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Research Article **Araştırma Makalesi**

Physicochemical Properties of Sprat (*Sprattus sprattus* L., 1758) Protein Hydrolysate and Usage as a Coating Material on Trout (*Oncorhynchus mykiss*, Walbaum, 1792) Fillets

Çaça (*Sprattus sprattus*, L., 1758) Protein Hidrolizatının Fizikokimyasal Özellikleri ve Alabalık (*Oncorhynchus mykiss*, Walbaum, 1792) Filetolarında Kaplama Malzemesi Olarak Kullanımı

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Abstract: Trout is the most widely cultivated and traded fish species in Turkey and many European countries, and a total of 471686 tons of production was realized in Turkey's seas and inland waters in 2021. In addition to its nutritive value, this fish takes a crucial position in the aquaculture sector due to its continuous and intensive production. In this study, innovative bioactive protein hydrolysates (PH) produced from sprat were used as a coating to increase the quality and shelf life of trout fillets stored at +4 ˚C. The yields of traditional hydrolysate (TH) and ultrasound-assisted hydrolysate (UH) were 8.66% and 12.63%, respectively. Protein values of TH and UH were 75.88 and 74.45%, respectively. Three fillet groups were prepared from trout: uncoated control group (C), traditional enzymatic protein hydrolysate coated group (THC), and ultrasound-assisted enzymatic protein hydrolysate coated group (UHC). TVB-N value, which was 12.96 mg/100g in fresh trout, exceeded the consumable limit with 38.52 mg/100g on the 9th day of storage in the C. This value reached 32.92 mg/100g and 33.62 mg/100g on the 12th day for THC and UHC, respectively. At the beginning of storage, 0.21 mg MA/kg TBA reached the consumable limit with 7.72 mg MA/kg in the C on the 9th day, while THC and UHC remained within the limit with 6.67 mg MA/kg and 6.79 mg MA/kg, respectively. Total aerobic mesophilic bacteria (TAMB) and total aerobic psychrophilic bacteria (TAPB) counts on day 0 of storage were 2.13 log cfu/g and 1.66 log cfu/g, respectively. These values were calculated as 6.90, 5.20, 5.04, 6.95, 3.48, and 3.22 log cfu/g for C, THC, and UHC on the 12th day of storage, respectively. The application of ultrasound during the production of protein hydrolysate significantly increased the yield and hydrolysis degree of UH compared to TH. The lipid ratio of UH was also found to be significantly lower than TH. In these respects, the application of ultrasound has provided an advantage. Although both coatings have provided significant protection compared to the C, generally similar results were obtained between the THC and UHC. PH coatings delayed the chemical, physical, and microbial spoilage of trout fillets and extended the storage period. The results showed that PH can be used as a coating on fish fillets stored in cold conditions.

Keywords

- Trout
- Protein hydrolysate
- Coating
- Sprat
- Ultrasound

Özet: Alabalık ülkemizde ve birçok Avrupa ülkesinde yetiştiriciliği ve ticareti en fazla yapılan balık türüdür ve 2021 yılında ülkemiz denizleri ve içsularında toplam 471686 tonluk üretimi gerçekleştirilmiştir. Besleyici değerinin yanısıra sürekli ve yoğun üretiminden dolayı bu balık su ürünleri sektöründe önemli bir yere sahiptir. Bu çalışmada +4 ˚C'de depolanan alabalık filetolarının kalite ve raf ömrünü uzatmak amacıyla çaçadan üretilen yenilikçi biyoaktif protein hidrolizatları (PH) kaplama olarak kullanılmıştır. Geleneksel hidrolizat (TH) ve ultrason destekli hidrolizat (UH) verimleri sırasıyla %8.66 ve %12.63 olarak hesaplanmıştır. TH ve UH'a ait protein değerleri sırasıyla %75.88 ve %74.45 olarak belirlenmiştir. Kaplamasız kontrol grubu (K), geleneksel enzimatik protein hidrolizatı ile kaplanmış grup (GHKS) ve ultrases destekli enzimatik protein hidrolizatı ile kaplanmış grup (UHKS) olmak üzere 3 fileto grubu hazırlanmıştır. Taze alabalıkta 12,96 mg/100g olan TVB-N değeri K grubunda depolamanın 9. gününde 38,52

Anahtar kelimeler

- Alabalık
- Protein hidrolizatı
- Kaplama
- Çaça
- Ultrason

mg/100g ile tüketilebilirlik sınırını aşmıştır. Bu değer GHKS ve UHKS için 12. günde sırasıyla 32,92 mg/100g ve 33,62 mg/100g'a ulaşmıştır. Depolamanın başında 0,21 mg MA/kg TBA değeri 9. günde K grubunda 7,72 mg MA/kg ile tüketilebilirlik sınırına ulaşırken GHKS ve UHKS de sırasıyla 6,67 mg MA/kg ve 6,79 mg MA/kg ile sınırın içerisinde kalmıştır. Depolamanın 0. gününde toplam aerobik mezofilik bakteri (TAMB) ve toplam aerobik psikrofilik bakteri (TAPB) sayıları sırasıyla 2,13 log kob/g ve 1,66 log kob/g olarak bulunmuştur. Bu değerler depolamanın 12. gününde K, GHKS ve UHKS için sırasıyla 6,90, 5,20, 5,04 ve 6,95, 3,48 ile 3,22 log kob/g olarak hesaplanmıştır. PH kaplamalar alabalık filetolarında kimyasal, fiziksel ve mikrobiyal bozulmayı geciktirerek depolama süresini uzatmıştır. Elde edilen sonuçlar PH'ın, soğuk koşullarda depolanan balık filetolarında kaplama olarak kullanılabileceğini göstermiştir.

1. INTRODUCTION

Today, the awareness of consumers has led to an increase in the consumption of functional foodstuffs, especially seafood. This necessitated the use of safe additives to extend the shelf life, improve color and textural properties, and increase flavor and nutritional value by preserving the sensory properties of the foods to be consumed at the industrial food production stages, without impairing the quality. In general, artificial acidity regulators, antioxidant and antimicrobial effects, color, texture, flavor enhancers, emulsifiers, and stabilizing agents can be used in the processing technologies to be applied to the product. In recent years, there has been a tendency to use chemical additives in foods in natural and near-natural forms as much as possible (Ghanbari et al., 2013; Erkan et al., 2015). There is a need for studies to develop alternative production models using natural raw materials to meet the demands for healthy and safe products, and to obtain products with improved nutritional value with different additives.

Fisheries are important sources in meeting the world's protein needs. The aquaculture sector is among the important sectors that provide continuous input to the national economies. But there are critical issues at all stages from the production to consumption of seafood faced by the aquaculture industry, both economically and environmentally. Namely, species that landed as discarded, species that traditionally have no demand for consumption, by-products and wastes generated during processing, and poor and/or inadequate processing, packaging, and storage conditions (FAO, 2020).

With the scientific research in recent years innovative and environmentally friendly production, processing, packaging, distribution, and storage techniques are being developed (Biji et al., 2015; Hoek et al., 2021). At the same time, from the raw material to the final product; by evaluating the discharged species and by-products and wastes, with added value, with functional features, Significant progress has been made in the development of natural products versus synthetic products (Šimat et al., 2021; Tiwari and Khawas, 2021).

Lorenzo et al. (2018) reported that people are aware of the food-health relationship and that they have started to use their food consumption preferences in favor of ingredients consisting of healthy ingredients free from synthetic additives. Protein hydrolysates demonstrate great potential as antioxidant additives to food because they can act in several ways: by reducing hydro-peroxides, scavenging free radicals, ROS inactivation, pro-oxidative transition metal chelation, and modifying the physical properties of the products (Sohaib et al., 2017). Loi, Eyres, and Birch, (2019) stated that showing surface-active properties, proteins derived from protein hydrolysates they can act as a physical barrier, minimizing the contact of lipids with oxidizing agents, which contributes to the reduction of lipid peroxidation in such foods. Barcellos et al., (2020) determined the impacts of the commercial fish gelatin hydrolysate added to rainbow trout fillets (*Oncorhynchus mykiss*) packed under vacuum. Their results were compared and correlations were observed regarding fish preservation assessments. The authors concluded that the application of protein hydrolysate as an additive is promising and can effectively control food quality for a longer period.

In the evaluation of world fishery products, live fresh and chilled products have the largest share among consumers' preferences (FAO, 2020). Usage areas of seafood products other than human consumption are agricultural usage from wastes, industrial usage as a nutrient-rich soil amendment and digest into fuel and fertilizer from by-products, and the highest and most preferred usage is food grade usage for human consumption as maintaining food grade standards, hydrolyzed proteins, nutraceuticals, pharmaceuticals, etc., (Anal, 2017).

In this study, sprat, which is the most cough fish after anchovy in Turkey's seas, but is not used for human consumption was used for the production of a protein hydrolysate, and some physicochemical properties of hydrolysate were determined. The enzymatic method was applied for Protein hydrolysate production, and it was used in different ways as conventional and ultrasound-assisted. Trout fillets were coated with sprat protein hydrolysate, with various functional properties, to reduce and/or delay the quality losses during the storage at +4 °C. The quality changes that occurred by coating with hydrolysate were investigated.

2. MATERIALS AND METHODS

2.1. Materials

In the study, whole sprat (*Sprattus sprattus* L., 1758). was used for the production of hydrolysates (TH and UH) (Figure 1a). Sprat was caught by a research vessel of Central Fisheries Research Institute, Trabzon, Turkey, stored in the freezer in the vessel, and reached the Food Technology Laboratory in iced box adjusted Styrofoam boxes. Trout (*Oncorhynchus mykiss*, Walbaum, 1792) was used for the production of coated fish fillets (Figure 1b). The fish were obtained from a local fish store (Trabzon, Turkey) and brought to the laboratory under a cold chain. After measuring their length and weight, the fish were washed under tap water and their internal organs and skins were removed, and the fish were filleted. The enzyme used in the production of hydrolysate was Alcalase 2.4 L (AU/kg Sigma Aldrich) (Novozymes, Bagswaerd, Denmark).

Food-grade glycerol; (99.96% purity W252506, Sigma-Aldrich) at 20% (w/w, based on biopolymer content) was purchased from Sigma Aldrich (Steinheim, Germany) and used as a plasticizer. All other chemicals, and solvents used in this work were of analytical grade.

2.2.Methods

The hydrolysis process was carried out in a shaking water bath at pH 8 and temperature of 60 °C, using Alcalase at 0.5% enzyme/substrate ratio for 1 hour according to Sathivel et al. (2005) with some modifications as expressed in Balçık Mısır and Koral, 2019. Ultrasound pretreatment was applied for UH production. A probe-type ultrasound (Bandelin, Sonoplus HD 4200, homogenizer, Germany) was used for this purpose; the device was set up with 40% amplitude, and the pulse/wait time was set up 10/10 seconds as given Rodriguez-Turienzo et al. (2012).

a b

Figure 1. Spratt (*Sprattus sprattus*)(a), rainbow trout (*Oncorhynchus mykiss*)(b)

2.2.1. Degree of Hydrolysis

The degree of Hydrolysis of protein hydrolysates was determined according to the pH-stat method. The pH of the solution was kept constant at 8 with 0.1 N NaOH. NaOH consumption was recorded every 5 minutes for 60 minutes. Results are expressed as % (Wrolstad et al., 2005).

2.2.2. Yield of Hydrolysate

Yields of fish protein hydrolysates were based on the initial substrate amount.

2.2.3. Proximate analysis

Standard methods were used for crude protein, and lipid, analysis (AOAC 1995). The amount of crude protein was calculated by multiplying the amount of N% obtained by a factor of 6.25 (AOAC, 1995). A crude lipid with Soxhlet extraction with diethyl ether. The moisture content of the samples

was determined according to Ludorff and Meyer (1973). Ash analysis was conducted according to Mattissek et al. (1988).

2.2.4. Antioxidant Activity

Free radical scavenging activities of hydrolysates were carried out according to the method of Brand-Williams et al. (1995).

2.2.5. Preparation of the coatings:

Coatings were prepared according to Rodriguez-Turienzo et al. (2012). FPH content of the coatings was adjusted to 8% and weighted values mixed with distilled water at 20 ºC for 30 minutes. By adding glycerol to the mixtures with a protein/glycerol ratio of 2:1, the pH was adjusted to 7 with 1N NaOH in a water bath at 60 ºC and shaken for 30 min (Figure 2).

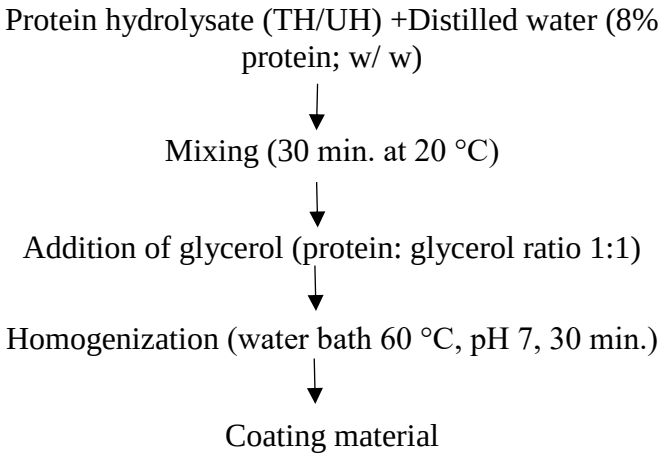

Figure 2. Preparation of coating materials

2.2.6. Coated Trout Fillets

2.2.6.1. Preparation of coated fillets:

The dipping method was used for the coating process. Fish fillets were coated with TH and UH as given by Balcik Mısır and Koral (2021). Briefly 5 min. immersing into the coating solution for 3 min. draining on the grill. The coating process of trout fillets is demonstrated in Figure 3.

Fillets were prepared by hand, washed, and drained. Drained fillets were divided into three treatments: control without any coating (C), TH coated group (THC) UH coated group (UHC).

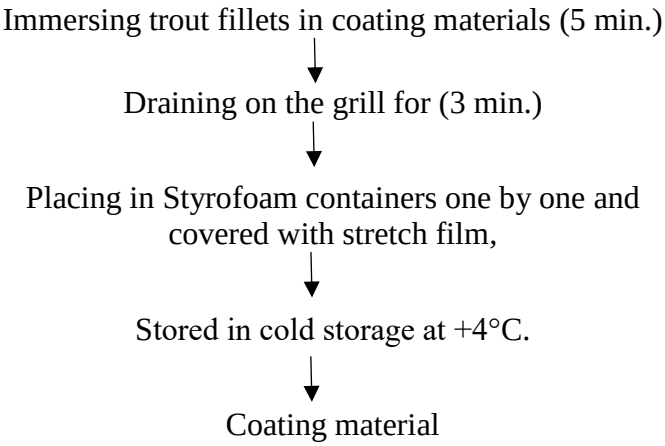

Figure 3. The coating process of trout fillets

In the study, samples were separated from each group for day 0 analyses. Analyzes were performed at +4 °C with an interval of 2 days.

2.2.6.2. Water activity

The water activities of the samples were measured with a water activity device (Aqualab 4TE, USA). The amount of water activity in the samples is placed in the measuring cups of the device and read automatically.

2.2.6.3. pH measurement

The pH values of the samples were determined using a pH meter (Mettler Toledo, FiveGo F2, Greifensee Switzerland).

2.2.6.4. Color Analysis

Color analysis was performed using Konika Minalto CR 10 (Inc., Japan) color analyzer. L*, a*, and b* values were determined according to the criteria given by the International Commission on Illumination CIELAB (Commission Internationalele de I'E Clairage), which is based on three-dimensional color measurement.

2.2.6.5. Total Volatile Basic Nitrogen (TVB-N) Analysis

The determination of total volatile basic nitrogen (TVB-N) was determined by the distillation method (Varlık et al., 1993) applied by Antonacopoulos and Vyncke (1989).

2.2.6.6. Thiobarbituric Acid (TBA) Analysis

TBA analysis was made according to Tarladgis et al. (1960).

2.2.6.7. Microbiological Analysis

All microbial counts were made using a 10 g sample. Nine times the weighed amount of dilution liquid was added to form a 10-1 dilution. Other decimal dilutions were prepared from the 10-1 dilution obtained by transferring 90 ml of 0.01 peptone water on the sample.

2.2.6.8. Total aerobic mesophilic bacteria count:

The total aerobic mesophilic bacteria count was made according to the AOAC, 990.12 method. 3M Petri films (3M^{TM} 7100039374) were used for the analysis and the Petri films were incubated at 35±1 ºC for 48±3 hours under aerobic conditions. The total number of aerobic mesophilic bacteria was determined by counting petri films at the end of incubation (AOAC, 1995).

2.2.6.9. Total coliform bacteria count

Total coliform bacteria counts were made according to the AOAC, 991.14 methods. 3M Petri films (3M^{TM} 7100126846) were used for the analysis. The colonies were counted after incubating for 24±2 hours at 35±1 ºC for total coliform by seeding on petri films in appropriate amounts from the prepared dilutions (AOAC, 1995).

2.2.6.10. Yeast-Mold count

Yeast-mold counting was done according to the method of AOAC, 997.02. 3M Petri films (3M^{TM} 7100039379) were used for the analysis. The colonies were counted after incubating at 20-25 ºC for 3-5 days by planting the prepared dilutions on petri films used in appropriate amounts (AOAC, 1995).

All experiments were performed in triplicate. All reagents used were of analytical grade.

2.2.6.11. Statistical analysis

In the comparison of days and groups, repeated measures of variance analysis were applied. The difference between measurement times for each group and the difference between groups at each measurement time was evaluated with a one-way ANOVA. Duncan's multiple comparison test was used to reveal the difference between the means. The results were considered significant at the $p<0.05$ significance level. MedCalc 17.9 package program was used for statistical analysis.

3. RESULTS AND DISCUSSION

3.1. Fish Protein Hydrolysates

The relationship between the degree of hydrolysis of TH and UH obtained from sprat is presented in Figure 4. Accordingly, the maximum hydrolysis degree was determined as 22.93 ±0.08% and 25.45±0.01% for TH and UH, respectively. A rapid increase in the hydrolysis degrees of both groups was observed from the beginning of the process until approximately 20 min. Afterward, a decrease in the reaction rate was observed with a certain decrease in the degree of hydrolysis of TH. The increase in UH continued until 35th min. Then, the reaction continued at a constant rate. While the difference between the hydrolysis degrees of the groups was insignificant between the 10th and 30th min. ($p>0.05$), it was significant in the other stages of hydrolysis ($p<0.05$).

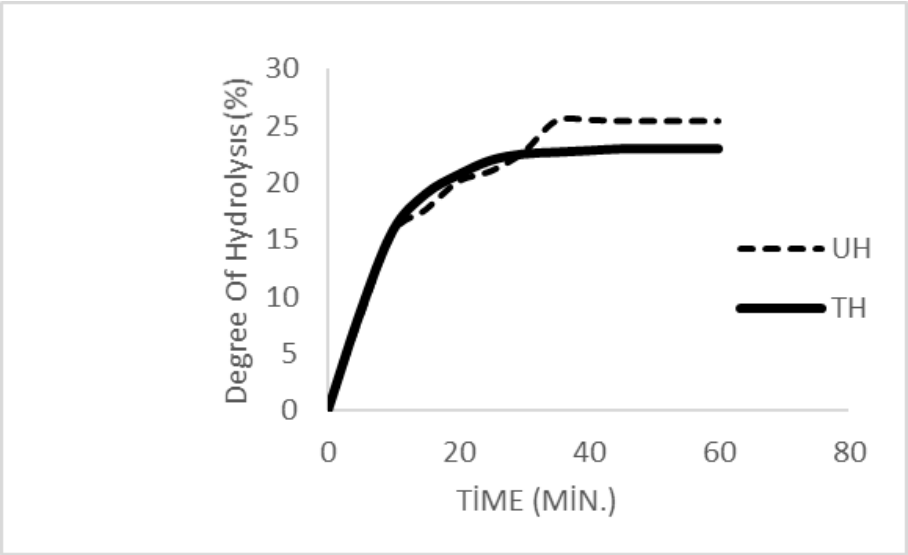

Figure 4. Degree of hydrolysis of TH and UH.

Koç (2016) calculated the hydrolysis degrees of protein hydrolysates obtained from anchovy meat and waste after 1 hour of hydrolysis; as 56.7% and 73.5%, respectively.

Huang et al. (2015) compared three different ultrasonic methods based on the production of antioxidative peptides from corn protein. The corn protein was sonicated with probe ultrasound (PU), flat plate ultrasound with sweeping frequency (FPUSF), and flat plate ultrasound with fixed frequency (FPUFF) before hydrolysis by acalase. As a result of the study, it was revealed that the hydrolysis degrees of all three ultrasound pre-applicated groups were higher than the control group.

The yield, pH, and color analysis results of TH and UH are presented in Table 1. The yields of TH and UH were 8.66±0.01% and 12.63±0.06%, respectively. The difference between these values was found to be statistically significant ($p<0.05$).

Arslan (2016) determined the yield of protein hydrolysate produced by using alcalase from trout waste as 9.05%. Hau et al. (2018) calculated the yield of yellow-striped horse mackerel (*Selaroides leptolepis*) protein hydrolysate produced by using alcalase after freeze and spray drying as 0.6%-1.6% and 12-16% for spray drying and freeze drying, respectively. Sinthusamran et al. (2020) determined the yield of protein hydrolysates obtained from salmon fillet using 3% alcalase and flavourzyme for 180 minutes, as 28.5% and 32.3% g/100g for alcalase and flavourzyme, respectively.

It can be thought that ultrasound application together with the alcalase used in the current study positively affected the activity of alcalase and increased the hydrolysate yield.

The pH values for TH and UH were measured as 8.82±0.01 and 8.66±0.01, respectively. The difference between these values is statistically significant ($p<0.05$).

Similar results were found in the study conducted by Hu et al. (2019). The researchers reported that the ultrasound application on cuttlefish mantle did not cause any difference in the pH values of the product. Arredondo-Parada et al. (2020) investigated the physicochemical and functional properties of ultrasound-treated *Dosidicus gigas* mantle hydrolysate. They explained that the application of ultrasound at various times is affected on pH value but the difference was reported as not statistically significant. Jambrak et al. (2009) determined that 20 kHz probes and 40 and 500 kHz ultrasound applications changed the pH values of soy proteins.

Compared to the literature, it is seen that ultrasound application has different effects on meats and hydrolysates produced from different raw materials. pH changes are dependent on applied frequency, time, and various factors such as sample conditions, protein concentration, sample geometry, or dimensions. It has been shown that when used in large samples such as tissue and muscle, the application can affect the cell membrane with a more aggressive effect and cause ionic imbalance, while it does not have such an aggressive effect when used in protein solutions.

L*, a*, and b* values of C, THC, and UHC are presented in Table 1. In the study, L* and b* values were lower, a* value was higher in TH than UH. The differences between the L*, a*, b* values of both groups were found to be statistically significant ($p<0.05$).

Mintah et al. (2020) investigated the effects of different ultrasound applications (constant and sweeping frequency ultrasound) on the structural, physical, and functional properties of protein isolates and hydrolysates obtained from the soldier fly (*Hermetia illucens*) by enzymatic method. The researchers found the L* values of the hydrolysates to be 74.71, 74.41and 73.52 for conventional enzymatic hydrolysate, fixed frequency pre-applied hydrolysate, and scavenging frequency ultrasound pre-applied hydrolysate, respectively. They revealed that ultrasound applications did not affect the L* values of hydrolysates significantly. The a* and b * values of the same samples differed in all 3 groups ($p<0.05$). Among these values, b* values of fixed and sweeping frequency ultrasound groups were statistically similar, while the other values differed.

Table 1. The yield, pH and color values of TH and UH.

	Yield	pH	Color		
			L*	a*	b*
TH	8.66±0.01[a]	8.82±0.01[a]	75.23 ±0.18[a]	2.66± 0.01 [a]	16.49±0.18 [a]
UH	12.63±0.06[b]	8.66±0.01[b]	79.08 ±0.04[b]	1.43±0.04 [b]	18.34 ±0.05 [b]

± standard error (n=3). Different letters (a, b) on the numbers in the same column indicate differences between the groups ($p<0.05$). TH: traditional hydrolysate; UH: ultrasound-assisted hydrolysate

The biochemical composition findings of the hydrolysates (TH and UH) produced from sprat are presented in Table 2.

Table 2. Biochemical composition findings of TH and UH

Hydrolysate groups	Protein	Lipid	Ash	Dry matter
TH	75.88±0.47[a]	2.05±0.07[a]	12.88±0.23[a]	96.51±0.03[a]
UH	74.45±0.28[a]	1.51±0.03[b]	14.97±0.08[b]	96.55±0.03[a]

± standard error (n=3). Different letters (a, b) on the numbers in the same column indicate differences between the groups ($p<0.05$). TH: traditional hydrolysate; UH: ultrasound-assisted hydrolysate

In the study, protein values of TH and UH were found to be around 75%. When compared with sprat used as raw material (the protein value of sprat is 14.43±0.03%, Dağtekin et al., 2021), it can be said that the protein value is due to the general characteristics of hydrolysates. It has been reported that during the hydrolysis process, its proteins become largely soluble (Chalamaiah et al., 2010). The lipid values of TH and UH lipid values were about 1.5 to 2%. This can be explained by separating the oil layer and removing it from the medium during the centrifugation and other separation processes performed after the hydrolysis process. Low lipid values are desirable in hydrolysates. Similarly, several researchers have reported the lipid content of different fish protein hydrolysates to be <5% (Benjakul and Morrissey, 1997; Kristinsson and Rasco, 2000; Wasswa et al., 2007).

It has been reported that low lipid value can prolong the shelf life of the product by delaying the deterioration due to lipid oxidation in fish protein hydrolysates (Kristinsson and Ingadottir, 2005). The ash value of UH was found to be higher than that of TH. It is thought that the high ash value may be caused by the salts coming from the alkali added to keep the pH constant during the hydrolysis process or the breakdown of raw materials such as bone and skin (Choi et al., 2009; Batista et al., 2010). Dry matter values of TH and UH were found to be very close to each other and very high since the product was dried after hydrolysis. According to these results, it was determined that ultrasound did not have a significant effect on the protein and dry matter values of hydrolysates. Similarly, Zou et al. (2016) reported that the protein values of ultrasound-treated porcine cerebral hydrolysate were higher than normal hydrolysate, but this value was not statistically significant, whereas the ash values increased statistically significantly. In the study of Koç (2016), protein, oil and ash and dry matter values based on dry matter in hydrolysates obtained from anchovy meat and waste are respectively; 83.36%, 15.38%, 10.3%, 97.98%, and 76.55%, 14.13%, 0.93% and 94.60%. Arslan (2016) determined the biochemical content of powdered protein hydrolysate made from trout wastes as 94.75±0.68% dry matter, 86.40±0.34% crude protein, 0.37±0.01% crude oil, and 6.34±0.01% raw ash.

According to the literature above, it has been reported that the protein content of protein hydrolysates obtained from various aquatic products and by-products varies between 70.0% and

90.0% on average. When evaluated in general, it is concluded that different process parameters such as raw material, enzyme type and rate, hydrolysis time, and temperature used during the production of hydrolysate can greatly affect the biochemical composition of the obtained hydrolysates. However, in the current study, similar to the literature, it was revealed that ultrasound application did not perform a statistically significant change on the chemical composition of protein hydrolysates.

Three different concentrations of hydrolysates were used in the analyzes made with the DPPH method. According to this, the DPPH radical scavenging activity levels of TH are as % inhibition for three different concentrations of 10%, 15%, and 20%, respectively; 11.83; 21.52 and 31.54; 23.96 for UH, respectively; It was determined as 27.90 and 30.96. It was observed that the antioxidant activity value increased as the hydrolysate concentration increased. It was determined that antioxidant levels of TH and UH showed a significant difference in all three concentrations ($p<0.05$). When the two hydrolysate groups were compared, it was determined that only 20% of concentration values showed similarity ($p>0.05$) (Figure 5).

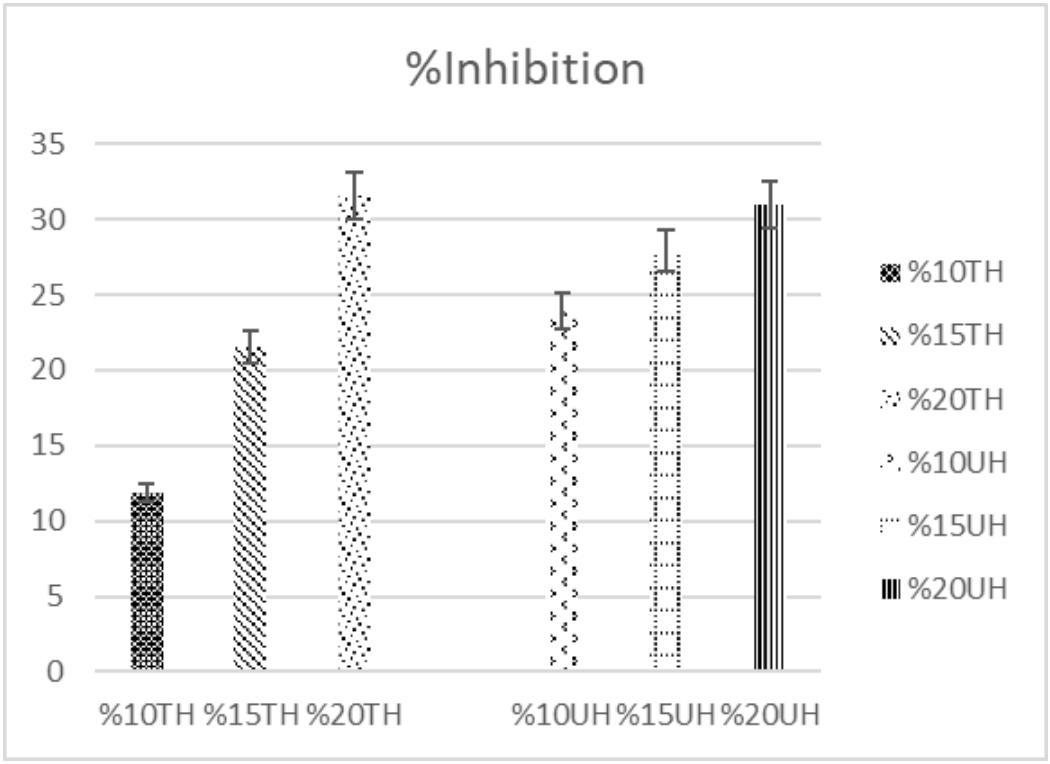

Figure 5. Antioxidant activities of TH and UH.

3.2. Coated Fillets

a_w and pH values of C, THC, and UHC are presented in Figure 6. According to the results, a_w value of fresh trout, which was 0.9981, decreased with similar values in all groups during storage. The lowest a_w value was recorded as 0.9680 on the 12th day in the THC. When a_w values of the groups were evaluated statistically, it was determined that the differences between the groups were significant ($p<0.05$). The differences between a_w values of C and UHC on the 12th day were insignificant ($p>0.05$), and significant on the other days ($p<0.05$).

Although these conditions allow many microorganisms to grow in the medium for all groups, the fact that the decreases are not significant also shows that no significant changes have occurred in the fillets' tissues. It has been reported that a_w value alone is not sufficient to reveal the quality status of the product (Karaçalı, 2002).

Linn et al. (2021) treated the catfish fillets with different coatings (distilled water, chitosan, alginate, and a combination of these materials). The water activity of fillets was increased gradually from 0.9916 to the maximum value of 0.9941 for the control sample. The researchers attributed the higher water activity of the control samples, compared to the coated groups, to moisture migration by edible coatings on the fish surface. When compared with the literature, it is seen that there are

similarities between the studies using FPH, and differences when polysaccharide-based products are used.

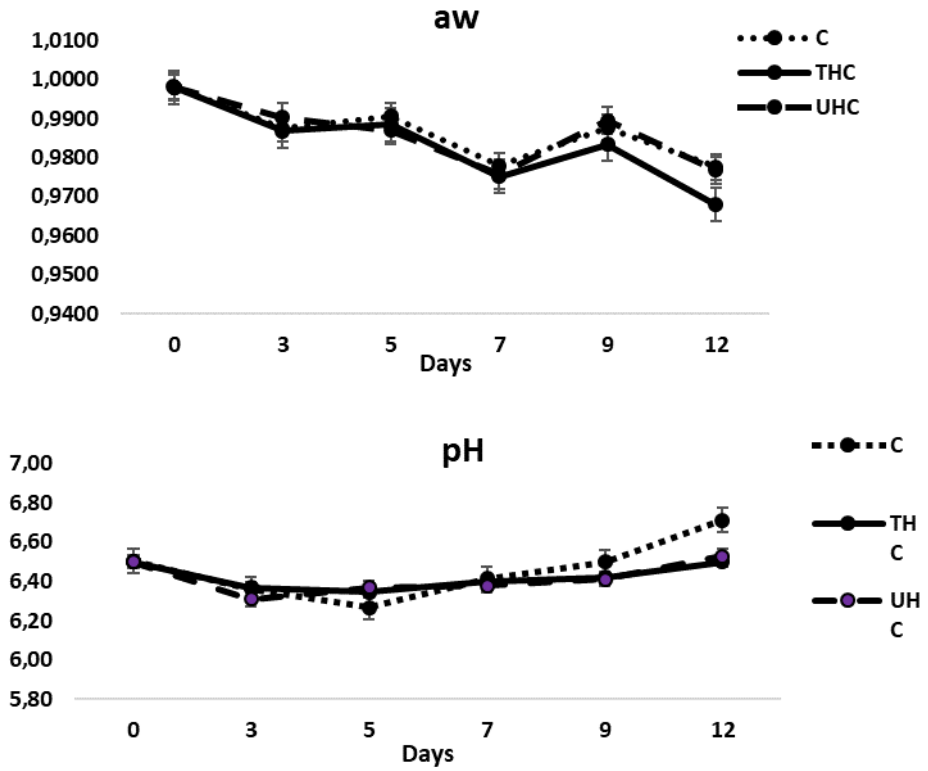

Figure 6. a_w and pH values of C, THC, and UHC

The pH value, which was 6.5 at the beginning, decreased until the 5th day and increased from this day to the 12th day. The minimum and the maximum pH value was determined at C as 6.26 and 6.71 on the 5th and 12th day, respectively. The differences between the pH values of C, THC, and UHC were insignificant on days 0, 3, and 7 ($p>0.05$). On the fifth, 9th, and 12th days, THC and UHC were insignificant ($p>0.05$), while C was significant ($p<0.05$).

The increase in pH values was higher in C, lower and close to each other in THC and UHC. However, this increase in pH remained within consumable limits throughout storage, including the last day. It is thought that the conversion of glycogen to lactic acid during storage may have caused the pH value to decrease. The formation of nitrogenous compounds such as ammonia, trimethylamine, and histamine, which are derived from both autolysis by endogenous enzymes and microbial enzymatic activities, may have caused the pH value to increase.

A similar trend was found by Sun et al. (2019). They investigated the effects of fish gelatin coating, enriched with curcumin/β-cyclodextrin (CUR/βCD), on grass carp fillets during storage at +4 °C. The authors expressed that the pH values of the fillets increased during the first 3 days and decreased rest of storage. However, while the decrease in the values of the control group was less, the increase was higher than the other groups. Linn et al. (2021) found that the pH value increased continuously during 21 days of storage in the control and all coated groups.

The L*, a*, and b* values for C, THC, and UHC are given in Figure 7. Accordingly, L*, a*, b* values of fresh trout were determined as 41.92, 10.15, and 20.25, respectively on day 0. The maximum L* value was 47.94, in C on the 12th day of storage; the minimum L* value was 39.33, recorded in UHC on the 7th day of storage.

The differences; in L* values were not significant in all groups on the 0th, 9th, and 12th days of storage ($p>0.05$); a* values were also found to be insignificant in all groups at 0, 5, 9, and 12 days of storage ($p<0.05$); and b* values were found to be insignificant in all groups on the 7th day of storage ($p>0.05$).

The L* value, which remained similar for the first 9 days in C, reached the highest value on the 12th day of storage and the fillets took on a darker color. In the case of THC and UHC L* values showed more fluctuations, although they did not change much. In general, L* values of all groups were close to each other during storage. The a* and b* values of the fillets followed a fluctuating trend with a similar value throughout storage. Increases in a* values indicate an increase in the redness of the fillets, while increases in b* values represent yellowing. It can be said that the increase in b* values may have occurred due to the lipid and protein oxidation of the fillets.

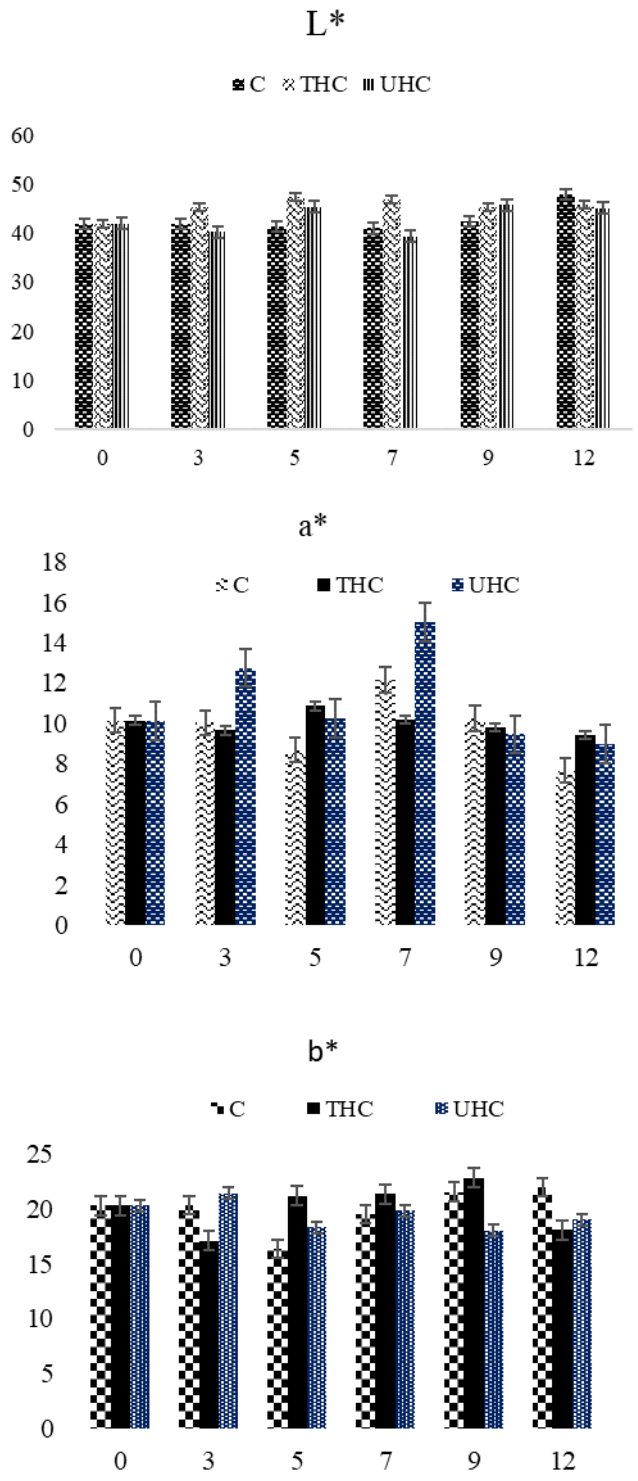

Figure 7. Color parameters (L*, a* and b*) of C, THC and UHC

The changes in TVB-N and TBA values for C, THC, and UHC, are given in Table 3 and Table 4, respectively. While the TVB-N value of fresh trout was 12.96 mg/100g, it increased over time in all three groups. In the C, it exceeded the consumable limit with 38.52 mg/100g on the 9th day of storage. It was determined that the differences between all days of TVB-N values of C during storage were significant ($p<0.05$).

TVB-N values of THC and UHC were statistically similar at 0, 3, 9, and 12 days of storage ($p>0.05$) but were significantly lower than C ($p<0.05$). It differed significantly in all groups on the 5th and 7th days ($p<0.05$), and the highest values on these days belonged to C.

In the current study, TVB-N values increased in all groups due to bacterial and enzymatic deterioration during storage, but this increase was lower in THC and UHC than in C. It exceeded the consumable limit in C on the 9th day of storage. While it did not reach the limit value in THC and UHC on the 12th day of storage. This can be explained by the fact that TFPH and UFPH may have effectively delayed the microbial degradation of protein and nitrogen-containing components. However, the absence of a significant difference between THC and UHC can be considered a sign that ultrasound application does not show a significant superiority over THC.

Table 3. TVB-N values of C, THC, and UHC during the storage at +4 °C

Days	TVB-N		
	C	THC	UHC
0	12.96 ± 0.23^{aA}	12.96 ± 0.23^{aA}	12.96 ± 0.23^{aA}
3	16.11 ± 0.43^{bB}	13.66 ± 0.18^{aA}	14.01 ± 0.05^{aA}
5	24.51 ± 0.37^{cC}	21.71 ± 0.43^{bB}	18.21 ± 0.72^{bA}
7	31.52 ± 0.37^{dC}	23.11 ± 0.37^{cA}	24.51 ± 0.37^{cB}
9	38.52 ± 0.37^{eB}	30.12 ± 0.37^{dA}	31.52 ± 1.16^{dA}
12	41.32 ± 1.18^{fB}	32.92 ± 0.37^{eA}	33.62 ± 0.20^{eA}

± Indicates the standard error (n=3). Different lowercase letters (a, b, c..) in the same column show the differences between different days of the same group ($p<0.05$), and different uppercase letters (A,B,C…) in the same row show the differences of different groups on the same days. C: uncoated control group, THC: traditional enzymatic protein hydrolysate coated group, UHC: ultrasound-assisted enzymatic protein hydrolysate coated group

Yu et al. (2021) investigated the effects of ultrasound-assisted chito-oligosaccharide coating (COS-UA) on the quality of chilled grass carp fillets. It was reported that the TVB-N value increased slowly in all groups until the 3rd day of the study and this increase was statistically insignificant, and the TVB-N value of the control group increased sharply in the following days. Barcellos et al. (2020) investigated the effect of protein hydrolysate obtained from commercial fish gelatin on rainbow trout fillets that were vacuum-packed and stored in refrigerator conditions. It was reported that although there was a difference in TVB-N values and bacterial deterioration between the control and hydrolysate groups, these differences were statistically insignificant, and optimization of the procedures was needed. In the study, the hydrolysate was applied by injecting it into the vacuum package. The application difference because the fish were not directly exposed to the hydrolysate solution suggests that the results may have been obtained in different directions.

While TBA values were calculated as 0.21 mg MA/kg at the beginning of storage, it reached the consumable limit with 7.72 mg MA/kg in C on the 9th day. On the same day, this value remained within the consumable limit with 6.67 mg MA/kg and 6.79 mg MA/kg for THC and UHC, respectively.

According to the statistical analysis between the groups, the TBA values of THV and UHC showed significant differences on the 3rd, 5th, and 7th days of storage ($p>0.05$), and insignificant on the 9th and 12th days ($p>0.05$). TBA values of C were significantly higher than THC and UHC during the whole storage ($p<0.05$).

Table 4. TBA values of C, THC and UHC during the storage at +4 °C

Days	TBA		
	C	THC	UHC
0	0.21 ± 0.00^{aA}	0.21 ± 0.01^{aA}	0.21 ± 0.00^{aA}
3	1.36 ± 0.50^{bC}	0.96 ± 0.02^{bB}	0.84 ± 0.02^{bA}
5	3.46 ± 0.50^{cC}	2.93 ± 0.01^{cA}	3.18 ± 0.01^{cB}
7	5.02 ± 0.53^{dC}	3.34 ± 0.02^{dA}	4.68 ± 0.05^{dB}
9	7.72 ± 0.57^{eB}	6.67 ± 0.02^{eA}	6.79 ± 0.05^{eA}
12	9.13 ± 0.165^{fB}	8.19 ± 0.06^{fA}	7.96 ± 0.01^{fA}

± Indicates the standard error (n=3). Lowercase letters (a, b, c) in the same column show the differences between different days of the same group ($p<0.05$), and uppercase letters (A,B,C…) in the same row show the differences of different groups on the same days. C: uncoated control group, THC: traditional enzymatic protein hydrolysate coated group, UHC: ultrasound-assisted enzymatic protein hydrolysate coated group

It has been reported that the increase in TBA value is associated with the presence of oxygen (Cai et al., 2014). It can be said that the coating layer formed on the fillet surface is effective in reducing the secondary products of lipid oxidation, so the increase in TBA values is lower than the C depending on the coating materials used. Although the antioxidant activity of UFPH was higher than GFPH in the antioxidant activity analyses performed on protein hydrolysates, it was observed that it did not provide significantly better protection than GFPH when applied to fillets.

Parvathy et al. (2018) reported that the TBA value of the control group reached 5.14 mg manolaldehyde/kg on the 5th day of storage. However, it was determined that the increase in TBA value in the coated groups was significantly less than in the control group and remained within the consumable limits until the 11th day.

It has been reported in the literature that coating and film materials, which can show higher potential in in-vitro studies, show lower activity when applied to foods. It has been stated that the reason for this is that a more complex matrix is formed when the coating material combines with food (Kulawik et al., 2019). This explains why newly developed coatings and films need to be tested on foods. de Abreu et al. (2011) also encountered similar results in their study.

In the study, TAMB, TAPB, coliform bacteria, and yeast mold (Figure 8) analyses were performed for C, THC, and UHC. While the number of TAMB was 2.13 log cfu/g on day 0, it increased during storage in all groups. The maximum TAMB value was calculated at 6.90 log cfu/g and C on the 12^{th} day, it was determined as 5.20 log cfu/g and 5.04 log cfu/g for THC and UHC on the same day, respectively.

It was determined that the differences between the TABM values of all three groups were significant on the same days during the entire storage period ($p>0.05$).

The number of TAPB, which was 1.66 log cfu/g at the beginning, increased during storage and reached a maximum of 6.95, 3.48, and 3.22 log cfu/g for C, THC, and UHC on the 12^{th} day, respectively.

The number of coliform bacteria, which was 1.66 log cfu/g on 0. day, increased in all groups during storage. This value reached 4.54 log cfu/g, 2.56 log cfu/g, and 2.86 log cfu/g, respectively, for C, THC, and UHC on the 12^{th} day. The differences between different days of each group were found to be significant ($p>0.05$).

According to the statistical analysis between the groups, it was determined that the differences between the Coliform bacteria values of all groups were significant on the same days during the entire storage period ($p>0.05$).

The yeast-mold count was 1.66 log cfu/g on 0. day, and reached its maximum values 4.60 log cfu/g, 2.74 log cfu/g, and 2.74 log cfu/g for C, THC, and UHC on the 12^{th} day of storage, respectively.

The differences between different days of each group were found to be significant ($p>0.05$).

When the statistical analysis between the groups was examined, it was determined that the differences between the yeast-mold values of all groups were significant on the same days during the entire storage period ($p>0.05$).

Throughout the study, increases were observed in the number of TAMBs of C, THC, and UHC. These increases were also in parallel with the TVB-N values and were higher in C than in THC and UHC (Table 3). As of the 12^{th} day of the study, the number of TAMBs determined for C reached the

consumable limit, whereas it remained around 5 log cfu/g for THC and UHC. However, the TAMB values of UHC were significantly lower than those of the THC.

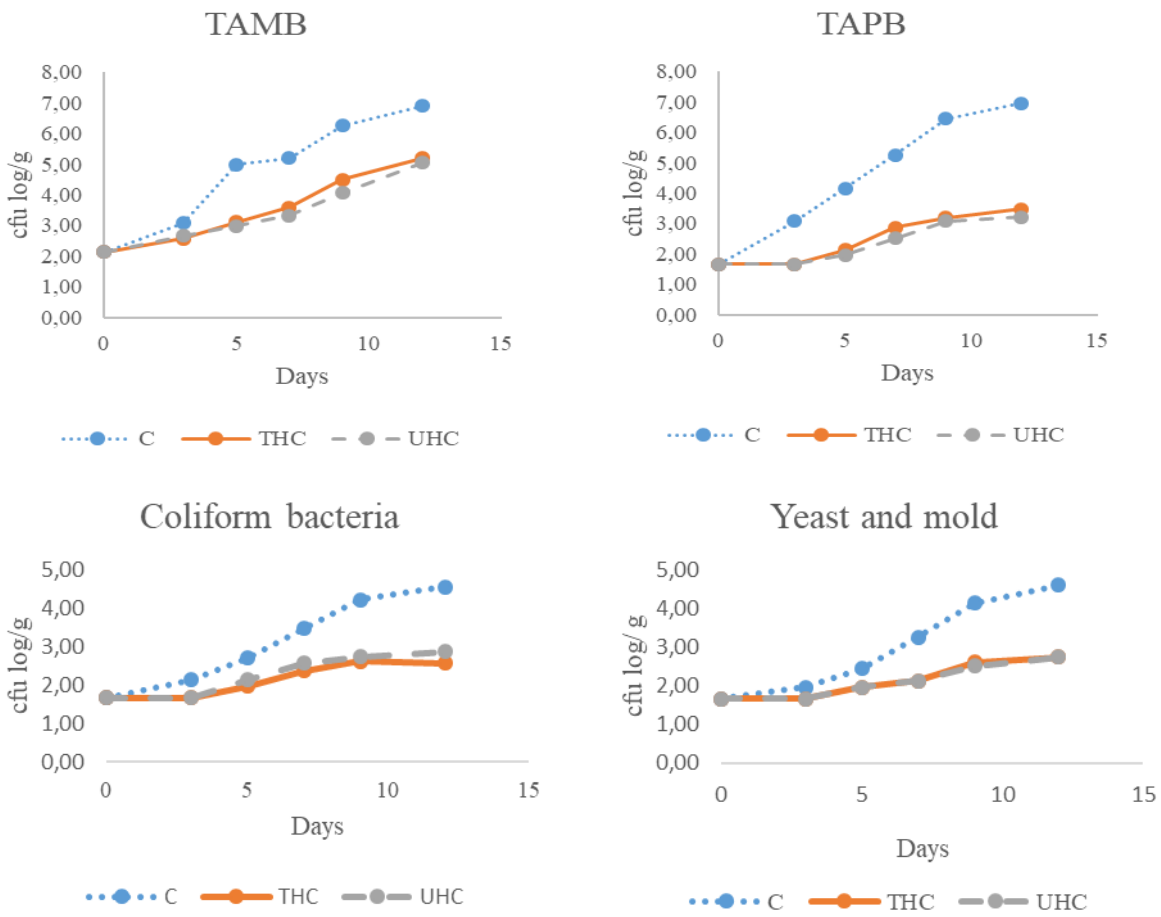

Figure 8. TAMB, TAPB, Coliform, Yeast, and Mold counts of C, THC, and UHC

A similar trend was obtained in TAPB numbers, reaching the consumable limit for C on the 12th day. On the same day, it remained within the acceptable limits in terms of consumables with 3.48 log cfu/g and 3.22 log cfu/g for THC and UHC, respectively. It can be said that the growth of microorganisms may have been prevented in this environment, where the coating materials cover the fish surface and create a barrier between the fillet and the external environment, preventing the passage of oxygen. A similar trend was also observed in coliform bacteria and yeast-mold numbers. However, the increases are significantly higher for C compared to other groups.

Gómez-Guillén et al. (2011) reported that fish gelatin hydrolysate showed bacteriostatic and bactericidal properties. However, it has also been reported that edible coatings can act as a rich nutrient medium for bacteria in some products and support microbial growth (Sardari and Nordberg Karlsson, (2018).

The low levels of coliform bacteria can be explained by the low storage temperatures during the fish slaughtering process and the time it takes to take their place on the shelves (Junior et al., 2014). On the other hand, high contamination with coliform bacteria is mostly associated with environmental factors related to fish farming, such as the degree of contamination of the water and the feeding of fish.

4. CONCLUSION

In the current study, it has been demonstrated that the protein hydrolysate produced from sprat can be used as a coating material in cold-stored fish fillets, which can extend the storage period of the fillets. Compared with the control group, it was revealed that the coating materials prepared with UH and TH can effectively protect the trout fillets microbiologically and chemically and prolong the

storage period, therefore the hydrolysates obtained from sprat can be used as a coating material for fillet fish. It is thought that different results can be obtained with studies that can be done by changing the ultrasound application parameters.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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AUTHOR CONTRIBUTIONS

GBM: Fiction, Literature, Performing the experiment, Manuscript writing, BBD: Performing the experiment, Data analysis, SK: Performing the experiment. All authors approved the final draft.

ETHICAL APPROVAL STATEMENTS

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of the present study are available from the corresponding author upon reasonable request.

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