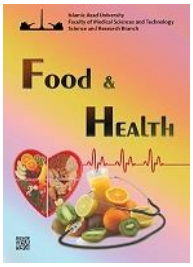

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Use of edible coating of alginate enriched with nettle extract to enhance the shelf life of rainbow trout fillet

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ABSTRACT

The present study was performed to investigate the effect of nettle hydroalcoholic extract (NE) with edible sodium alginate (SA) coating on the quality of rainbow trout fillet (*Oncorhynchus mykiss*) during a 12-day refrigeration period (4±1˚C). NE was extracted by ethanol solvent and the chemical compounds of NE were determined. Experiments were performed on 4 treatments (T1: control treatment, T2: SA 2%, T3: SA+NE 0.5% and T4: SA+NE 1%) in three replications. The results of chemical tests performed in comparison with the control sample showed that the samples containing the SA and NE were able to control the increase of chemical and microbial parameters ($p<0.05$). At the end of the storage period, the lowest amount of peroxide value, thiobarbituric acid and total volatile basic nitrogen, and the lowest amounts of lactic acid-producing bacteria, total bacterial count, psychrotrophic values were observed in SA+NE 1% ($p<0.05$). The results of the sensory evaluation showed that the mentioned treatment maintained the quality of the fillet until the end of the storage period. In general, edible coating of SA with NE can delay the process of lipid oxidation and microbial spoilage and maintain or improve the sensory properties of refrigerated fish fillets.

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1. Introduction

Fish is a valuable source of protein and contains large amounts of unsaturated fatty acids ω-3 (PUFAs) which play an important role in a healthy diet (1). Rainbow trout (*Oncorhynchus mykiss*), a major member of the Salmonidae family, is a cold-water fish that has been an important part of the diet in Iran and the world due to its aquaculture suitability, nutritional quality, and delicious taste (2). Raw trout have some protective issues such as non-enzymatic lipid oxidation, microbial growth and high levels of unsaturated fatty acids, the presence of biogenic amine (BA) autolysis enzymes, have a shorter shelf life than other meat products. Subsequently, they are considered very vulnerable foods (3,4). Maintaining the freshness and quality of fresh products is a main challenge in the food industry around the world. Chemical preservatives are one of the most universal and reliable ways to maintain the quality of seafood and increase its shelf life during storage (1, 5). Nowadays, the use of these chemicals is limited due to their destructive effects on DNA and their toxicity. A safe and acceptable approach is to use plant extracts as natural additives in foods to protect them against oxidation and prevent the growth and proliferation of microorganisms (3,7). *Urtica dioica* L., commonly known as nettle, is a wild annual plant of the *Urticaceae* family. This plant is widely known for its extraordinary biological activity and beneficial effect on human health. Various studies have confirmed the antioxidant, antimicrobial, anti-inflammatory, antibacterial, and analgesic properties of nettle extract (NE) (8-10). Although the use of plant extracts and essential oils in products is increasing, their use is limited due to cost and other disadvantages such as severe perfume and in some cases potential toxicity. An approach to overcome these disadvantages could include the combination of plant extracts in the formulation of edible

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coatings, which reduces their dose with the same protective effects (3,11). Coatings and edible films have been considered because of their advantages over synthetic films. The most important advantage of these compounds is their ability to be consumed with food compounds (12). Alginates are isolated from the cell walls of brown algae (*Laminaria digtata*/ *Ascophyllum nodosum*), where they are found in the form of sodium, calcium, and magnesium salts of alginic acid (13). Sodium alginate is a nontoxic, biodegradable, biocompatible, and cheap hydrocolloid. It may have and has many applications in the food industry as a packaging material for portioned products, limiting the dehydration of meat, as a thickening agent, in gel formation, and as a colloidal stabilizing agent in the beverage industry, in the textile, pharmaceutical, and paper industries, and being used to obtain polymeric matrices used in the encapsulation of drugs, proteins, cells, and DNA (13, 14, 15). Edible alginate coating and film alone or with various antioxidant additives have been used by other researchers to preserve fish fillets (13, 15,16). Therefore, this study aimed to produce a biodegradable edible coating based on sodium alginate (SA) containing different concentrations of NE and to investigate its effect on the quality and shelf life of rainbow trout fillets at a temperature of 4 °C in the refrigerator.

2. Materials and methods

2.1. Raw materials

The nettle plant was prepared from the Doehezar area of Tonekabon city, located in Mazandaran province, Iran, and the leaves of the plant were washed, then stored in suitable conditions (dark and dry) and completely dried and ground for extraction. All the chemicals used were prepared by the German company Merck and had a degree of decomposition.

2.2. Preparation and analysis of nettle extract (NE)

For extraction, 70% ethanol was used by the soaking method. 50 g of plant leaf powder was weighed separately and poured into the decanter and then ethanol was added step by step. The addition of ethanol was continued until the entire volume of the plant in the decanter was soaked and some ethanol was placed on the sample surface. After 72 hours of extraction, the solvent was separated from the extract by a rotary device using a vacuum pump (17). One gram of extract was injected into the GC/MS (Trace GC-ISQ mass spectrometer, ThermoScientific, USA) The relative percentage of each of the ingredients of essential oils was determined according to the area under the curve of each of the chromatogram peaks of the device and its comparison with the total area under the curve (18).

2.3. Measurement of total phenolic compounds

Total phenolic content was measured using the Folin-Ciocalteu reagent. Gallic acid is used as a standard for drawing calibration curves. Total phenolic content was reported based on the equivalent of mg gallic acid/ g extract (19).

2.4. Preparation of SA/ NE

To produce the coating, the mixture was prepared by dissolving SA in distilled water (20 g/ l) and then stirring at 50 °C until the mixture became clear (13), then the viscous solutions are cooled to 20 °C and 0.1 ml of glycerol monostearate as a plasticizer was added to increase the strength and flexibility of viscous solutions. For active edible coating, different concentrations of NE (0.5 and 1%) were mixed on a magnetic stirrer at 55 °C. The final solution was homogenized with a homogenizer at 7000g for 2 minutes and the final coating was prepared (20).

2.5. Preparation of coatings of SA/ NE on fish fillets

The fish fillets were prepared and quickly transported to the food laboratory by boxes containing ice. Then, to create a coating on the surface of the fillets, the fillets were first immersed in the prepared solutions for 1 minute. After that, they were removed from the solution and after about 2 minutes, they have placed in the coating solution again for another 1 minute. The control was left uncovered. To dry the fillets, they were hung on sterile mesh plates for 5 hours and subjected to a gentle stream of air (at 10 °C) to form a coating on the fillets. The samples were placed in sterile polyethylene bags and stored at 4 °C until testing. Microbiological and chemical evaluation of fillets was performed continuously from the 3rd to the 12th day and compared with control samples prepared in water solutions without coating material. In total, the present study included 4 treatments: (1) Control (without coating); (2) SA 2%; (3) SA+ NE 0.5%; (4) SA+ NE 1%.

2.6. Chemical analysis

The peroxide value (PV) in the samples was determined according to the Pearson method (21). The amount of thiobarbituric acid (TBA) was determined by the colorimetric method described by Valipour et al. (11) and expressed as mg malondialdehyde/kg sample). The total volatile base nitrogen (TVB-N) of fish fillets was determined using the micro-diffusion method according to Valipour et al. (11).

2.7. Microbiological analysis

For microbiological experiments, 10 g of fillet meat sample was sterilized under sterile conditions with 90 ml of 0.85 sterile physiology serum for 60 seconds in a laboratory mixer. Each sample was sampled three times separately. Homogenized samples were used for culture in the following microbiological media: Plate count agar (total viable count (TVC) and psychrotrophic bacteria (PTC), de Man, Rogosa, and Sharpe (MRS) agar for lactic acid bacteria (LAB). The inoculated plates were incubated at 37 ºC for 2 days for TVC, 7 ºC for 10 days for PTC, and 30 ºC for 2 days for LAB (20).

2.8. Sensory analysis

Uncoated and coated fillets were examined after cooking at 185 °C for 60 minutes based on taste, odor, color, texture, and general acceptance characteristics, and the results were expressed on a 5-point hedonic scale. A sensory evaluation of the samples was performed after 3 days of storage.

2.9. Statistical Analysis

Data analysis was performed using analysis of variance (ANOVA) according to the normality of the data and the homogeneity of variance. Duncan's test at the 5% level was used to compare the mean of the data. All data were reported as mean standard deviation and evaluations were performed in 3 replications. The parametric bread method of the Chi-square test was used for statistical analysis of the sensory test. Software (SPSS version 25) was used for data analysis and Excel for drawing graphs.

3. Results and discussion

3.1. Investigation of the chemical compounds of NE

According to the results related to the chemical compounds of NE (Table 1), a total of 18 compounds with a total of 99.61% were identified. The most common components of the extract were Neophytadiene (26.69%), Phtaleic acid (13.12%), and Dibutylphtaleate (9.39%). Habibi Lahigi et al. (22) stated that the most constituents of NE were Neophytadiene (25.21%), Phtaleic acid (8.15%), 1, 2- benzenocli carboxylic acid (7.62%), and Dibutylphtaleate (7.37%), respectively. Moradi et al. (23) stated that the most constituents of NE were Neophytadiene (25.21%), Phtaleic acid, (8.15%), Dibutyl phtaleate, (7.37%) Bis (2ethyl hexyl) maleate (6.32%), and 1.2 -benzenocli carboxylic acid (7.62%). The chemical compounds in the mentioned studies were consistent with the present study.

3.2. Evaluation of phenolic compounds of NE

The phenolic compounds of NE were 91.11±2.03 mg gallic acid/g extract. Bourgeois et al. (25) reported the phenolic compounds of NE as 482.34-86.90 mg/g gallic acid/g. Mzid et al. (26) reported the phenolic compounds of NE extracted with aqueous and ethanol solvents as 29.56 and 31.41 mg gallic acid/ g extract, respectively. Differences in the values of phenolic compounds in the extract can be observed in various studies, the content of phenolic compounds in plants may vary during the processing stages such as growth, harvesting, storage, and technological methods used (26, 27).

3.3. Chemical evaluation of coated fish fillets

Lipid oxidation development measures are based on the formation of primary (hydroperoxides) and secondary oxidation compounds (aldehydes) (14). The results of the present study showed that with increasing time, the values of PV (Fig. 1a) increased in all treatments and these changes were more in the control treatment. The use of edible coatings slowed the upward trend of PV. In general, biodegradable films and coatings have very low permeability to oxygen and carbon dioxide. Therefore, the coating formed on the surface of the fillets significantly reduces the rate of contact of the product with oxygen, which reduces the rate of initial oxidation of fats and subsequent formation of hydroperoxides (11).

Table 1. Percentage composition of the chemical compounds of extract (NE).

No	Compound	Percentage (%)
1	2,4-dichlorophenol	4.37
2	Dibutylphtaleate	9.39
3	Phosphoric acid tributylester	4.59
4	4-chloro-3-methyl phenol	3.60
5	Neophytadiene	26.69
6	2-nitrophenol	3.21
7	Unknown	1.04
8	Bis(2-ethyl hexyl)maleate	7.84
9	1,2,-benzenedicarboxylic acid	8.94
10	Phtaleic acid	13.12
11	Eicosane	3.11
12	Unknown	1.85
13	Pentacosane	2.75
14	Hexacosane	2.25
15	Olean-18-ene	1.85
16	1-Heptadecene	2.21
17	Heneicosane	1.75
18	8-methylheptadecane	1.05
	Total	99.61

Also, the combination of SA could more effectively slow down the process of PV increase. The lower PV is due to the phenolic compounds in the extract because phenolic compounds prevent oxidation by inactivating fat-free radicals and peroxy radicals. Medicinal plants have different compounds but mainly contain polyphenols, which have antioxidant properties and therefore can increase the shelf life of meat (28). Increasing the concentration of the extract had a positive effect on this process. So that on the 12th day of storage, the lowest amounts of PV were observed in the treatment of SA+ NE 1% (3.52 mEq/kg fat) and the highest values in the control treatment (4.65 mEq/kg fat). Numerous studies have reported that the antioxidant effect of plant extracts is dependent on the concentration used and with increasing concentration; their antioxidant properties also increase (24, 28, 29). Zhang et al. (6) also reported that the combined coating of sodium SA-agar with ginger essential oil slows down the process of increasing beef PV. TBA values increased in all treatments with increasing time and these changes were more in the control treatment. Increased TBA levels in rainbow trout fillets have also been reported by other researchers (13, 30-32). The use of SA coating slowed the upward trend of TBA (Fig. 1b). Some researchers have found the effect of edible SA coating on fish fillets to control lipid oxidation (13, 31). They believed that SA coatings, which are placed on the surface of food, are also resistant to oxygen release. Lipid oxidation can be initiated and accelerated

through various mechanisms such as the production of single oxygen, enzymatic and non-enzymatic free radicals, and reactive oxygen species (31). Also, the use of NE slowed down the upward trend of TBA and had a positive effect on this process by increasing the concentration of the extract. Better results were observed in most of the storage times in the combined treatment of edible coatings and extract so that on the 12th day of storage, the lowest value of TBA was in the treatment of SA+ NE 1% (0.81 mg malondialdehyde/kg fat), and the highest values were in the control treatment (1.49 mg malondialdehyde/kg fat). It is possible to use dried plants and their extracts effectively to reduce the oxidation of fats in meat products. The compounds in the extracts are good donors of electrons and protons, and their intermediate radicals are very stable due to the phenomenon of electron movement in the benzene ring and the lack of a sensitive site to oxygen attack. Compounds in NE, including Neophytadiene, have the property of neutralizing free radicals and are also able to inhibit metal ions such as Fe^{2+}, thus reducing the rate of formation of reactive oxygen molecules (26, 33).

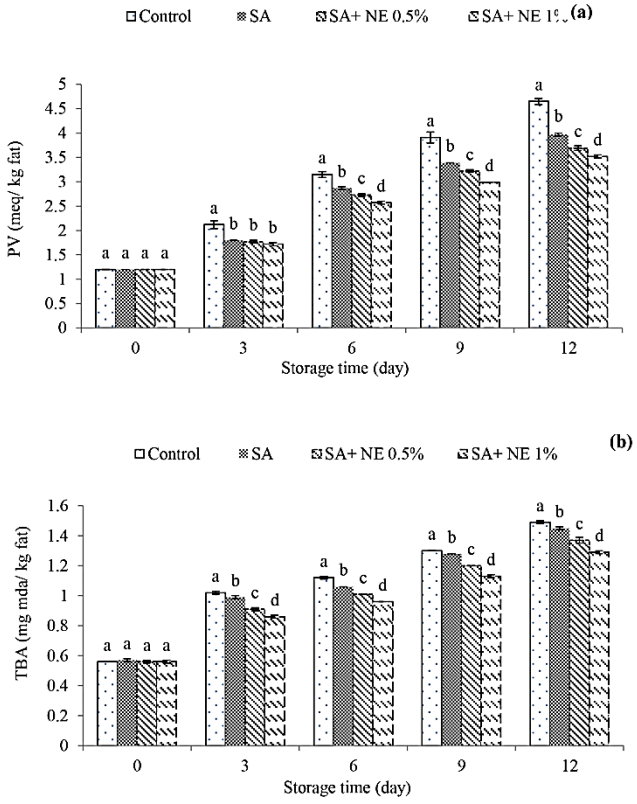

Fig. 1. Changes in peroxide value (a) and thiobarbituric acid (b) fish fillet coated by sodium alginate (SA) and SA+ nettle extract (NE). Values on the same day with different small letters are significantly different at p<0.05.

These results are in agreement with those of Heydari et al. (15), who reported that sodium alginate coating enriched with horsemint (*Mentha longifolia*) essential oil was effective against lipid oxidation in bighead carp fillets stored at 4±1°C, as well as confirmed the obtained results by Sáez, et al., (13), who reported that alginate coating enriched with tannins was effective in retarding of lipid oxidation reactions in rainbow trout fillets stored at 4±1°C). TVB-N is one of the most important parameters to determine fish quality and freshness (14). TVB-N values (Fig. 2) in all treatments increased with increasing time. The increase in TVB-N in fish may be due to various enzymatic processes such as the deamination of free amino acids, decomposition of nucleotides, and oxidation of amines (11). TVB-N values increased in all treatments with increasing time and these changes were more in the control treatment. At the end of the storage period in the control, treatment was 38.08 mg/100 g. The use of edible coatings slowed the increase in TVB-N. Edible coatings can reduce the water loss of the fillets or act as a barrier to the entry of oxygen, thus it may also affect the TVB-N content of the fillets (34).

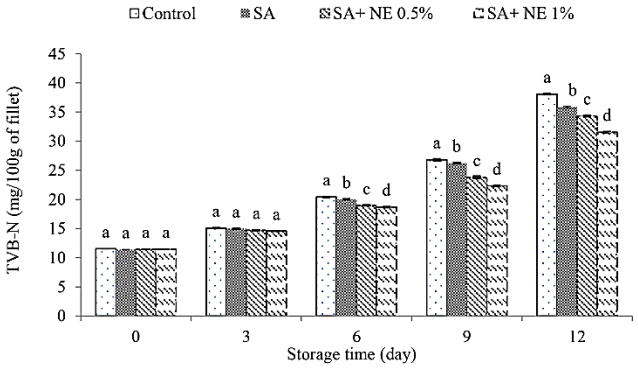

Fig. 2. Changes in total volatile base nitrogen of fish fillet coated by sodium alginate (SA) and SA+ nettle extract (NE). Values on the same day with different small letters are significantly different at p<0.05.

It may, therefore, be claimed that alginate beads are capable of detecting microbial changes and fish spoilage (14). The addition of the extract slowed down the increasing process of TVB-N and increasing the concentration of the extract had a positive effect on this process better results were observed in most of the storage times in the combined treatment of edible coatings and extracts so that on the 12th day of storage, the lowest values of TVB-N in the treatment of SA+ NE 1% (31.54 mg/100 g). The lower TVB-N value in this treatment than other treatments can be due to the reduced bacterial population of these treatments or the reduced oxidative ability of bacteria to separate amines from TVB-N compounds or both due to the effect of the extract on the bacteria in Fillet. With increasing the concentration of the extract due to the increase of phenolic compounds, its antibacterial effect also increased. Therefore, in the treatment containing higher concentrations of essential oil, the amount of TVB-N was lower (3). Heydari et al. (15) investigated the effect of sodium alginate coating enriched with horsemint (*Mentha longifolia*) essential oil on TVB-N of bighead carp fillets, a positive trend was observed due to the addition of essential oil to the sodium alginate coating in reducing of TVB-N.

3.4. Microbial evaluation of coated fish fillets

TVC values (Fig. 3a) increased in all treatments with

increasing time and these changes were more in the control treatment. At the end of the storage period, the control treatment was equal to 8.28 CFU/g log. The use of SA edible coatings slowed the increase in TVC, SA has unique colloidal properties through cross-linking with calcium by becoming treated with calcium chloride solution and is capable of producing insoluble polymers or forming strong gels. These types of biopolymer-based films can maintain good quality and increase food shelf life by preventing microbial contamination, preserving flavor, reducing wrinkles, and reducing fat oxidation. SA also acts as a barrier to oxygen transport and inhibits the growth of aerobic bacteria (31,35).

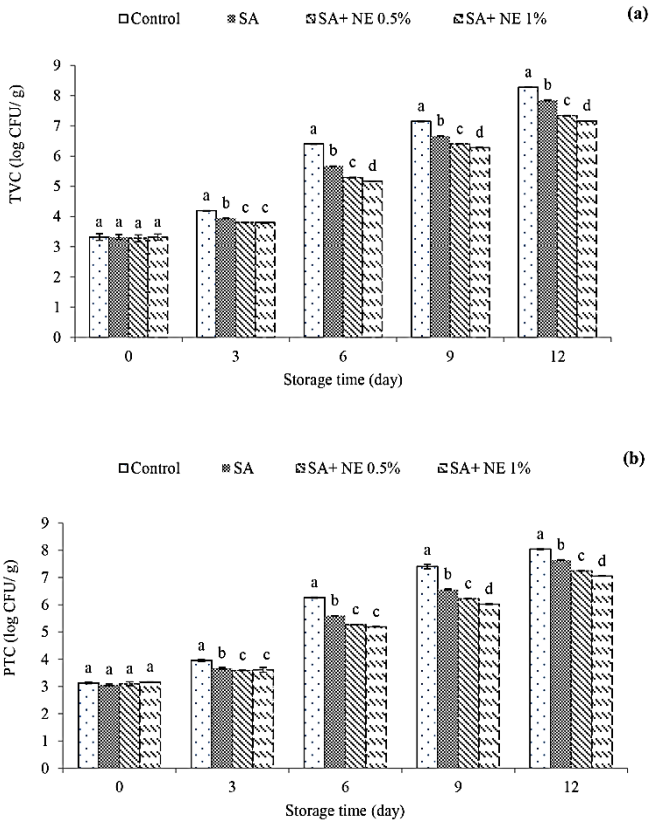

Fig. 3. Changes in total viable count (a) and psychrotrophic bacteria (b) of fish fillet coated by sodium alginate (SA) and SA+ nettle extract (NE). Values on the same day with different small letters are significantly different at p<0.05.

Also, the use of extract slowed down the increasing process of TVC, and increasing the concentration of the extract had a positive effect on this process and better results were observed in most of the storage times in the combined treatment of food coatings and extract. The lowest value of the TVC number was in SA+ NE 1% treatment (7.16 log CFU/g). Low TVC load in extracts containing extracts can be due to phenolic compounds. Phenolic compounds in plant extracts degrade the outer membrane of microorganisms and cause the release of liposaccharides and increase the permeability of the cytoplasmic membrane to ATP. ATP release leads to the depletion of cell energy storage and cell death (36). PTC values (Fig. 3b) increased in all treatments with increasing time and these changes were more in the control treatment. At the end of the storage period, the control treatment was equal to 8.04 log CFU/g. The use of edible coatings and extracts slowed down the increasing process of PTC number and increasing the concentration of extract had a positive effect on this process. The minimum amount of PTC was in the treatment of SA+ NE 1% (7.05 log CFU/g). The chemical structure of phenolic compounds affects their antimicrobial mechanism and the hydroxyl groups in phenolic compounds have an important effect on the antimicrobial properties of essential oils and plant extracts. The presence of an active hydroxyphenyl group has made these compounds easily able to form hydrogen bonds with active sites of enzymes (37). These results are in agreement with those of Kazemi and Rezaei, (16), who reported that gelatin–alginate film containing 1.5% oregano essential oil was effective against microbial spoilage in rainbow trout stored at 4±1°C, as well as confirmed the obtained results by Sáez, et al., (13), who reported that alginate coating enriched with tannins was effective in retarding of TVC and PTC in rainbow trout fillets stored at 4±1°C. LAB values (Fig. 4) increased in all treatments with increasing time and these changes were more in the control treatment. At the end of the storage period, the control treatment was equal to 7.69 CFU/g log. The use of edible coatings and extracts slowed down the increasing process of LAB and increasing the concentration of extract had a positive effect on this process. The lowest LAB value was in SA+ NE 1% treatment (6.23 log CFU/g). LAB is reported to be the most resistant gram-positive bacterium against antimicrobial agents. SA coatings act as oxygen barriers and prevent the growth of lactic acid bacteria. Neophytadiene has also been reported to have antibacterial activity, which is effective in the process of reducing lactic acid bacteria (1,38). The same results were observed by Kazemi and Rezaei, (16), who stated that rainbow trout fillet packed by gelatin–alginate film containing 1.5% oregano essential oil had lower LAB compared to the control treatment.

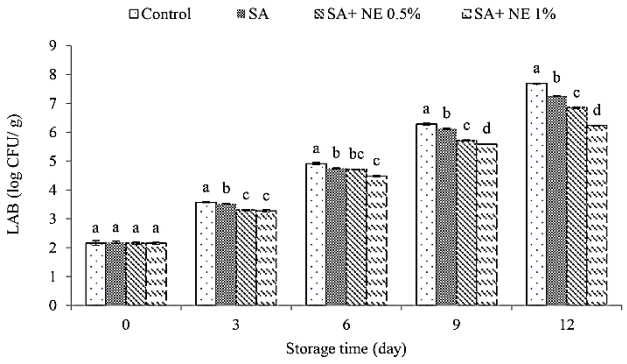

Fig. 4. Changes in lactic acid bacteria of fish fillet coated by sodium alginate (SA) and SA + nettle extract (NE). Values on the same day with different small letters are significantly different at p<0.05.

3.5. Sensory evaluation of coated fish fillet

Determination of food spoilage is done based on product quality evaluations by various sensory, chemical, and

microbiological methods. Sensory evaluation is a suitable method for evaluating the quality and freshness of fish during the storage period and is used as a simple and fast method. It should be noted that this method alone cannot be accepted as a fixed standard in the laboratory. Over time, the results of sensory properties (Fig. 5) were significantly reduced in all treatments. The use of edible coatings and extracts had a minor adverse effect on fish fillet samples.

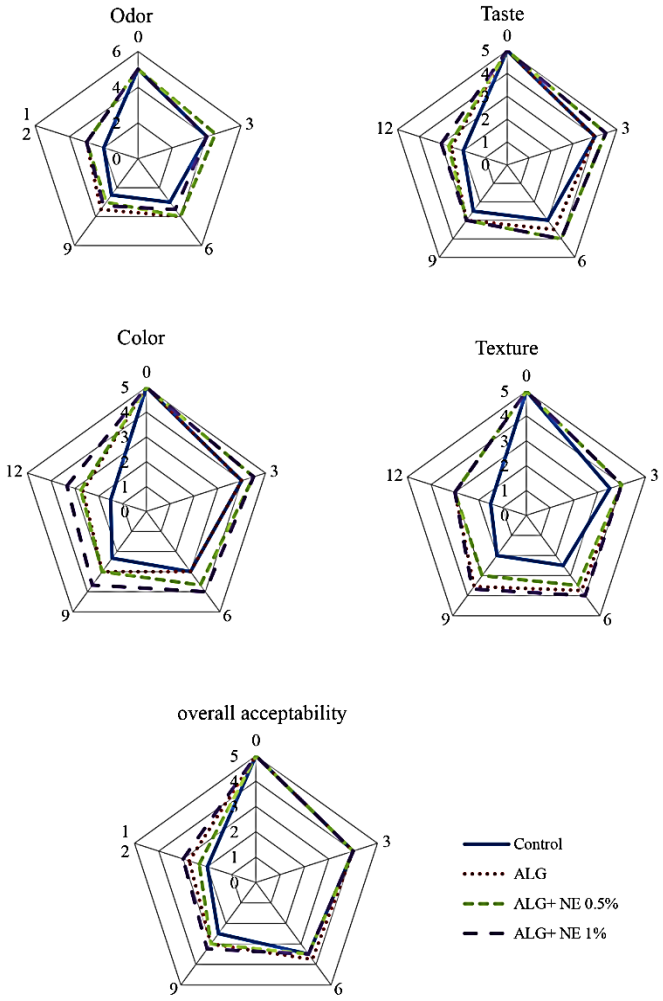

Fig. 5. Sensory evaluation of fish fillet coated by alginate (ALG) and ALG+ nettle extract (NE).

Also, in the control group, unpleasant sensory properties could be inhaled after 6 days of storage at refrigerator temperature and the control samples were completely unusable after 6 days. Changes in sensory properties were consistent with chemical and microbial results so that on the 12th day of storage, the highest values of sensory points were observed in SA+ NE 1%. This can be said to be because fat oxidation leads to degradation and loss of sensory quality and reduce the amount of nutrients, including PUFA, and the production of toxic oxidation products (11). On the other hand, increasing fat hydrolysis and accumulation of free fatty acids leads to a decrease in some product acceptance indicators, because free fatty acids have been proven to affect the stability of proteins and cause tissue destruction by reacting with proteins. Oxidation of proteins in this state occurs faster than fats that are high molecular weight fats (such as triglycerides and phospholipids) due to increased protein access to oxygen and other peroxide molecules. Also, the coherence between changes in the process of bacterial spoilage and sensory evaluation has already been proven (39), which may be related to the activity of microorganisms responsible for food spoilage.

4. Conclusion

The results of the chemical analysis showed that in general, SA coating along with the NE slowed down the increasing trend of oxidative spoilage indices compared to the control treatment and the results of the microbial analysis indicate that in all treatments, there is an increase in microbial load over time, but this increase occurred slower in treatments containing extracts and better results were observed with increasing concentration. The results of the sensory evaluation were consistent with chemical and microbial results. In general, the SA coating containing NE 1% has unique properties that can be used as an edible coating to create long shelf life for food products such as rainbow trout fillets.

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