significantly ( $P \leq 0.05$ ); So that this increase in samples covered with film containing 10% quercetin nanoliposome and 6% zinc oxide nanoparticles was less than the control sample; that the cause of this case can be due to the synergistic and positive effect of quercetin nanoliposome and zinc oxide nanoparticles on reducing this index during the shelf life of packaged fish meat; On the other hand, the result was consistent with the results of the antioxidant activity of the edible film, and the reduction of this index can be attributed to the extraordinary activity of inhibiting free radicals of the prepared edible film and reducing the rate of oxidation of lipids, unsaturated fatty acids and the production of secondary metabolites (secondary oxidation of lipids). Ratio during storage time [14, 15]. Similar results in this regard were reported by Zheng et al. (2022), who stated that the increase in thiobarbituric acid index was reduced in pork samples packaged with chitosan/coix seed starch based films containing zinc oxide nanoparticles and [16]. Also, Lou Artemisia annua essential oil et al. (2021) stated that films containing quercetin effectively reduced the thiobarbituric acid index in cod fish during storage, compared to films based on kaferin and pure polyethylene [17].

#### 3-4- Index of volatile nitrogen compounds

Volatile nitrogen compounds are one of the main factors for evaluating the freshness of meat. Changes in the index of volatile nitrogen compounds of fish samples coated with nanocomposite film based on wheat gluten during 6 days of storage are shown in graph1-b. According to the results of statistical analysis, with the passage of time, the amount of volatile nitrogen compounds in the samples increased significantly ( $P \leq 0.05$ ). On the other hand, with the addition of 10% quercetin nanoliposome and 6% oxy-nanoparticles, a significant decrease in the amount of nitrogen compounds was observed compared to the control sample

( $P \leq 0.05$ ). The results indicate the fact that the compounds used in the formulation of the prepared edible film, in addition to having antimicrobial properties, lead to the reduction of nitrogenous compounds and substances and also reduce the accumulation of non-proteinaceous compounds such as ammonia, dimethylamine and trimethylamine (primary and secondary amines). And third) during maintenance [15, 18]. In this regard, Khan et al. (2023) reported favorable results for chicken meat using a film based on *Artemisia sphaerocephala* Krasch containing zinc oxide nanoparticles and forsythia essential oil. Also, Lou et al. (2021) also expressed similar results in films containing quercetin compared to films based on kaferin and pure polyethylene in cod fish [19].

#### 4-4- Peroxide value

Peroxide is a good indicator to identify the lipid oxidation process in meat. The results of measuring the peroxide value of rainbow trout samples wrapped in nanocomposite film are presented in graph 1-c. As can be seen, with the passage of time up to 6 days, the peroxide index of fish samples increased significantly ( $P \leq 0.05$ ). But this increase in the sample, containing 10% quercetin nanoliposome and 6% zinc oxide nanoparticles was less than the control sample; So that this is mainly due to the reduction of hydroperoxides formation, reduction of oxidation and high antioxidant activity by these two compounds used [20]. Similar results presented in this context by Feng et al. (2019) stated that the effect of antioxidant and antimicrobial coating based on whey protein nanofibrils with TiO<sub>2</sub> nanotubes led to the reduction of cold meat peroxide compared to other samples [21]. In addition, Tongdeesontorn et al. (2021) reported similar results regarding peroxide reduction in antioxidant films based on cassava starch/gelatin biocomposite enriched with quercetin and TBHQ in pork [22].

#### 4-5- fat

The results of fat measurement of fish meat samples packed with nanocomposite film are given in graph 1-d. As can be seen,

there was no significant difference in the amount of fat in the control sample and other fish samples packed with films containing 10% quercetin nanoliposome and 6% zinc oxide nanoparticles ( $P > 0.05$ ).

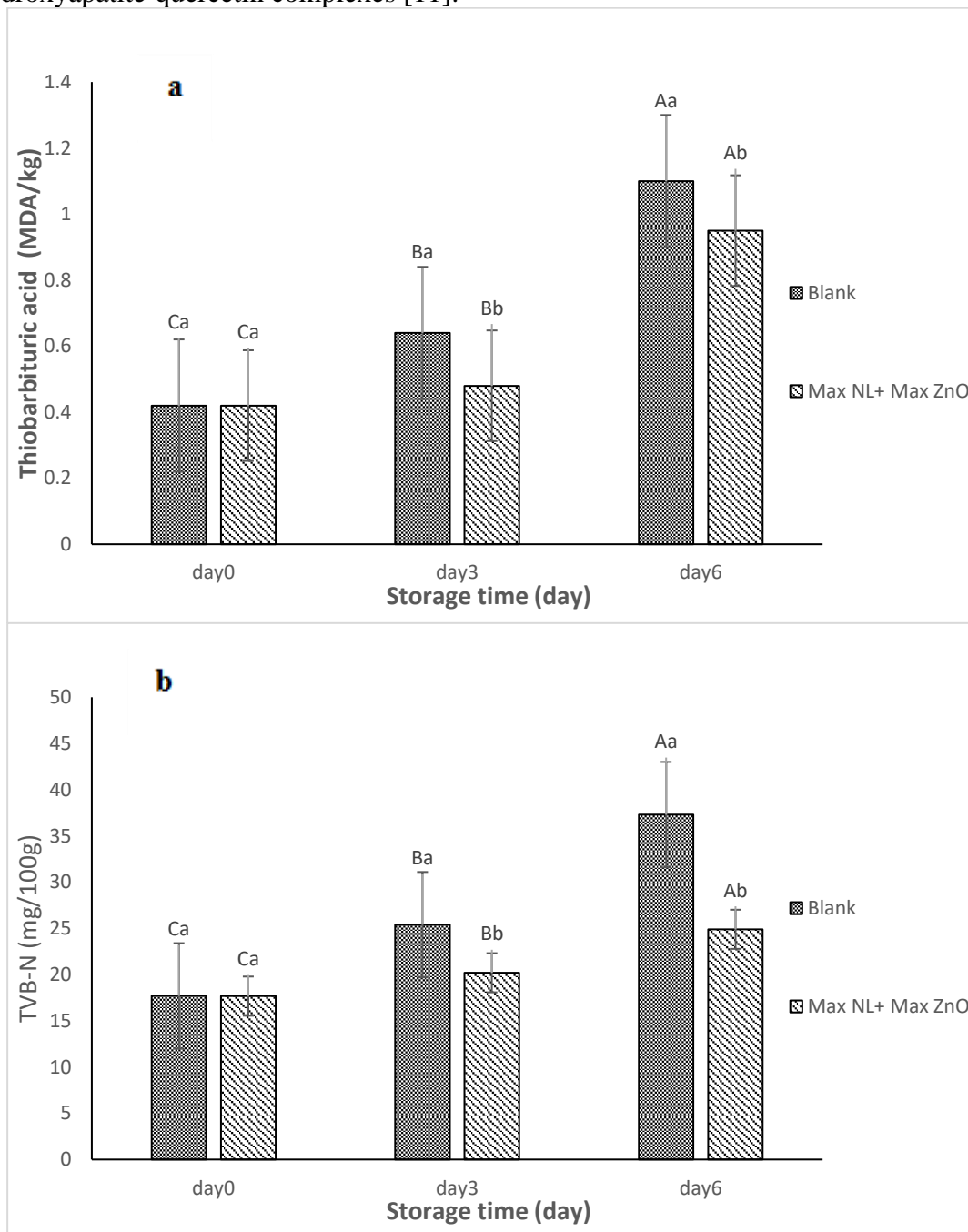
#### 6-4- Colorimetry

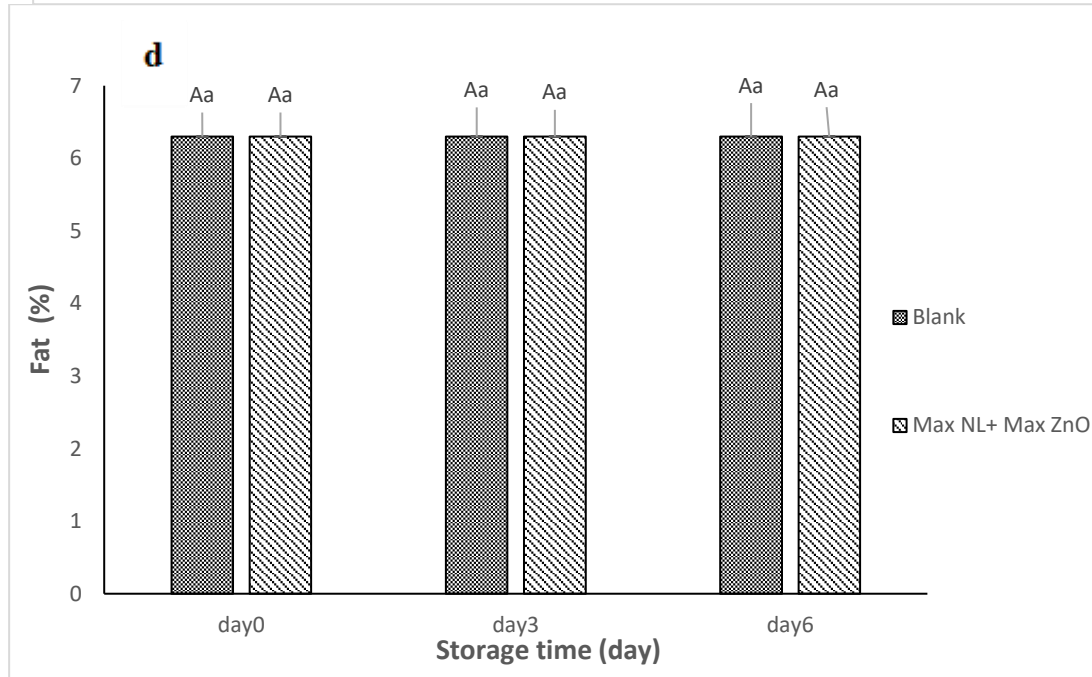
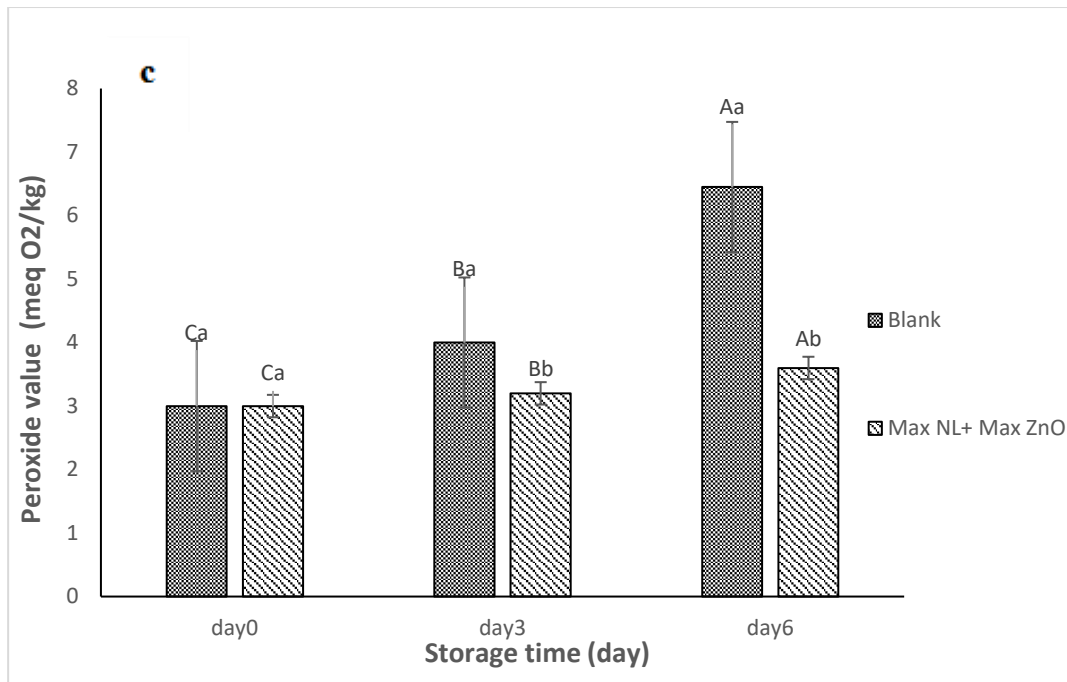
The results of measuring the color of fish meat samples are shown in graphs 1-e to 1-g. Based on the results, with the passage of storage time (6 days), the amount of brightness ( $L^*$ ) and the amount of redness ( $a^*$ ) of the samples decreased significantly, and the amount of yellowness ( $b^*$ ) of the fish samples increased significantly ( $P < 0.05$ ). ; So that the changes in the color of the samples in the fish sample covered with a film containing 10% quercetin nanoliposome and 6% zinc oxide nanoparticles were less than the control sample; which is prepared due to the high property of inhibiting free radicals and reducing the oxidation of lipids in the compounds added to the edible film [23]. Similar results were reported by Shabahang et al. (2022) for rainbow trout meat covered with a film based on whey protein isolate/pectin containing oxidase and betanin nanoparticles [24].

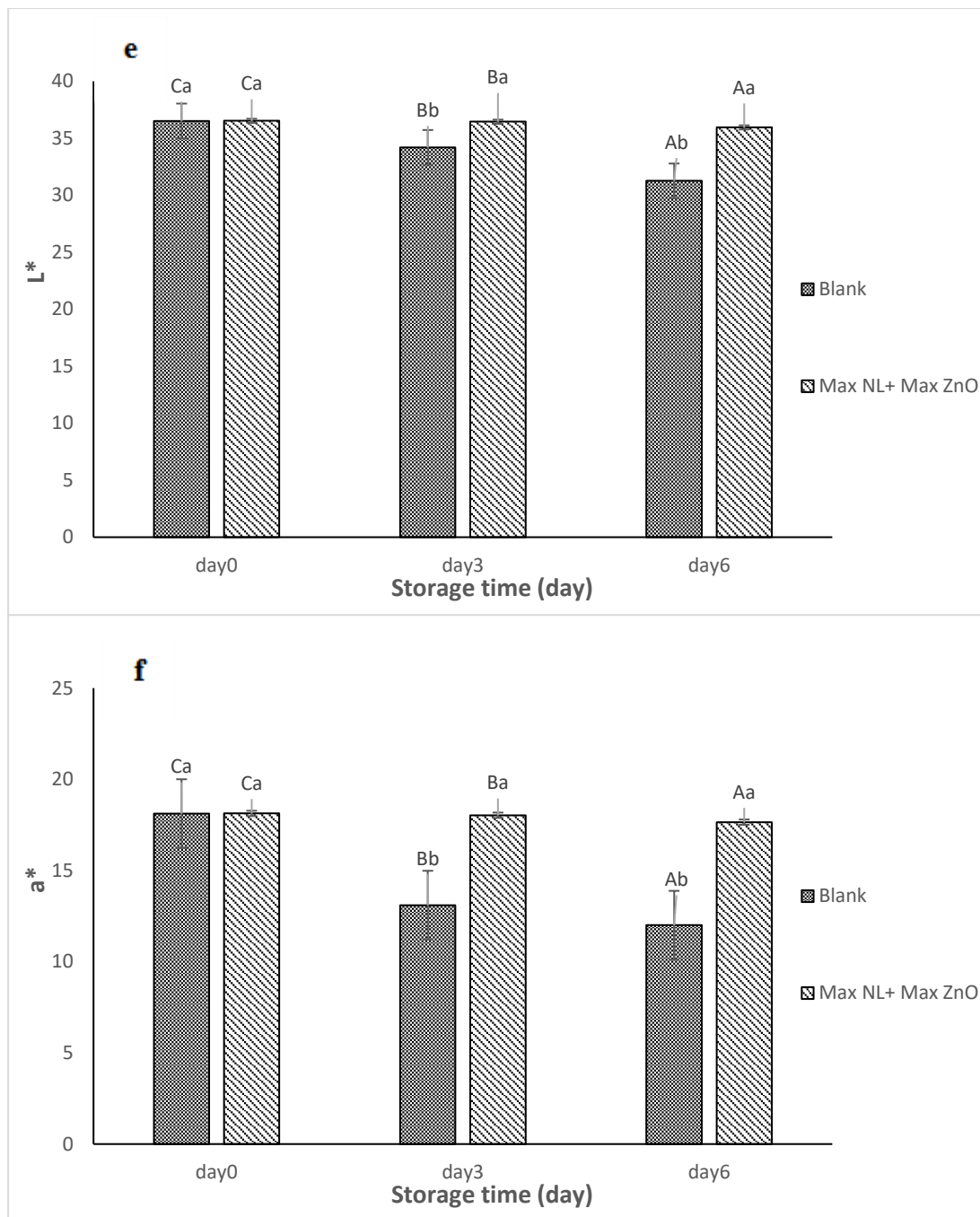
#### 4-7- Total microbial count

The total microbial count of the fish samples packed with nanocomposite film is shown in diagram 1-h. As presented, the microbial count of samples increased significantly with the passage of storage time up to 6 days ( $P < 0.05$ ); So that the microbial growth rate in the sample packed with wheat gluten-based film containing 10% quercetin nanoliposome and 6% zinc oxide nanoparticles was lower than the control sample; which is due to the high antimicrobial properties of the compounds used (quercetin and zinc oxide nanoparticles) and the synergistic effect of these two compounds in the formulation of the nanocomposite film [25]. Similar results were reported by Sayadi et al. (2022) for fresh meat coated with alginate-based nanocomposite film with cumin essential oil and TiO<sub>2</sub> nanoparticles [26]. Also, Malvano et al. (2022) presented favorable results for fresh chicken fillet packed with active film

based on alginate enriched with hydroxyapatite-querctetin complexes [11].









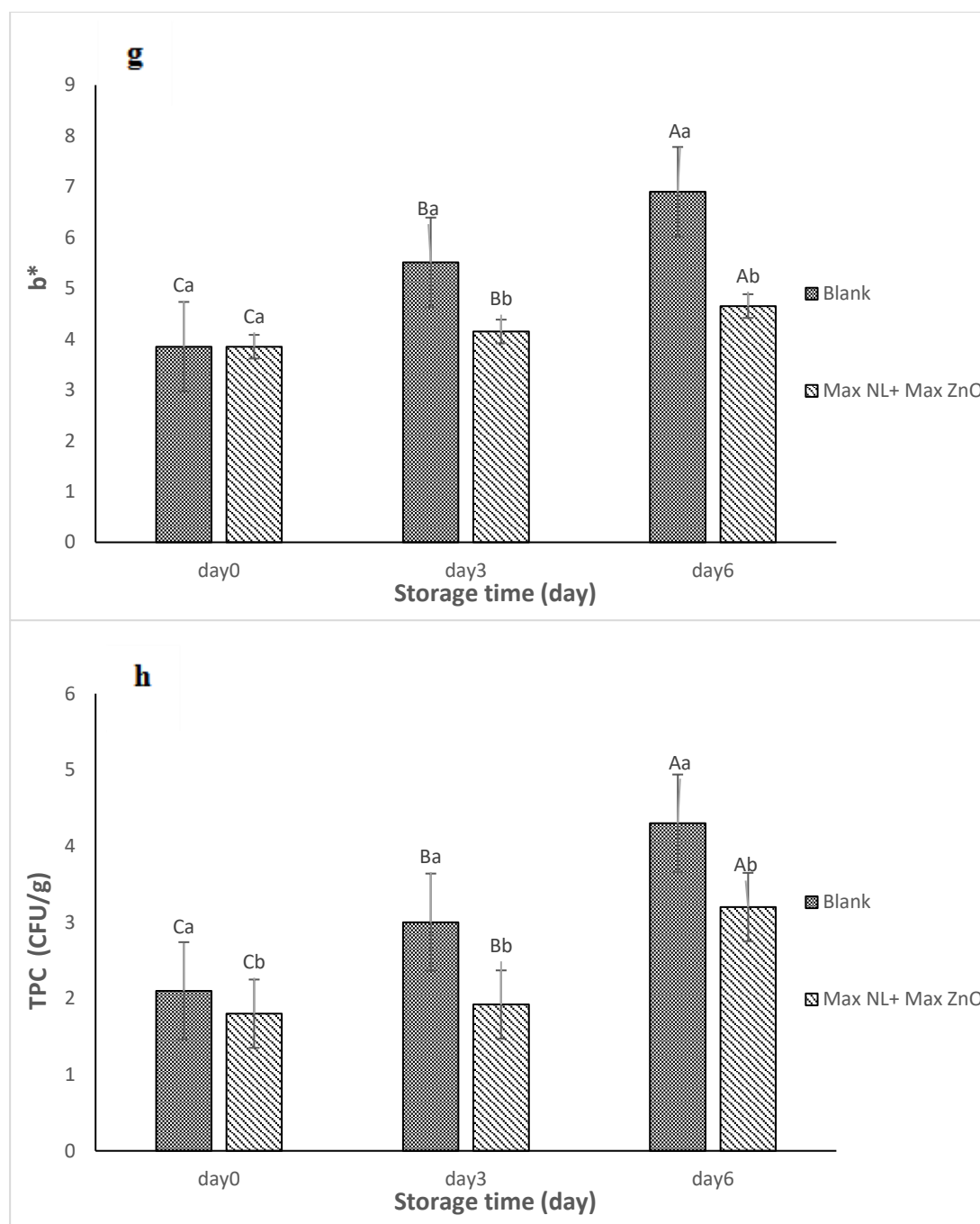


Figure 1(a-h). Physicochemical and microbial tests of optimal films for packaging Rainbow trout meat

products with the absence of any side effects, they have focused on health. In addition, today, researchers and industrialists are looking to increase the nutritional value of these types of preservation products; In the meantime, one of these new storage protocols is the use of active and intelligent edible films and coatings containing antimicrobial and antioxidant compounds as a combination to increase the nutritional value of packaging; Therefore, in this research, we investigated the physicochemical and structural properties of

## 5- Conclusion

In recent years, food preservation technologies have faced important challenges in relation to increasing the shelf life of perishable food products. So, in an effort to realize two important goals, including the appropriateness of the processes used and the production of environmentally friendly maintenance

nanocomposite films based on wheat gluten vegetable protein containing additives with high antioxidant properties, quercetin nanoliposome and zinc oxide nanoparticles, and then the optimal and suitable nanocomposite film for packaging rainbow trout meat. was used, and based on the results, the sample containing 10% quercetin

nanoliposome and 6% zinc oxide nanoparticles can be introduced and used as an optimal and suitable sample in perishable food packaging.

## 5-Resources

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مقاله علمی-پژوهشی

تهیه فیلم نانوکامپوزیت حاوی نانو ذرات اکسید روی و نانولیپوزوم کوئرستین بر پایه گلو تن گندم جهت بسته بندی گوشت ماهی قزل آلی رنگین کمان

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چکیده

اطلاعات مقاله

هدف از این پژوهش تهیه فیلم نانوکامپوزیت بر پایه گلو تن گندم حاوی نانولیپوزوم کوئرستین و نانوذرات اکسیدروی و استفاده از آن بسته بندی ماهی قزل آلی رنگین کمان بود؛ که بدین منظور در این تحقیق اثر نانولیپوزوم کوئرستین در سطوح (۰، ۵، ۱۰٪ حجمی / حجمی) و نانوذره اکسیدروی در سطوح (۰، ۳، ۶٪ وزنی / وزنی) با استفاده از روش سطح پاسخ در قالب طرح مرکب مرکزی بر ویژگی های فیزیکوشیمیایی فیلم نانوکامپوزیت مورد بررسی قرار گرفت و در نهایت نمونه بهینه در بسته بندی گوشت ماهی قزل آلی رنگین کمان برای بررسی خواص آن در طی مدت زمان نگهداری (۰، ۳ و ۶ روز) استفاده شدند. همچنین نتایج بررسی های گوشت ماهی بسته بندی شده نشان داد که استفاده از فیلم نانوکامپوزیت گلو تن گندم حاوی ۱۰٪ نانولیپوزوم کوئرستین و ۶٪ نانوذرات اکسیدروی منجر به کاهش اندیس پراکسید، شاخص ترکیبات ازته فرار، شاخص تیوباریتوریک اسید و شمارش کلی میکروبی در طی مدت زمان نگهداری شد. همچنین در چربی نمونه های مورد بررسی تفاوت معناداری مشاهده نگردید و رنگ گوشت ماهی بسته بندی شده نسبت به نمونه شاهد کدرتر بود. در نهایت با توجه به نتایج حاصله و بررسی های صورت گرفته افزودن ۱۰٪ نانولیپوزوم کوئرستین و ۶٪ نانوذرات اکسیدروی در فرمولاسیون فیلم نانوکامپوزیت گلو تن گندم منجر به بهبود خصوصیات فیلم تولیدی و خصوصیات ماهی بسته بندی شده در طول مدت زمان نگهداری گردید.

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کلمات کلیدی:

گلو تن گندم،  
نانوذرات اکسیدروی،  
نانولیپوزوم کوئرستین،  
ماهی قزل آلی رنگین کمان

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