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Evaluation of antibacterial and antioxidant effect of gelatin-chitosan bilayer edible coating containing nanoemulsion of *Perovskiaabrotanoides* Kar. essential oil on growth control of *Aeromonas hydrophila* inoculated into rainbow trout fillet

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ABSTRACT

In this study, the effect of bilayer edible coating of gelatin-chitosan containing nanoemulsion of *Perovskiaabrotanoides*Kar. essential oil on growth control of *Aeromonas hydrophila* inoculated into rainbow trout fillets over a 12-day period at 4 °C was investigated.The chemical composition of the essential oil was evaluated by GC-MS, its antibacterial properties by disk diffusion, plate well and micro-dilution broth, and its antioxidant properties by DPPH.Treatments included control, gelatin, chitosan, gelatin+essential oil, chitosan+essential oil and gelatin-chitosan+essential oil.After preparation, the treatments were packed in sterile polyethylene bags in laboratory conditions and stored for 12 days at 4 °C and their microbial, chemical and sensorial properties were evaluated every 3 days. The results of GC-MS showed that eucalyptol (24.51%) and camphor (22.02%) are the main components. The MIC and MBC of *Perovskiaabrotanoides*Kar. essential oil nanoemulsions were reported to be 0.0625 and 0.125%, respectively. The amount of TVB-N and POV increased during the study period. According to the results of POV and pH measurements, there was a significant difference between all treatments and control treatment (P<0.05). The mean reduction log of bacteria was significantly different between all treatments and the highest antibacterial effect was observed in the gelatin-chitosan coating containing nanoemulsion of *Perovskiaabrotanoides*Kar. essential oil. According to the results,*Perovskiaabrotanoides*Kar. essential oil has good antioxidant and antimicrobial properties and according to the test results, it was found that chitosan-gelatin coating containing nanoemulsion of *Perovskiaabrotanoides*Kar. essential oil has an effective antimicrobial effect against *Aeromonas hydrophila* and can protect sensory properties and increase shelf life of rainbow trout fillets as well as useful coating to increase the shelf life of food products.

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1. Introduction

Today, fish meat is considered as one of the important sources of human diet. The biggest concern in consuming fish meat is the high rate of corruption and the loss of its initial quality; Therefore, the storage conditions and the type of packaging are of special importance [1-3]. rainbow trout[1] It is known as one of the hyper-consumption species of farmed fatty fish in most parts of the world and even in Iran [3]. Methods such as freezing and adding preservatives do not have the ability to control fish spoilage reactions. According to recent studies, the use of natural preservative compounds and suitable packaging methods not only protects the product against physical damage and microbial spoilage and increases its shelf life, but is also important in terms of marketability and attracting customers. has a special [1, 4, 5]. Nowadays, due to the greater demand for smaller, more convenient and safer packages, the use of edible films and coatings has become popular in order to increase the shelf life of fish. Edible coatings[2], thin layers with a thickness of less than 0.1 mm are consumable and safe materials for humans, which are directly used to cover the external part of all kinds of products, and in addition to antioxidant and antimicrobial properties, many capabilities In preserving the smell, color, taste and aroma, they prevent the entry of moisture and oxygen into it, which increases the shelf life of the product. Edible coatings can be used alone or in combination. Gelatin is a protein obtained from the hydrolysis of collagen found in bone or skin, and chitosan is a polysaccharide that is obtained through acetylation of chitin and has various applications in food, agriculture and medicine industries. The mechanical properties of gelatin coatings or films are weak only because of the feature of moisture absorption during contact with the surface of food. Therefore, by adding polymers such as chitosan and preparing a mixed film, food coatings with suitable physical and mechanical properties can be prepared [1, 6-8]. Until now, chemical preservative compounds were used to increase the storage time of food products, and due to the health risks of these compounds, in the last two decades, the use of natural compounds such as plant essential oils has increased [7]. By adding essential oils, the antimicrobial properties of food coatings increase. Plant essential oils are aromatic and hydrophobic compounds that are extracted from different parts of plants by physical methods, and their antimicrobial and antioxidant properties have been proven today [10]. furniture plant[3] With the local name of blue flower belonging to the mint family[4] It is possible that it grows in cold and dry climate in Iran and some neighboring countries. Due to the different therapeutic properties of this essential oil, such as reducing rheumatic pains, Salk's disease, analgesic, cooling and anti-inflammatory effects, it also has economic value. The extract of this plant contains compounds such as sesquiterpenes and monoterpenes, which cause antimicrobial properties against pathogens [11, 12]. To improve the antimicrobial and antioxidant properties and solve the problem of volatility and high consumption of essential oils, their nanoemulsions can be used [13]. bacteria*Aeromonas hydrophila*[5]It is widely present in the aquatic ecosystem and is considered a natural part of the intestinal flora of aquatic animals, which becomes pathogenic during stress and is of great importance in the fish farming industry.*Aeromonas hydrophila* It is a ubiquitous, opportunistic, gram-negative, rod-shaped, mainly motile, facultative anaerobic, oxidase and catalase-positive, glucose-fermenting bacterium and can cause wound contamination, blood infection, and gastroenteritis in humans [14]. . The purpose of this study is to investigate the antibacterial effect of edible gelatin-chitosan coating containing nanoemulsion of Brazambel essential oil in order to control the growth of bacteria.*Aeromonas hydrophila* It was inoculated into rainbow salmon fillets kept at 4 degrees Celsius.

2- Materials and methods

2-1- Banking materials

1. *Oncorhynchusmykiss*
2. ediblecoating
3. *Perovskiaabrotanoides*What.
4. lamiaceae
5. *Aeromonas hydrophila*

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Brazambel plant was obtained from the northern regions of Razavi Khorasan province and approved by Ferdowsi University of Mashhad. bacteria*Aeromonas hydrophila*(ATCC7966) was obtained from the Department of Food Hygiene, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad. Agar heart-brain broth culture medium[6] From Kiolab company[7]Chitosan with a molecular weight of $10^5$1.6x and degree of deacetylation 85%, tween 80, methanol, reagent[8]DPPH, synthesis[9]BHT, triphenyltetrazolium chloride reagent[10]Cold water fish skin gelatin[11] From Sigma Aldrich[12]was purchased

2-2- Essential oil extraction and preparation of essential oil nanoemulsion

Extraction of essential oil from Brazamble plant by water distillation method[13] And it was done with the help of the Cloninger machine. The prepared essential oil was stored in light-proof jars at refrigerator temperature for use in food coating. The components of the essential oil were determined by Agilent model 6890 gas chromatography device connected to Agilent 5973 mass spectrometer (GC-MS) [15]. Nanoemulsion of Brazamble essential oil with a concentration of 10% (10 grams of Brazamble essential oil + 5 grams of Tween 80 + deionized distilled water, brought to a volume of 100 ml) was prepared with the help of an ultrathorax machine (IKA T18digital) with 10000 rpm for 5 minutes and an ultrasound machine (200W HF-power, Bandelin, Germany) was prepared with a frequency of 20 kHz for 15 minutes at a temperature of 20 degrees Celsius. The prepared nanoemulsion was stored in a light-proof container at a temperature of 4 degrees Celsius [16].

2-3- Preparation of bacteria and microbial suspension

bacteria*Aeromonas hydrophila*Agar culture was given in heart-brain broth culture medium and incubated in an anaerobic jar at 25 degrees Celsius for 24-48 hours. A spectrophotometer was used to prepare the microbial suspension. The concentration equivalent to half McFarland (10 CFU/mL).^1.5 ×) was prepared from bacteria grown for one night and then from that concentration equal to 10 CFU/mL[5] It was prepared for inoculation in fish fillet [9].

2-4- Determination of particle size (PSA) of nanoemulsion in assembly

The size of nanoemulsion particles was determined using a particle size spectrometer (DLS) (Dynamic Light Scattering) (NanoS, Malvern, England)[17].

5-2- Determining the minimum inhibitory concentration (MIC) and the minimum lethal concentration (MBC) of Brazambel essential oil nanoemulsion

To determine the minimum inhibitory concentration, broth microdilution method was used in heart and brain broth culture medium using 96-well microplate. Different concentrations of essential oil nanoemulsion (0.007, 0.015, 0.03, 0.06, 0.12, 0.25, 0.50, 1%) were prepared. To each of the wells, 160 microliters of heart and brain culture medium and 20 microliters of bacterial suspension with a concentration equivalent to 10 CFU/mL[5] And 20 microliters of different concentrations of essential oil nanoemulsion were added. A number of wells were considered as positive and negative controls. The cultured microplate was placed in an anaerobic jar at a temperature of 25 degrees Celsius for 24-48 hours. Control of bacterial growth or lack of growth was done using triphenyltetrazolium chloride reagent and visual observation. The lowest concentration in which there was no bacterial growth or no red color change was considered as the minimum inhibitory concentration (MIC). To determine the minimum lethal concentration, higher concentrations than the minimum inhibitory concentration were cultured in heart and brain agar culture plates. The lowest concentration of

6. Brain Heart Infusion(BHI) agar

7. Q-LAB

8. 2,2-diphenyl-1-picrylhydrazil (DPPH)

9. Butylated hydroxytoluene (BHT)

10. Triphenyltetrazolium)TTC(chloride

11. Gelatin from cold water fish skin

12. SIGMA-Aldrich

13. Hydro distillation

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essential oil nanoemulsion in which bacteria did not grow (less than five cells), was considered as the minimum lethal concentration [18].

2-6- Evaluation of the antioxidant capacity of Brazambel essential oil

Antioxidant capacity was determined by free radical reduction (DPPH) method. Different concentrations of Brazamble essential oil and 0.004% solution of DPPH reagent in methanol were prepared. Then, 5 ml of the mentioned solution was added to different concentrations of essential oil and it was placed in a dark greenhouse for 30 minutes at a temperature of 25 degrees Celsius. After this period, the optical absorbance of the samples was read in comparison with the blank at a wavelength of 517 nm. BHT synthetic antioxidant was also used as a positive control [19]. DPPH radical inhibition percentage is calculated based on the following formula and a concentration of essential oil that has 50% radical inhibition $(IC)_{50}$) was determined to be

$$\text{DPPH radical inhibition percentage} = 100 \times \frac{\text{The amount of light absorption except essential oil}}{\text{The amount of light absorption except for essential oil - the amount of light absorption of different concentrations of essential oil prepared}}$$

2-7- Preparation and inoculation of bacteria to rainbow trout fillet

Rainbow salmon fillets were purchased from a reliable supply center and transported to the laboratory in ice cubes. After washing with sterile distilled water, 10 g pieces were prepared and sterilized in 70% alcohol. 100 microliters of bacterial suspension prepared with a concentration of 10 CFU/mL[5] The prepared parts were inoculated superficially and spread with the help of a sterile L-shaped glass rod and kept for 30 minutes to stabilize the bacteria [20].

8-2- Preparation of gelatin-chitosan mixture coating

In order to prepare the coating, a 2% (w/v) chitosan solution in 1% acetic acid and a 3% (w/v) gelatin solution in sterile distilled water were prepared with glycerol and nanoemulsion of Brazamble essential oil and Tween 80, then with Bain Marie It reached a temperature of 50±2 and was stirred with an ultrathorax device (IKA T18digital) at 10000 rpm for 10 minutes [21].

2-9- Preparation of different treatments

In order to create a coating on the fish fillet, the pieces were immersed for 2 minutes in different treatments according to table number 1. Then, the pieces were removed from the solution and after drying, they were coated in 3% (weight/volume) calcium chloride aqueous solution for 3 minutes and then in gelatin solution in two layers. In the laboratory environment, the parts were packed in sterile polyethylene bags and kept at 4 degrees Celsius for 12 days. Different microbial and chemical tests were performed at regular intervals (0, 3, 6, 9 and 12 days) in 3 repetitions on the stored parts.

Table 1 The treatments studied

NO	Traetments	Description
1	Control (With)	Uncoated fish pieces
2	CH	Fish fillets + 2% chitosan solution
3	G	Fish fillets + 3% gelatin solution
4	CH+1 % BREAKDOWN	Fish fillets + 2% chitosan solution+ 1% PA[1]nanoemulsion of EO (NEO[2])
5	G+ 1% BREAKDOWN	Fish fillets + 3% gelatin solution+ 1% PA nanoemulsionof EO (NEO)
6	CH+G+1%BREAD	Fish fillets + 2% chitosan + 3% gelatin + 1% PA nanoemulsionof EO (NEO)

[1]PA (*Perovskiaabrotanoides*), [2]NEO (nanoemulsion of essential oil)

2-10- bacterial count

In order to investigate the growth status of the inoculated Aeromonas hydrophila bacteria, on days 0, 3, 6, 9 and 12, each of the 10-gram pieces corresponding to each treatment was mixed with 90 ml of sterile 0.1% peptone water and kept for 5 They were completely uniform using a bag mixer. Then successive dilutions were prepared from the obtained suspension and in a special culture medium*Aeromonas hydrophila* It was

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cultivated and placed in a greenhouse at 25 degrees Celsius for 24-48 hours.

2-11- Chemical tests

2-11-1- pH measurement

10 grams of fish sample was mixed with 90 milliliters of distilled water and homogenized, and then with the help of a pH meter (Metrohm, Switzerland), the pH of the samples was measured in 3 repetitions [22].

2-11-2- Measurement of volatile nitrogen bases

Volatile nitrogen bases were measured using the Keldahl distillation system and expressed as milligrams of nitrogen per 100 grams of fish sample [23].

Volatile nitrogen bases (mg percent) = 100 x 14 x acid concentration x amount of acid/10

2-11-3- Measurement of peroxide index

n-hexane was used as a solvent to separate the fat of fish samples, then the amount of released iodine was calculated by titration and using the following formula, the amount of peroxide in terms of milliequivalents of peroxide per 1000 grams of fat was calculated [22].

= peroxide index

ml of thiosulfate for titration × thiosulfate normality × 1000/ fat weight (g)

2-12- Sensory evaluation of samples

Rainbow salmon fillets were procured from reliable supply centers and after sterilization, they were divided into 10 gram pieces. The treatments were prepared according to table number 1 and placed in sterile polyethylene bags. The control and coated treatments were evaluated for 12 days with a time interval of 3 days, in raw form and under suitable conditions. Sensory evaluation of the samples by 8 trained evaluators in terms of color, smell, texture and overall acceptance indicators with A 9-point hedonic scoring scale was used. A score of 1 was equivalent to extremely unpleasant and 9 was equivalent to extremely pleasant. The acceptable limit of 5 points was determined to be equivalent to neither pleasant nor unpleasant (indifferent). Samples with a score of less than 4 were considered unusable[24].

13-2- Statistical analysis

All experiments were performed in 3 repetitions. Statistical analysis of data was done using SPSS16 software. Data analysis and comparison of average data in different treatments was done based on repeated measure ANOVA test. In all evaluations ($0.05P<$) was considered as a significant level.

3- Results and discussion

3-1- The chemical composition of Brazambel essential oil

Brazambel essential oil was analyzed after extraction using a gas chromatography device connected to a mass spectrometer. According to this study, 21 chemical compounds were identified in this essential oil (Table 2). Among the identified compounds, Eucalyptol with 24.51%, Camphor with 22.02%, Alpha-Pinene with 17.35% and 3-Carene with 11.05%. percent were recognized as the most effective compounds of this plant. In a study by Safai Qomi and Betoli (2010), the chemical compounds of Brazambel essential oil were isolated, and alpha-cadinol, 1,8-cineole and camphor were its main components[25]. . In the study conducted by Morteza-Semanani (2004), the chemical composition of Brazambel essential oil was investigated by mass spectrometer, and according to the results, 21 types of components were isolated from this essential oil, and camphor with 34.1%, 8.1-cineol with 18%, b- Caryophyllene with 8.2% and a-homolene with 6.5% were the most important constituents, and the amount of camphor as an important component in this essential oil was consistent with the results of the present study [26]. The difference in the type and amount of these compounds depend on factors such as genetics, growing place, different parts of the plant, and the method of extracting essential oils, which can be the reasons for the difference between the results of the present study and other studies [12].

Table 2 Chemical composition of *Perovskiaabrotanoides*Kar.essential oil

No	Phytochemicals	Percent	RT
1	α-Pinene	17.35	10.37
2	Camphene	5.88	11.17
3	β-Pinene	0.50	12.61

4	β-Myrcene	0.83	13.32
5	3-Hulls	11.05	14.27
6	o-Cymene	1.45	15.24
7	Limonene	2.09	15.44
8	Eucalyptol	24.51	15.64
9	γ-Terpinenes	0.26	17.11
10	Terpinolene	0.18	18.62
11	Linalool	0.27	19.59
12	Camphor	22.02	22.28
13	endo-Borneol	0.77	23.65
14	α-Terpineol	1.08	25.02
15	Bornyl acetate	2.81	29.91
16	3-Methyl-4-isopropylphenol	0.89	30.86
17	Thymol	1.17	33.27
18	β-Guaiene	0.15	34.59
19	Caryophyllene	3.24	36.83
20	Humulene	2.46	38.64
21	tau.-Cadinol	0.94	46.24
22	Total	99.90	

2-3- Minimum inhibitory concentration (MIC) and minimum lethal concentration (MBC)

To determine the minimum inhibitory concentration, broth microdilution method was used, the results of which are shown in Table 3. According to the obtained results, bacteria*Aeromonas hydrophila* It was highly sensitive to Brazambel essential oil nanoemulsion, and Brazambel essential oil has been effective in preventing the contamination of this microorganism, and these results are consistent with the study of Safai Qomi and Betoli (2010) [25].

Table 3 MIC and MBC of Nanoemulsion of *Perovskiaabrotanoides*essential oil against*Aeromonas hydrophila*

MBC(%)	MIC(%)	Essential oil nanoemulsion	Microorganism
0.125	0.0625	Nanoemulsion of *Perovskiaabrotanoides* Kar.essential oil	*Aeromonas hydrophila*(ATCC 7966)

3-3- The particle size of Brazambel essential oil nanoemulsion

The size of the nanoemulsion particles of Brazambel essential oil was measured with 5 repetitions, which is reported in Table 4. Hatanaka[14]et al. (2010) as well as Severino[15]et al. (2015), in their studies, they considered the range of less than 500 nm in nano dimensions, which was consistent with the results of the present study [27, 28].

Table 4 Particle size (PSA) of *Perovskiaabrotanoides* EO nanoemulsion

PDI	Z-average(nm)	Formulation
0.26±0.03	162.32±11.43	Nanoemulsion of *Perovskiaabrotanoides* Kar.essential oil

3-4- Determination of antioxidant capacity by free radical reduction method

Inhibition of DPPH free radical by bramble essential oil was investigated in laboratory conditions. According to the findings, with the increase in the concentration of the essential oil, the free radical inhibition power also increases. The concentration of the essential oil that inhibited 50% of radicals compared to the synthetic antioxidant BHT was 46.44 mg/ml. Based on the results obtained, Brazamble

[14] .Satanaka

[15] .Severino

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essential oil has good antioxidant properties, which is in line with the results of the studies. by Ghafourian and Mazandarani (2017) andAshraf et al. (2014) corresponds [29, 30].

5-3- Microbial count test

Average logarithm of bacterial count*Aeromonas hydrophila* Fish fillets were kept for 12 days at refrigerator temperature every three days and the results are shown in Figure 1. According to the results, in different treatments, the growth of bacteria increased, but the chitosan-gelatin treatment along with nanoemulsion Essential oil has well controlled the growth of bacteria. Chitosan treatment containing brazamble essential oil nanoemulsion has more ability to control bacterial growth than chitosan coating alone. The above findings are consistent with the study results of Ajago et al. (2010) and Yagan Mohammadi et al. (2016) [5, 31]. Gelatin coating along with essential oil nanoemulsion was more effective in controlling bacterial growth compared to gelatin coating alone, which is consistent with the results of the study by Fazal Ara et al. (2017) [32]. A comparative study of chitosan and gelatin coatings has been able to control the growth of bacteria more effectively due to the antimicrobial property of chitosan, which is consistent with the results obtained in the study of Fan et al. (2009)[4]. The treatment containing gelatin alone did not have an effect on the control of bacterial growth, which is consistent with the study of Fazal Ara et al. (2017) and Rezaei and Taghizadeh Andvari (2011) [32, 33].Bey Mohammadi et al. (2021), workEdible film based on chitosan-gelatin containing Chovir essential oil.*Angle the ferulago*)investigated the characteristics of turkey meat at 4 degrees Celsius. According to the obtained results, adding chuir essential oil to the chitosan-gelatin film increases the shelf life of turkey meat in the refrigerator [34].Saki et al. (2017) alsoThey investigated the effect of coating and film of edible chitosan-gelatin mixture on the characteristics of fish kept at refrigerator temperature. The results obtained in the present study showed that the gelatin-chitosan coating containing 1% nanoemulsion of Brazambel essential oil had a good effect in controlling the growth of Aeromonas hydrophila bacteria inoculated into rainbow trout meat, which shows the good antimicrobial effect of the essential oil and the appropriate characteristics of the coating. It is a gelatin-chitosan mixture [35].

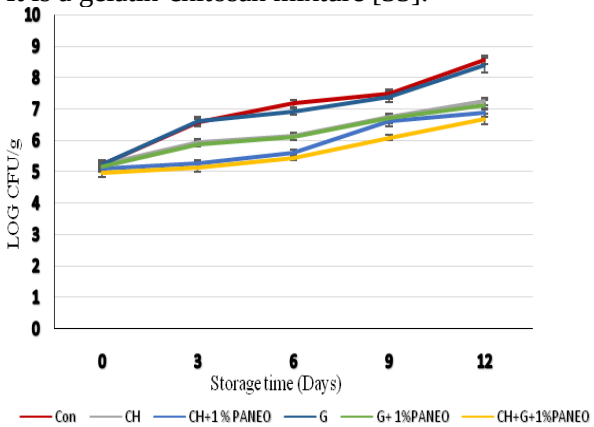

Fig 1 Changes of *Aeromonas hydrophila* in different treatments

Pairwise comparison of the mean reduction of the logarithm of bacterial count*Aeromonas hydrophila* inoculated into the fish fillet during the storage period in the refrigerator is shown in Table 5. Based on the results obtained, there is no significant difference between the mean of the logarithm of bacterial growth reduction*Aeromonas hydrophila* It was not observed in control treatments with gelatin coating alone and chitosan treatments alone with gelatin containing 1% nanoemulsion of essential oil.*P*> 0.05) (Table 5).

Table 5 Double comparison of logarithm reduction of *Aeromonas hydrophila* in the studied treatments

Treatment I	Mean difference I-J				
	Treatment J				
	CH	CH+ 1% BREAKDOWN	G	G+ 1% BREAKDOWN	CH+G+ 1% BREAKDOWN
Control	0.76*	1.11*	0.10	0.80*	1.33*
CH		0.35*	-0.65*	0.04	0.57*
CH+1 %BREAKDOWN			-1.01*	-0.31*	0.22*
G				0.69*	1.23*
G+ 1% BREAKDOWN					0.53*

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* The difference is significant (P<0.05)

6-3- Chemical tests

3-6-1- pH measurement

Figure 2 shows the average pH changes of different treatments during the storage period.

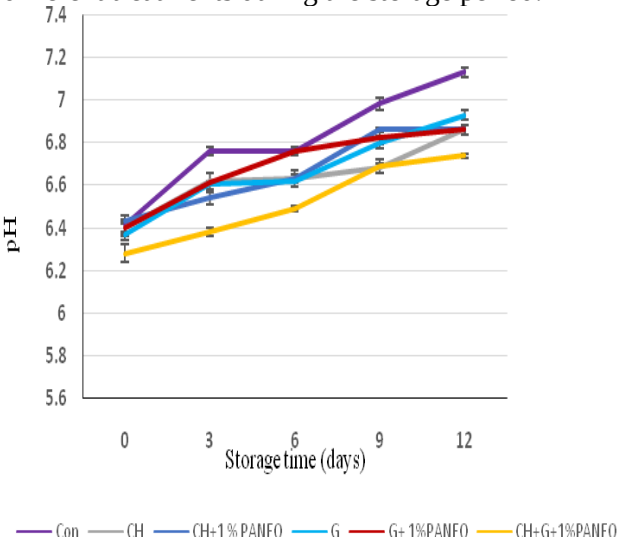

Fig 2 pH changes in different treatments

Fan et al. (2009), in a study, investigated the effect of chitosan coating on the shelf life of silver carp fish kept in freezer temperature and reported that during the storage period, the amount of volatile nitrogenous bases gradually increased and the pH in the samples first decreased and Then it was increased that these results are consistent with the findings of the present study [4]. In a study by Gomes-Staca et al. (2010), they evaluated the effect of oral coating containing clove essential oil on a number of bacteria and showed that the pH first increased and then remained constant. In this study, the amount of volatile nitrogen bases generally had an increasing trend until the end of the period. These results are consistent with the findings of the present study [36].

3-6-2- Measurement of peroxide index

The average changes of peroxide index in different treatments are reported in Figure 3.

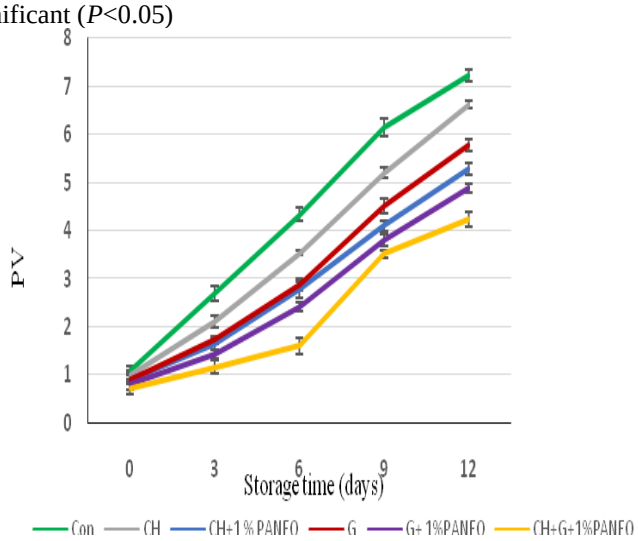

Fig 3 PV changes in different treatments

In a research by Saki et al. (2017)They investigated the effect of coating and edible chitosan-gelatin film on the characteristics of a type of fish kept in the refrigerator. According to the findings, the tests of the index of fat oxidation (free fatty acid) indicated that the amount of oxidation was lower in the samples with film and gelatin chitosan coating compared to the control sample (fillet without coating and film), which is consistent with the findings of the present study. has [35].

3-6-3- Measurement of volatile nitrogen bases

The average changes of volatile nitrogen bases in different treatments are reported in Figure 4.

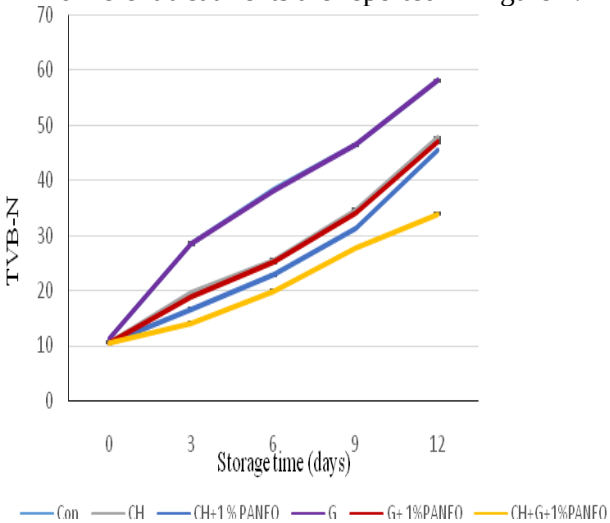

Fig 4 TVB-N changes in different treatment

Farajzadeh et al. (2016), in a research,

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investigated the effect of chitosan-gelatin coating on the quality of a type of shrimp in refrigerated conditions. According to the findings, the formation of total nitrogen in 57% of shrimps with this coating was less. Also, this coating reduced the amount of oxidation and peroxide index during the storage period [37]. Fazal Ara et al. (2017) investigated the effect of gelatin coating containing Shirazi thyme on the chemical properties of ostrich fillet at 4 degrees Celsius during a period of 15 days. According to the findings, in terms of chemical factors, Shirazi gelatin-thyme treatment showed a lower amount of total volatile nitrogen and lower pH during the storage period than the other three groups, which are consistent with the findings of the present study [32].

3-7- Sensory evaluation

The results of the sensory evaluation of cooked fish fillet pieces during the storage period in the refrigerator are reported in Table 6. As can be seen, the characteristics of texture, smell, color and general acceptance of all treatments decrease with time. The chitosan-gelatin coating containing the nanoemulsion of Brazambel essential oil preserves most of its properties

The bag has been fished. In the treatments without essential oil, the gelatin coating, due to its mechanical properties, kept the tissue strength of the samples. In the samples containing essential oil, due to the antimicrobial property of the essential oil, compared to the samples without essential oil, the characteristics of the samples were preserved to a greater extent.Saki et al. (2017)The effect of coating and film of edible chitosan-gelatin mixture on the characteristics of salted belang fish fillet kept at refrigerator temperature for 16 days was investigated. According to their report, the used films and coatings increased the shelf life of fillets stored in the refrigerator by 4 days and increased the shelf life and preserved the sensory properties of food products, which is consistent with the findings of the present study [35]. In a study conducted by Fan et al. (2009), the effect of chitosan coating on the quality and shelf life of silver carp was investigated in conditions of storage at freezer temperature for 30 days and it was found that these coatings increased the shelf life of the samples. which is consistent with the findings of the present study [4]. Gomez-Staca et al. (2010) investigated the effect of edible coating containing clove essential oil on the sensory properties of a type of fish and showed that the sensory properties of the samples were preserved, which is consistent with the findings of the present study [36]. Also, Farajzadeh et al. (2016) investigated the effect of chitosan-gelatin coating on the sensory characteristics of a type of shrimp in refrigerated conditions and showed that the shelf life and preservation of the sensory properties of the samples increased for 6 days [37]. Fazal Ara et al.(2017) also investigated the effect of gelatin coating containing Shirazi thyme on the sensory characteristics of ostrich fillet at refrigerator temperature and showed that treatments containing essential oils increased the shelf life of ostrich fillet, which is in line with the findings of the study. The present one corresponds [32].

Table 6 Sensory evaluation values of fish fillets stored at 4 ° C

Storage time (day)					Treatment	Sensory attributes
12	9	6	3	0		
2.46±0.33 Ed	3.85±0.22 Db	5.51±0.25 Cc	6.44±0.51 Bc	7.36±0.29 Ab	Control	
3.51±0.31 Dc	5.39±0.17 That	6.53±0.23 Not	7.21±0.26 Oops	7.66±0.35 Ab	CH	
2.82±0.30 Ed	4.11±0.21 Db	5.90±0.25 Cbc	6.64±0.30 Bbc	7.73±0.25 Ab	G	**Color**
4.06±0.16 Eab	5.57±0.23 And	6.27±0.25 Cab	7.15±0.29	8.51±0.35 Aa	CH+1 %	
3.91±0.11 Ebc	5.44±0.25 And	6.26±0.26 Cab	Chapter	8.42±0.37 Aa	PANEOG+	

4.48±0.28 Yes	5.79±0.20 And	6.48±0.36 That	7.22±0.35 Chapter 7.67±0.38 Not	8.53±0.41 Aa	1%PANEO CH+G+1%BREAD	
2.87±0.32 Dc 3.32±0.32 Ebc 3.10±0.11 Ebc 3.53±0.22 Eb 3.37±0.24 Eb 4.13±0.26 And	4.10±0.22 Cd 5.20±0.20 Db 4.73±0.25 Dc 5.29±0.28 Db 5.31±0.19 Db 5.78±0.22 That	5.80±0.30 Bc 6.41±0.29 Cab 5.77±0.30 Cc 6.53±0.23 Cab 6.15±0.16 Cbc 6.83±0.19 Not	7.08±0.18 Ad 7.27±0.25 Bd 8.02±0.16 Bbc 7.79±0.26 Bc 8.28±0.33 Chapter 8.66±0.17 Aa	7.50±0.50 Ab 8.59±0.52 Aa 8.81±0.27 Aa 8.82±0.28 Aa 8.84±0.17 Aa 8.88±0.14 Aa	Control CH G CH+1 % PANEOG+ 1%PANEO CH+G+1%BREAD	**Texture**
2.43±0.30 Dd 3.38±0.37 Ec 2.80±0.20 Ed 4.09±0.21 Eab 3.76±0.25 Ebc 4.43±0.19 Yes	4.50±0.33 This 5.27±0.25 Dcd 5.00±0.11 Dd 5.96±0.16 Ghost 5.71±0.25 Dbc 6.40±0.36 And	6.10±0.30 Bd 6.96±0.15 Cbc 6.66±0.20 Cc 7.13±0.18 Cab 6.98±0.22 Cbc 7.45±0.34 That	7.22±0.27 Acd 7.53±0.32 Bd 7.17±0.18 Bbcd 7.76±0.25 Chapter 7.70±0.24 Bbc 8.20±0.26 Not	7.68±0.28 Ab 8.51±0.23 Aa 8.37±0.37 Aa 8.58±0.37 Aa 8.73±0.24 Aa 8.79±0.26 Aa	Control CH G CH+1 % PANEOG+ 1%PANEO CH+G+1%BREAD	**Odor**
2.83±0.32 Dc 3.51±0.27 Eb 3.50±0.18 Db 3.76±0.25 Eab 3.78±0.13 Ghost 4.19±0.22 And	3.61±0.45 Cc 4.05±0.27 Dbc 3.86±0.31 Dbc 4.28±0.15 Ghost 4.16±0.17 Dbc 4.76±0.25 That	5.20±0.26 Bc 6.71±0.20 Cb 6.51±0.18 Cb 6.81±0.26 Cab 6.52±0.26 Cb 7.23±0.25 Not	6.76±0.25 And 7.77±0.25 Bb 7.68±0.30 Bb 7.74±0.25 Bb 7.72±0.24 Bb 8.71±0.28 Aa	7.26±0.36 Aa 8.31±0.20 Aa 8.27±0.17 Aa 8.66±0.28 Aa 8.60±0.32 Aa 8.75±0.25 Aa	Control CH G CH+1 % PANEOG+ 1%PANEO CH+G+1%BREAD	**Overall**

Means within the same row (A, B, C, D) and the same column (a, b, c, d, e, f) with different letters are significantly different ($P < 0.05$).

4- Conclusion

The results of this study showed that the use of chitosan-gelatin coating containing brazamble essential oil nanoemulsion reduced the number of bacteria.*Aeromonas hydrophila in*Rainbow salmon fillet was stored in the refrigerator. This result was more effective in the double-layer coating with Brazambel essential oil nanoemulsion than any combination alone, and it was found that this edible coating can be used as an active packaging in the food industry.

5- Resources

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مقاله علمی_پژوهشی

بررسی اثر ضد باکتریایی و آنتی اکسیدانی پوشش خوراکی دولایه ژلاتین-کیتوزان حاوی نانوامولسیون اسانس برازمبل روی کنترل رشد باکتری *آئروموناس هیدروفیلا* تلقیح شده به فیله ماهی قزل آلای رنگین کمان

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ژلاتین، ماهی قزل آلا،

پوشش، بسته بندی.

چکیده

در این مطالعه اثر پوشش خوراکی دو لایه ی ژلاتین-کیتوزان حاوی نانوامولسیون اسانس برازمبل بر روی کنترل رشد باکتری *آئروموناس هیدروفیلا* تلقیح شده به فیله ماهی قزل آلای رنگین کمان طی یک دوره ی ۱۲ روزه در دمای ٤ °C بررسی شده است. ترکیبات شیمیایی اسانس با دستگاه طیف سنج جرمی، خاصیت ضد باکتریایی به روش های انتشار در دیسک، چاهک پلیت و میکرو دایلوشن براثوخاصیت آنتی اکسیدانی با روش احیاء رادیکال آزاد مورد ارزیابی قرارگرفت. تیمارها شامل گروه های کنترل، پوشش ژلاتین، کیتوزان، ژلاتین+اسانس، کیتوزان+اسانس و ژلاتین-کیتوزان+اسانس بودند. نمونه ها پس از آماده سازی در کیسه های استریل پلی اتیلنی در شرایط آزمایشگاه بسته بندی و ۱۲روز در ٤°C نگهداری و به فاصله ۳ روز مورد ارزیابی میکروبی، شیمیایی و حسی قرار گرفتند. نتایج آنالیز اسانس نشان داد که بیشترین ترکیبات تشکیل دهنده اسانس شامل اکالیپتول (۲٤/۵۱ درصد) و کامفور(۲۲/۰۲ درصد) می باشند. حداقل غلظت مهارکنندگی و کشندگی نانوامولسیون اسانس برازمبل به ترتیب ۰/۰٦۲۵ و ۰/۱۲۵ درصد گزارش گردید. میزان بازهای ازته ی فرار و اندیس پراکسید در طول دوره ی مطالعه روند افزایشی داشته است. طبق نتایج حاصل از اندازه گیری شاخص پراکسید و pH، بین تمام تیمار ها و تیمار کنترل اختلاف معنی داری وجود داشت($P<۰/۰۵$). میانگین لگاریتم کاهش باکتری در بین گروه ها تفاوت معنی داری داشت و بیشترین اثر ضد باکتریایی در پوشش کیتوزان-ژلاتین حاوی نانوامولسیون اسانس برازمبل مشاهده شد. بر اساس نتایج بدست آمده مشخص گردید که پوشش خوراکی دولایه ژلاتین-کیتوزان حاوی نانوامولسیون اسانس برازمبل دارای اثر ضدمیکروبی مؤثری بر علیه باکتری *آئروموناس هیدروفیلا* داشته و سبب حفظ ویژگی های حسی و افزایش ماندگاری ماهی قزل آلای رنگین کمان شده و می تواند پوشش مناسبی جهت افزایش عمر ماندگاری محصولات غذایی باشد.

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