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- RESEARCH ARTICLE-

Effect of Active Packaging Films Containing Natural Antioxidant Essential Oils on the Oxidative Stability of the African Catfish (*Clarias gariepinus*)

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Abstract

The main function of food packaging is to delay natural processes that lead to food spoilage. To this end, antioxidants are often included in food packaging materials during processing and released into packaged food through a controlled diffusion mechanism. The use of antioxidants in food packaging helps delay both lipid oxidation and protein denaturation. In recent years, natural antioxidant packages have been preferred in food packages rather than synthetic ones. In this study, it was aimed to determine the effect of using packaging containing natural antioxidant essential oils on delaying the decay time of *Clarias gariepinus*. Films containing natural antioxidant essential oils (*Thymus sp., Mentha piperita, Foeniculum vulgare, Laurus nobilis, Fructus cumini*) were prepared. Then, the effect of prepared films on the chemical quality of C. gariepinus was investigated during refrigerated storage for 15 days. The preservative effect of films was assessed periodically by a free fatty acid, thiobarbituric acid value, peroxide value, and total sulfhydryl content analyses. Obtained results showed that the use of active packaging films containing natural antioxidant essential oils improved the oxidative stability of *C. gariepinus*.

Keywords:

Active packaging, antioxidant essential oil, films, African catfish, oxidative stability **Article history:**

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Introduction

The packaging is one of the main ways of ensuring food quality and safety. The purpose of packaging is to provide consumers with safe, high quality, and high nutritional value products. Quality loss in packaged foods decreases, shelf life is prolonged, and thus food waste is reduced.

Nowadays, food packaging does not only have passive tasks such as protection and marketing of food products. With their active and intelligent packaging concepts, they offer several innovative solutions such as extending or preserving the shelf life of food, improving or monitoring food quality and safety (Dobrucka, 2013).

The presence of pathogenic or spoilage organisms is an important factor in food spoilage as well as effective in increasing the risk of foodborne disease. On the other hand, lipid oxidation is a very complex process in which many reactions occur, causing physical and chemical changes in lipids. Lipid oxidation is one of the most important forms of spoilage in foods as it causes the formation of potentially toxic components and undesirable taste and aroma. It is possible to reduce lipid oxidation by using suitable packaging technology or by adding antioxidants directly to foods. If antioxidant compounds are added directly to the food surface, protection is terminated when the active compounds are exhausted and the food breaks down (Mastromatteo et al., 2010). Therefore, the use of packages containing antioxidants may be more beneficial. Foods are protected for a longer time thanks to the slow migration of antioxidants from the packaging material to the surface of the product.

Synthetic antioxidants such as butyl hydroxyanisole, butylated hydroxytoluene and propyl gallate are commonly used in the food industry (Pereira de Abreu et al., 2010). In recent years, it has been emphasized that synthetic antioxidants used in foodstuffs can be cancer promoters (Dance-Barnes et al., 2009). Due to its antioxidant properties, great attention is paid to natural products such as tea, rosemary, oregano, spices, herbs, clove, blueberries, mustard, red wine (Alen-Ruiz et al., 2009; Beddows et al., 2000; Bhale et al., 2007; Houhoula et al., 2004; McCarthy et al., 2001; Murphy et al., 2009; Ramos et al., 2014).

In the literature, there are several studies on active packaging developed to protect the quality of fish and prevent microbial growth (Haghighi & Yazdanpanah, 2020; Azizi-Lalabadi et al., 2020; Chen et al., 2020; Ehsani et al., 2020; Albertos et al., 2019; Cardoso et al., 2017; Souza et al., 2010; Ojagh et al., 2010). However, there is no study conducted with African catfish.

Clarias gariepinus is a fish species with a geographical distribution specific to the region extending from the Middle East to the Orange River in the south. It is a species that can live in freshwater lakes, rivers and even swamps in temperate climates in Africa and the Middle East. This species, which is mostly spread around Syria, Palestine and Egypt, has also entered our country from our southern borders and is especially found in the fresh waters of Adana, Antakya and Mersin regions. The fact that it is only in a very small part of the world and therefore is very difficult to access in non-existent areas makes it even more special in this respect (Koca, 2017). Clarias gariepinus have a large head and a slender long body (70-100 cm). It has a round body section. It has mustaches on its upper and lower jaws. Thanks to an additional respiratory tissue it has; it can live outside of water (Narin, 2003). The fact that African catfish is rich in omega 3, delicious and has less bones increases its preference. In our country, the number of studies on the African catfish is quite low.

In this study, films containing natural antioxidant essential oils were prepared and the effect of these films on the oxidative stability of fresh *Clarias gariepinus* during refrigerated storage was determined.

Material and methods

All chemicals were obtained from Merck (Darmstadt, Germany). All chemicals used were of analytical reagent grade and were at least 99.5% pure. Isolated soy protein with 90% protein content, and commercial essential oils (Thymus sp., Mentha piperita, Foeniculum vulgare, Laurus nobilis, Fructus cumini) were supplied by Mecitefendi Health Products and Chemicals Corp., (Izmir, Turkey).

Clarias gariepinus samples were purchased from the local fish markets in Hatay, Turkey. Fish organs, heads, scales and bones were removed using a clean knife and cutting board. Then, fish samples were finely chopped.

Edible films based on isolated soy protein were prepared using the methods described by Emiroğlu et al., (2010). The film solution was prepared by slowly dissolving it in distilled water while mixing 5% isolated soy protein (w/v). Glycerol (3.5 %, (w/v)) was used as a plasticizer. The pH of the solution was adjusted to 10.0 with 0.1 N NaOH. It was heated to 90 ° C in a water bath for 30 minutes, then cooled to 40 ° C and then filtered through four layers of cheese cloth with a vacuum pump. Essential oils were included in the film solution at a concentration of 5% (v / v). The film solution was poured into sterile plastic petri dishes (9 cm diameter), then dried at 30 ° C for 72 hours in an oven. The films were separated from petri dishes and stored in a vacuum desiccator for later use. Film thicknesses were measured with a digital micrometer (Loyka, Turkey) to the nearest 0.001 mm. Measurements were taken at six random locations of the films. The average thickness was calculated.

Quanta 450 FEG model scanning electron microscopy (SEM) was used for examining the morphology of the films. The properties of the films such as dense skin layer thickness, porosity and pore size were measured with the help of software on the computer connected to SEM.

Finely chopped fish was divided into seven groups. The groups were as follows: Untreated samples (control groups, CG); samples coated with isolated soy protein films without addition of essential oil (ISP); samples coated with films containing 5% Thymus sp essential oil (TO); samples coated with films containing 5% Mentha piperita essential oil (MPO); samples coated with films containing 5% Laurus nobilis essential oil (LNO); samples coated with films containing 5% Fructus cumini essential oil (FCO).

Samples in each group were weighed in portions of 50 ± 0.5 g. The film materials prepared as described above were applied to the upper and bottom surfaces of the samples except for control groups. Samples were placed in plastic bags and vacuumed. Then, samples were stored at 4 °C for 15 days. After refrigeration for the duration of 0, 1, 3, 6, 8, 10 and 12 days samples were taken and free fatty acid contents and peroxide values were determined.

The method described by Bligh and Dyer, (1959) was used in the extraction of lipids. Fifty grams of the sample was homogenized by adding 150 ml of chloroform: methanol (1:2) mixture. Then, 100 ml of chloroform: water (1:1) mixture was added to the homogenate with stirring. The

mixture was filtered through a Büchner funnel. The solvent evaporated at 40 °C on a rotary evaporator. Total lipid amounts were determined by standing in a desiccator for constant weighing.

Five grams from extracted lipids were taken and dissolved in a mixture of ethyl alcohol: diethyl ether (1:1). The solution was titrated with 0.1 N NaOH until a pink color accompanied by phenolphthalein indicator. Results were calculated in % oleic acid (Kodal, 2008).

The peroxide value (PV) is expressed as meq oxygen/kg fat. It was determined from the lipid extract according to method Cd 8-53 of the American Oil Chemists' Society (AOCS, 1998). The weighed 2 g lipid was mixed with 30 ml of acetic acid/chloroform (3:2, (v/v)) mixture. It was continuously stirred to dissolve the lipids. After the potassium iodide solution was added, it was allowed to stand for 1 minute. It was kept in the dark for 5 minutes with occasional agitation. Then, 30 ml of distilled water and 1% starch were added and titrated against 0.002 N Na₂S₂O₃. The same procedure was applied for the blank containing no lipid.

The method described by Vyncke (1970) was used to calculate the TBA value. TBA is expressed as mg malondialdehyde/kg sample. Twenty grams of fish were homogenized with 100 ml of 7.5% trichloroacetic acid for one minute. Then, it was filtered and 5 ml of filtrate was added to 5 ml of thiobarbituric acid reagent (0.02 M). The mixture was immersed in boiling water for 40 min. After cooling to room temperature, the absorbance was measured at 538 nm.

The total sulfhydryl value was determined according to the method developed by Monahan et al., (1995). One gram of sample was mixed in 9 mL of SDS solubilization buffer. It was heated at 100 °C for 5 min. Then, it was centrifuged at 10000 rpm for 15 min. 0.01 ml of Ellman's reagent was added to 1 mL aliquot of the supernatant. The mixture was incubated at 40°C for 25 min. (Yongsawatdigul & Park, 2004). Total SH groups were calculated by measuring absorbance at 412 nm. The extinction coefficient was used as 13600 $M^{-1}cm^{-1}$. Results were calculated as μ mol sulfhydryl / g protein (Ellman, 1959).

All analyses were performed with SPSS version 22.0 for Windows (Chicago, II., USA), and p < 0.05 was considered as statistically significant. Data were expressed as the mean \pm standard deviation (SD). Data were analyzed with variance (ANOVA) and then with Bonferroni's post hoc test. The calculation of the Pearson correlation coefficient was used to find the relationship between factors that were studied.

Results

The film thickness varied from 0.169 ± 0.018 mm to 0.194 ± 0.007 mm. The differences in thickness were due to differences in amounts of film solutions that were cast onto the petri dishes.

The micrographs obtained from the surfaces of films with different antioxidant contents are presented in Figure 1. The absence of cracks or fractures on the surfaces of the films indicated that the surface morphology was uniform. There was no significant difference between the SEM observations of the films with different essential oil contents.

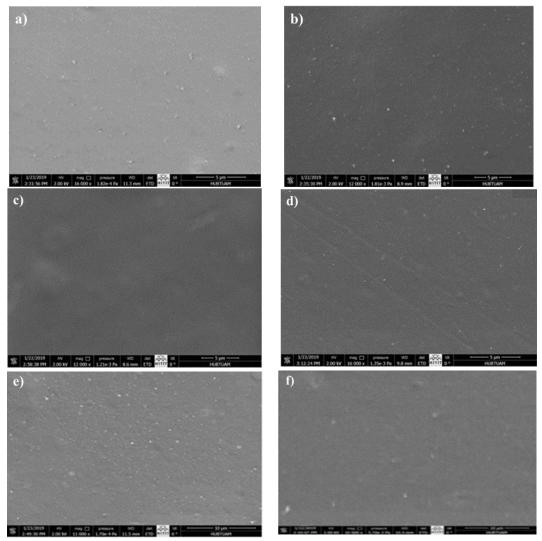


Figure 1. The micrographs obtained from the surfaces of films with different antioxidant contents (a) 5% *Fructus Cumini* essential oil added films (FCO); (b) 5% *Foeniculum Vulgare* essential oil added films (FVO); (c) 5% *Laurus Nobilis* essential oil added films (LNO); (d) 5% *Mentha Piperita* essential oil added films (MPO); (e) 5% *Thymus sp* essential oil added films (TO); (f) Control groups (CG)

Lipid hydrolysis produces free fatty acids. The degree of deterioration of foods is determined by measuring free fatty acid content (Barthet et al., 2008). The levels of free fatty acids during refrigerated storage of *Clarias gariepinus* are shown in Table 1. The free fatty acid contents of the samples packed with antioxidant films showed a gradual increase during the refrigerated storage period. The levels of free fatty acids showed a positive correlation with refrigerated storage times (Table 1). The free fatty acid content of the control samples was significantly higher than the treated samples. The lowest free fatty acid contents were obtained in samples coated with 5% Mentha piperita and Thymus sp. essential oil added films. The results showed that the hydrolytic activity was affected by the antioxidant type and storage time.

0.006; 0.005

Refrigerated Oleic Acid (%) p values storage time (days) **Control** LNO TO **FCO FVO MPO** 0 2.21 ± 0.02 2.21 ± 0.02 2.21 ± 0.02 2.21 ± 0.02 2.21 ± 0.02 2.21 ± 0.02 0.017; 1 2.81 ± 0.03 1.73 ± 0.01^{a} 1.69 ± 0.04^{b} 1.78 ± 0.01^{c} 1.86 ± 0.01 $1.94\pm0.03^{\rm d}$ 0.005; 0.049; 0.007 3 3.74 ± 0.01 1.91 ± 0.02^{a} 1.75 ± 0.03^{b} 1.84 ± 0.01 $1.99\pm0.02^{\rm e}$ 2.05 ± 0.01 0.005; 0.010; 0.001 6 4.38 ± 0.01 3.11 ± 0.01 2.86 ± 0.01^{b} $3.06\pm0.02^{\rm c}$ 3.18 ± 0.01 3.23 ± 0.02 0.004; 0.019 8 5.86 ± 0.01 3.54 ± 0.01^a $3.03\pm0.03^{\text{b}}$ $3.14\pm0.03^{\rm c}$ 3.25 ± 0.01 0.002; 3.34 ± 0.04 0.011; 0.017 0.019; 10 7.07 ± 0.03 4.61 ± 0.03 4.31 ± 0.01^{b} $4.47\pm0.01^{\rm c}$ $4.72\pm0.01^{\rm e}$ 4.16 ± 0.01^{d} 0.007; 0.047; 0.031 12 7.91 ± 0.01 5.07 ± 0.01 4.88 ± 0.01^{b} 5.19 ± 0.03^{c} 5.62 ± 0.01^{e} 4.57 ± 0.01 0.003:

Table 1. The levels of free fatty acids during refrigerated storage of *Clarias gariepinus* (N=3)

0.991

Correlation

coefficients

Peroxide values (PV) during refrigerated storage of *Clarias gariepinus* are shown in Table 2. In both control and samples of the catfish packed with antioxidant films, the peroxide values increased significantly up to 12 days during storage and then decreased gradually until the end of the study. After reaching the maximum peroxide value, a decrease in peroxide value levels was observed. This decrease is a sign of advanced oxidation. Peroxide values showed a positive correlation with refrigerated storage times (Table 2). During 12 days of storage, the PV in the samples wrapped in the film containing antioxidants was significantly lower than in the control samples. There are studies in the literature stating that reductions in peroxide values during advanced degradation stages may result from the instability of peroxide molecules (Abreu et al., 2011).

0.930

0.938

0.941

0.966

Table 2. Peroxide values during refrigerated storage of *Clarias gariepinus* (N=3)

Refrigerated							
storage time (days)	Control	LNO	ТО	FCO	FVO	MPO	p values
0	7.81 ± 0.01						
1	8.15 ± 0.01	8.04 ± 0.01	8.09 ± 0.01	8.12 ± 0.01	8.06 ± 0.01	8.07 ± 0.01	
3	8.52 ± 0.01	8.21 ± 0.02	8.39 ± 0.01	8.44 ± 0.02	8.34 ± 0.01	8.46 ± 0.01	

^{0.959} a: groups control and LNO; b: groups control and TO; c: groups control and FCO;

d: groups control and MPO; e: groups control and FVO

6	12.28 ± 0.01	10.03 ± 0.01	9.76 ± 0.01^a	10.33 ± 0.01	10.41 ± 0.01^{b}	$9.88 \pm 0.02^{\circ}$	0.011;0.022; 0.001
8	14.95 ± 0.01	13.64 ± 0.01	$13.55 \pm 0.01^{\rm a}$	13.05 ± 0.01^{d}	13.18 ± 0.01	13.47 ± 0.02	0.020; 0.011
10	16.63 ± 0.01	$15.26\pm0.01^{\text{e}}$	14.33 ± 0.01	$15.88\pm0.01^{\text{d}}$	15.91 ± 0.02	16.11 ± 0.01	0.007; 0.023
12	19.16 ± 0.02	17.76 ± 0.01	17.19 ± 0.01	$18.57\pm0.02^{\text{d}}$	19.04 ± 0.01	19.08 ± 0.01	0.021
14	15.90 ± 0.01	$14.72\pm0.01^{\text{e}}$	$14.35\pm0.01^{\mathrm{a}}$	$14.56\pm0.01^{\mathrm{d}}$	14.88 ± 0.01	14.23 ± 0.02	0.033; 0.004;
16	13.86 ± 0.01	13.50 ± 0.01	13.01 ± 0.01	13.09 ± 0.01	13.12 ± 0.01	13.24 ± 0.01	0.007
Correlation coefficients	0.987	0.966	0.962	0.965	0.963	0.957	

a: groups control and TO; b: groups control and FVO; c: groups control and MPO;

TBA value is an important indicator of oxidation, which gives the amount of malonaldehyde that occurs as a result of lipid oxidation. The concentrations of TBA in samples of *Clarias gariepinus* stored at 4 °C are shown in Table 3. The concentrations of TBA in both control and the catfish samples packed with antioxidant films increased constantly during the twelfth days of the study. TBA values showed a positive correlation with refrigerated storage times (Table 3). The significant increase in TBA values indicated oxidative deterioration. The concentrations of TBA in the control group were significantly higher than the treated samples. This indicates that the antioxidants have a protective effect on secondary oxidation.

Table 3. Thiobarbituric acid values during refrigerated storage of *Clarias gariepinus* (N=3)

Refrigerated	Thiobarbituric acid values (mg malondialdehyde / kg sample)						
storage time (days)	Control	LNO	то	FCO	FVO	MPO	
0	0.476 ± 0.01	0.476 ± 0.01	0.476 ± 0.01	0.476 ± 0.01	0.476 ± 0.01	0.476 ± 0.01	
1	0.504 ± 0.01	0.498 ± 0.01	0.481 ± 0.01	0.499 ± 0.01	0.480 ± 0.01	0.482 ± 0.01	
3	0.613 ± 0.01	0.533 ± 0.01^{a}	$0.525 \pm 0.01^{\rm b}$	0.552 ± 0.01^{c}	0.492 ± 0.01^{d}	0.497 ± 0.01	0.015; 0.046; 0.025; 0.028
6	0.676 ± 0.02	0.564 ± 0.01^{a}	$0.540 \pm 0.01^{\rm b}$	$0.575 \pm 0.01^{\circ}$	$0.507 \pm 0.01^{\rm d}$	0.515 ± 0.01^{e}	0.027; 0.017; 0.047; 0.039; 0.001
8	0.762 ± 0.02	0.572 ± 0.01	$0.548 \pm 0.01^{\rm b}$	0.622 ± 0.01	0.535 ± 0.01	0.540 ± 0.01	0.008
10	0.769 ± 0.02	0.620 ± 0.02^{a}	0.564 ± 0.01	0.661 ± 0.02^{c}	0.544 ± 0.01	0.552 ± 0.01	0.004; 0.032
12	0.863 ± 0.02	0.659 ± 0.02^{a}	$0.579 \pm 0.01^{\rm b}$	0.684 ± 0.02^{c}	0.555 ± 0.01	$0.564 \pm 0.01^{\text{e}}$	0.001; 0.001; 0.047; 0.006
Correlation coefficients	0.988	0.986	0.972	0.993	0.990	0.996	

d: groups control and FCO; e: groups control and LNO

a: groups control and LNO; b: groups control and TO; c: groups control and FCO;

With the oxidation of proteins, sulfhydryl groups form disulfide bonds within or between molecules. The amount of sulfhydryl groups is gradually decreasing due to the progression of oxidation. Therefore, determining the total amount of sulfhydryl in foods is useful in understanding the level of oxidation occurring in proteins (Batifoulier et al., 2002). The content of sulfhydryl in both control and the catfish samples packed with antioxidant films decreased constantly during refrigerated storage. Sulfhydryl contents showed a negative correlation with refrigerated storage times (Table 4). In the control groups' reduction in the total sulfhydryl content was higher than in the samples. By assimilating the SH groups of proteins to antioxidants, they can trap free radicals generated and prevent further greater oxidation during storage.

Table 4. The levels of sulfhydryl during refrigerated storage of . (N=3)

Refrigerated storage time	Sulfhydryl content (µmol/g protein)						
(days)	Control	LNO	ТО	FCO	FVO	MPO	
0	0.747 ± 0.01	0.747 ± 0.01	0.747 ± 0.01	0.747 ± 0.01	0.747 ± 0.01	0.747 ± 0.01	
1	0.486 ± 0.01	0.628 ± 0.01	$0.703 \pm 0.01^{\rm a}$	0.698 ± 0.01^{b}	0.672 ± 0.01^{c}	0.629 ± 0.01	0.006; 0.027; 0.003
3	0.478 ± 0.01	0.590 ± 0.01	0.519 ± 0.01	0.518 ± 0.01	0.581 ± 0.01	$0.537 \pm 0.01^{\text{d}}$	0.027
6	0.456 ± 0.01	0.521 ± 0.01	$0.513 \pm 0.01^{\mathrm{a}}$	0.474 ± 0.01	0.506 ± 0.01	$0.529 \pm 0.01^{\rm d}$	0.004; 0.002
8	0.454 ± 0.01	0.502 ± 0.01^{e}	0.488 ± 0.01	0.467 ± 0.01	0.484 ± 0.01	0.498 ± 0.01	0.003
10	0.415 ± 0.01	0.493 ± 0.01^{e}	0.486 ± 0.01	0.450 ± 0.01	0.448 ± 0.01	0.425 ± 0.01	0.013
12	0.389 ± 0.01	0.411 ± 0.01^{e}	0.477 ± 0.01	0.439 ± 0.01	0.426 ± 0.01	0.407 ± 0.01	0.001
Correlation coefficients	-0.757	-0.945	-0.854	-0.885	-0.960	-0.933	

a: groups control and TO; b: groups control and FCO; c: groups control and FVO;

Discussion

It is known that fish have many benefits from brain development to cardiovascular diseases, from diabetes to strengthening the immune system. Fish meat, which is a source of protein with high biological value, contains sufficient amounts of the mineral, vitamin, and fatty acids, which are important in terms of nutrition, as well as their taste characteristics. However, fish meat, which is so important for health, can be spoiled by undergoing some changes in improper storage conditions. Active packaging films and coatings, which were previously used to prevent moisture loss during storage and transportation, today it is used to improve the quality characteristics of foods and to extend the shelf life.

d: groups control and FVO; e: groups control and MPO

d: groups control and MPO; e: groups control and LNO

There are studies in the literature that many polysaccharides, lipids and proteins of vegetable and animal origin are used in the production of edible films and coatings, alone or in mixtures (Robertson, 2013). Generally, lipids are used to reduce water transfer, polysaccharides to control the passage of oxygen and other gases, while proteins are used to give films mechanical resistance (Tural et al., 2017). Edible films and coatings are very promising systems for the future in preserving and improving product quality. However, studies on edible films and coatings are very new and are still at the research level.

Various active packages have been developed to extend the shelf life of fish. Azizi-Lalabadi et al. (2020) prepared polypropylene-based films containing 4A zeolite, ZnO nanoparticles and green tea extract. They found that the PV, TBA and TVBN (total volatile basic nitrogen) values of salmon wrapped in these films increased significantly during storage. They stated that PP nanocomposite packages containing ZnO NPs / 4AZ / GTE increased the shelf life, quality and safety of salmon. Haghighi and Yazdanpanah (2020) studied the effects of chitosan films combined with cinnamon and tea extracts on the quality parameters of fish fillets. They stated that FFA, pH, TBA and TVBN values remained within the standard range during storage and they extended the shelf life of fish fillets. Ehsani et al. (2020) investigated the effect of active biodegradable films containing sage essential oil or lactoperoxidase system on the shelf life of fish burgers. Fish burgers were prepared from minced carp meat and wrapped in chitosan, alginate and gelatin films containing sage essential oil or lactoperoxidase system. As a result of this study, they found that the chitosan film containing lactoperoxidase system extended the shelf life of fish burgers the most. Cardoso et al. (2017) developed active films poly (butylene adipate co-terephthalate) with oregano essential oil to increase the durability of fish fillets. They analyzed the bioactivity, antioxidant and antimicrobial activities of the films. Furthermore, they stated that the mechanical, thermal and water barrier properties of the films are suitable for food packaging. Albertos et al. (2019) developed edible films with seaweed to extend the shelf life of fresh fish burgers. They observed a decrease in lipid oxidation and an increase in antioxidant capacity of trout burgers during storage.

The edible films based on isolated soy protein were applied on the fish and the free fatty acidity, peroxide content, thiobarbituric acid content and sulfhydryl content were examined for 15 days. When all the analyzes were evaluated, more positive results were obtained in the fish samples that were wrapped with edible films with essential oil addition compared to the control groups without essential oil. The results showed that the active packaging developed with natural antioxidant essential oils delayed lipid oxidation and increased oxidative stability in the catfish samples during refrigerated storage.

Clarias gariepinus is a very rare fish in the world although it is abundant in Hatay. With this study, it will be easier to transport the catfish to different regions freshly and naturally.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical approval

For this type of study formal consent is not required.

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