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RESEARCH ARTICLE

Protective role of bio-based coating of ultrasound-improved trout (*Oncorhynchus mykiss* Walbaum, 1792) waste protein hydrolysate for bonito (*Sarda sarda* Bloch, 1793) fillets at storage at -18±1°C

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ABSTRACT

A novel and rich protein source was utilized in bonito fillets to prevent/ delay deterioration during frozen storage at -18±1°C. Accordingly, trout wastes protein hydrolysates (PH); produced enzymatically traditional protein hydrolysate (TPH) and ultrasound–treated protein hydrolysate (UPH) containing 86.40 g/100 g and 86.75 g/100 g protein respectively used as a coating. Hydrolysates were mixed with glycerol (2:1) to form coating materials. Three groups of fillets were prepared as control fillets (C) without coating, TPH coated fillets (TPHCF), and UPH coated fillets (UPHCF). pH, color, TVB-N, TBA, and TMA, and sensory analyzes were performed in all groups. The L* value of all groups reached a maximum the highest value at 6 months and was 54.56±0.27, 53.74±0.23, and 54.83±1.26 for C, TPHCF, and UPHCF, respectively. TVB-N was 18.08±0.10, 17.71±0.09, and 17.36±0.12, for C, TPHCF, and UPHCF, respectively, in the first month of storage. The values reached 32.18±0.29, 26.61±0.12, and 25.72±0.08 at 7th month for C, TPHCF, and UPHCF, respectively. TBA value of the C group samples of the frozen bonito fillets reached 7.53 in the 7th month, it remained within the consumable limits, and it remained within the consumable limits in the coated groups. Significant increases occurred in TMA values of all groups between months during the seven-month storage period. Accordingly, the TMA values for C, TPHCF, and UPHCF were 2.56±0.04 mg/100 g, 2.12±0.04mg/100 g and 2.16±0.06mg/100 g, respectively, at the 7th month. The mean values of sensory parameters were 9.15±0.08; 9.51±0.12 and 9.46±0.13 for C, TPHCF, and UPHCF, respectively, at the 1st month of storage. While they were 5.29±0.09, 6.23±0.06 and 6.24±0.09, in THE same order, respectively. Results showed that TPHCF and UPHCF have a potential as a coating for bonito fillets at frozen conditions, prolonging the shelf life.

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Introduction

Freezing is extensively and effectively applied for the conservation of fishery products. It prevents or minimizes undesirable chemical changes in fresh fish to preserve seafood and its properties.

Although microbial spoilage can be prevented to a large extent by freezing, due to temperature fluctuations caused by physical and biochemical changes and repetitive freeze-thaw processes during frozen storage, the distribution of water in muscle tissue and water holding capacity were found to be affected. The changes occur as color changes and tissue destruction by protein denaturation and lipid oxidation (Sriket & Laongnual, 2018).

In frozen fish, edible coatings can reduce quality losses with their features such as control of moisture, prevention of oxygen permeability, prevention on flavor, and can extend shelf life (Dehghani et al., 2018).

In recent years, as the people's awareness increased about the utilization of synthetic additives has potential risks both for health and environmental issues, the food sector is trying to use science and technology to add value to animal by-products to produce natural agents for prevention purposes on food (Aliotta et al., 2019).

As the range of processed products in seafood has increased, there have been significant increases in by-products and wastes, which are often become trash or utilized for animals such as feed, fertilizer and etc. However, having a significant nutritional value. Using innovative technologies, the evaluation of waste as functional components is an increasing focus of attention. (Al-Khawli et al., 2019).

Fish protein hydrolysates (FPH) are produced by breaking down proteins into small chain peptides and amino acids by chemical or biological means. Chemical hydrolysis is generally applied in industrial applications. On the other hand, biological hydrolysis used preferred by using enzymes for it results in products with high functionality and nutritional value (Krisstinson & Rasco, 2000). Essential amino acids are occurred with functional properties that can be evaluated in the fields of food and pharmacy. Due to their high content of essential amino acids, these products can be used as protein enhancers, as a substitute for milk powder, as a fat binder in meat products, as an emulsifier, and as an emulsifier with a high water binding capacity in foods (Nikoo et al., 2014; Karnjanapratum & Benjakul, 2015; Shen et al., 2022).

Some studies were conducted on FPH and peptides used as a coating in frozen foods. They have been applied as antioxidant and antimicrobial agents in the prevention of quality losses during storage (Gokoglu, 2019). FPH forms a physical barrier, that prevents the product from physical, chemical, and microbiological effects (Loi et al., 2019).

The aim of present research to evaluate the potential of ultrasound-improved trout wastes protein hydrolysates as coating for frozen trout fillets stored at -18±1 ±1°C for 7 months.

Material and Methods

Materials

TPH and UPH were produced from *Oncorhynchus mykiss* wastes enzymatically and characterized previously. The biochemical composition and antioxidant activity of PHs were determined in Balcik Misir & Koral (2019a). Provided from a local fishery market in Trabzon, Türkiye, a total of 30 bonito (totally 25 kg, with average weight and length 832.21±42.17 g and 38.94±1.08 cm, respectively) were prepared. Food-grade glycerol (99.96% purity W252506, Sigma-Aldrich, Steinheim, Germany) and analytically pure chemicals and reagents were used in the research.

Methods

Bio-based coatings were prepared from TPH and UPH. The preparation of the coating solution was given in Figure 1.

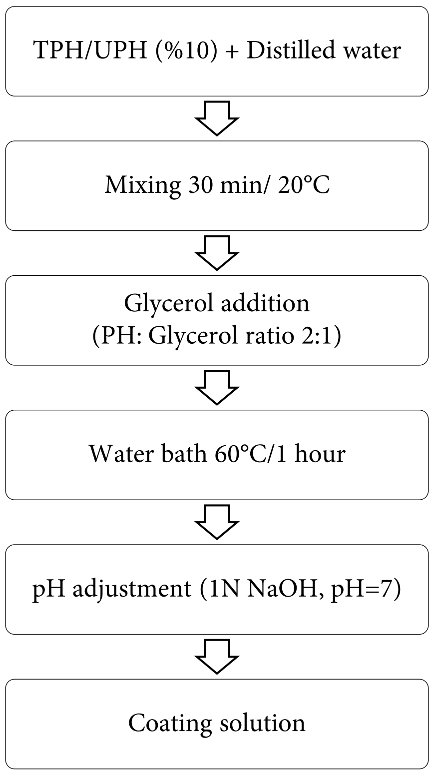

Figure 1. Preparation of coating material from protein hydrolysate (PH)

Bonito fillets were immersed in coating solution under aseptic conditions. After draining on the grill fillets were separated into three: 1) (C) without coating, 2) CH (10% w/v) coated fillet (TPHCF), and 3) UH (10% w/v) coated fillet (UPHCF). The coating process is given at Figure 2.

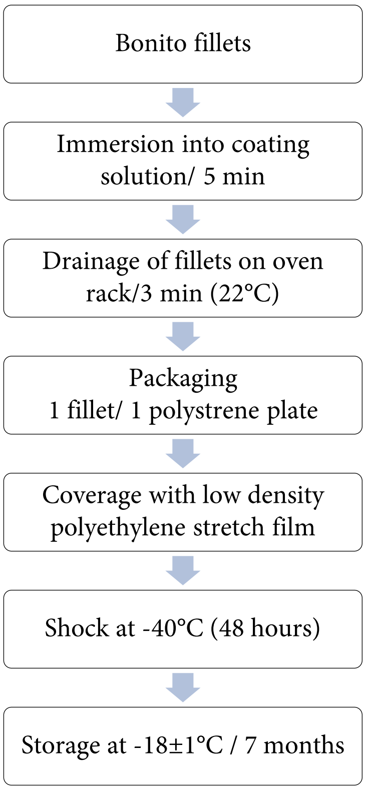

Figure 2. Coating of bonito fillets

Physical Analysis

pH measurement was made by immersing the probe of device (Mettler-Toledo AG, Seven Compact pH meter, 8603 N, Switzerland) into the homogenate (Koral., 2012).

The water activity was determined with the Aqualab 3TE (0.100- 1.000 ± 0.003, Aqualab, USA) working at 25°C (±0.2°C). The amount of water activity in the samples placed in the measuring cups of the device was automatically read and recorded.

Before measuring the L*, a* and b* of the fillets, the device (Konica Minolta, CR 10, Tokyo, Japan) was standardized using a white plate and values recorded from 3 points.

Chemical Analysis

TVB-N analysis was done according to the Lücke & Geidel (1935) method modified by Antonacopoulus and reported by Inal (1992). TVB-N results were given as mg/100g.

TBA values, were estimated using the method described by Tarladgis et al. (1960), results were expressed as mg malonaldehyde/kg.

TMA-N values were analyzed according to Boland & Paige (1971), results were given as mg/100g.

Chemical analyzes were applied as described in detail in Balcik Misir & Koral (2019b). Analyzes were done in triplicate. All reagents were analytical grade.

Sensory Analysis

Six trained panelists performed the sensory changes during the storage of bonito fillets. Panelists gave scores out of 10 based on odor, texture and appearance and average for raw fillets. 10-9 excellent, 8-7 good, 6-5 moderate, 4 acceptable limits, 3.9-1 unacceptable. Sensory parameters were evaluated according to Fisheries Regulation of Türkiye (URL-1, 2011), and Varlık et al. (1993).

Statistical Analysis

One-way ANOVA was utilized for all data. Tukey and Mann Whitney U test was applied if significant differences are found, (data not provided in the normality of assumptions) under the program called JMP5.0.1 (SAS Institute Inc., Cary, NC, USA) and SPSS 18.0 (SPSS Inc., Chicago, IL, USA) (Sokal & Rohlf, 1987). A significance level of 95% ($P < 0.05$) was used for all the analyses.

Results and Discussion

pH value of fresh bonito fillets was measured as 6.16. This value followed a fluctuating course during frozen storage conditions as seen in Figure 3. C showed a significant decrease in the 2nd month this decrease can be attributed to the release of inorganic phosphate, a product of lactic acid formation and ATP breakdown during anaerobic glycolysis. After that with a continuous increase it reached 6.58±0.04 in the 7th month. Similar values were obtained for the TPHCF and UPHCF at the 2nd, 3rd and 4th months. The increase can be dependent on the increase of some elements such as ammonia and trimethylamine due to autolytic and microbial activities. Considering the pH 7 is taken as the limit for consuming, all groups were lower than this limit during the storage.

Kaba et al. (2013) investigated the shelf life of fish meat ball produced with smoked bonito was during refrigeration conditions (4°C). They stated that the pH value of fresh bonito meat was 6.09 at the beginning of the study. Morachis-Valdez et al. (2017) coated carp fillets with chitosan and stored at -18±1°C and monitored the changes in their biochemical, physicochemical, textural, microbiological and nutritional characteristics. The researchers worked with an uncoated control group and a chitosan-coated group. In the study in which five months of storage was carried out, significant decreases were detected in the pH of the control, coated group up to the 2nd month. Karsli et al. (2021) found that the pH fluctuated barely for all groups during the 180-day study, and the pH of the control group were above the coated groups. They attributed this situation to acetic acid and aspartic acid, which they used together with chitosan in the coating material.

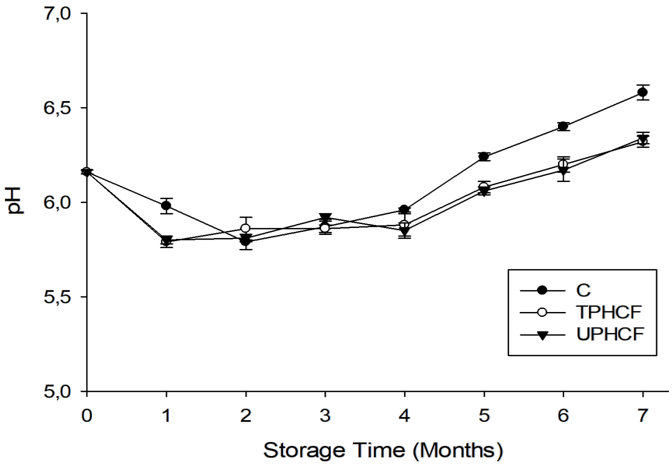

Figure 3. pH values of C: Control; TPHCF: Traditional Protein Hydrolysate Coated Fillet; UPHCF: Ultrasound-treated Protein Hydrolysate Coated Fillet during the storage of bonito fillets stored at -18±1°C

Color index (L*, a*, b*) of bonito fillets during storage at -18±1°C shown in Figure 4. At 0 day the L* value was 46.48±1.13; it was 51.42±0.24, 51.90±0.47 and 52.13±0.13 for C, TPHCF and UPHCF, respectively. There is no statistical difference between the values on the 1st month of storage ($p<0.05$). While statistically similar values were detected in the 2nd, 3rd, 4th and 7th months in the C group, a significantly lower value was found in the 5th month and a significant increase in the 6th month ($p<0.05$). The L* of all groups reached the highest value at 6th and it was 54.56±0.27, 53.74±0.23 and 54.83±1.26 for C, TPHCF and UPHCF, respectively.

While a* value of fresh bonito fillets was 7.80±0.88, it decreased to 4.94±0.05 4.85±0.06 and 4.75±0.17 for the 1st month C, TPHCF and UPHCF, respectively. The a* value of the UPHCF showed a similar increase and decrease trend with the TPHCF. C, TPHCF and UPHCF reached the highest a* values as of 6.86±0.42, 6.20±0.19 and 6.34±0.13, respectively, in the 7th month.

The b* of fresh bonito was measured as 11.06±0.63. This value increased in all groups at 1 month and was 12.00±0.08 for the groups covered with C, TPHCF and UPHCF, respectively; It was measured as 12.90±0.08 and 12.78±0.13. The highest values for all groups were obtained in the 7th month and were 17.86±0.07, 17.46±0.06 and 17.50±0.08 for C, TPHCF and UPHCF, respectively.

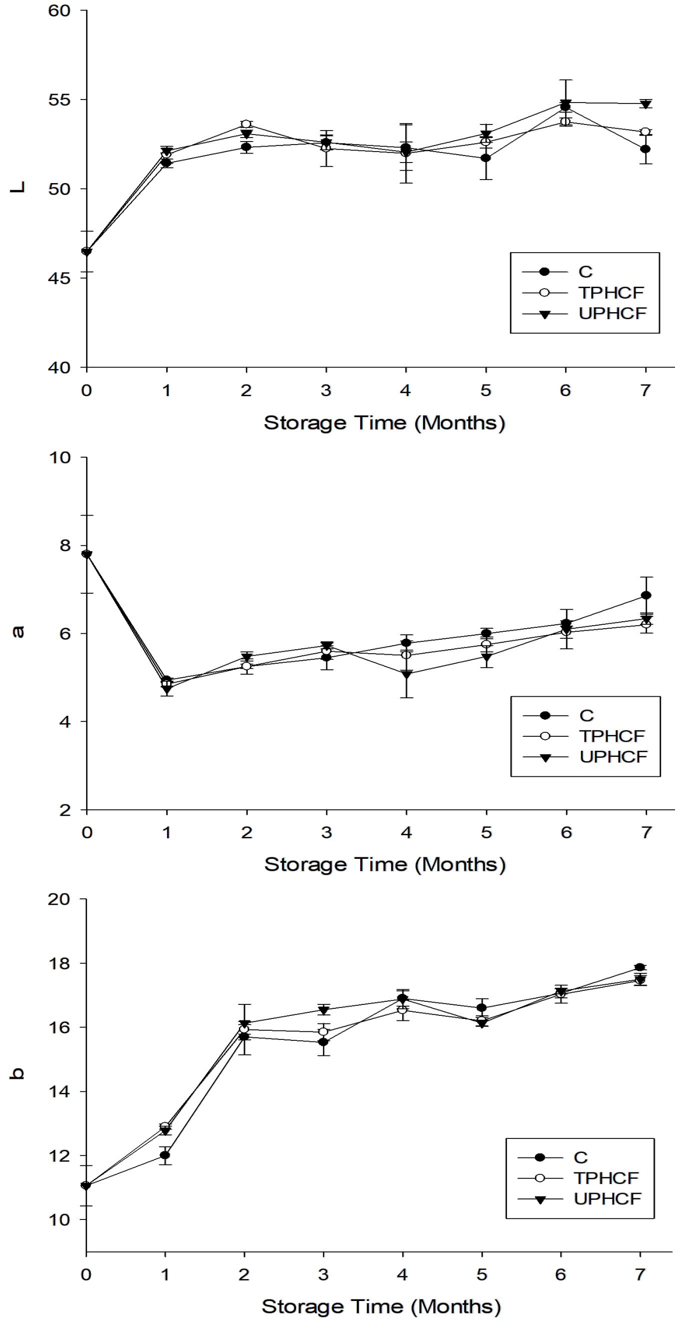

Figure 4. Color index (L*, a* and b*) of C: Control; TPHCF: Traditional Protein Hydrolysate Coated Fillet; UPHCF: Ultrasound-treated Protein Hydrolysate Coated Fillet during the storage of bonito fillets stored at -18±1°C

Karsli et al. (2021) examine the effects of chitosan on catfish fillets stored at -20°C, and they prepared a control group and 3 different chitosan coating groups. They reported insignificant change in L* values during storage, small fluctuations were determined in the study. They obtained similar results in a* values ($p>0.05$). Regarding the b* value they reported that the b* value was higher after 6 months of frozen storage compared to day 0 in all fillets except the control group.

Rodriguez-Turienzo et al. (2012) searched the impact of ultrasound-treated whey protein coatings on frozen Atlantic salmon and determined the L* value as 45.37 from the color index. They measured the a* value of the fillets as 19.49 and the b* value as 18.10.

Wang et al. (2022) compared the effect of sodium alginate coating or chitosan on the large yellow croaker after a 3-day frozen storage. The coatings showed better muscle color acceptability.

Figure 5 illustrated the TVB-N values of C, TPHCF and UPHCF during the storage of bonito fillets stored at -18±1°C.

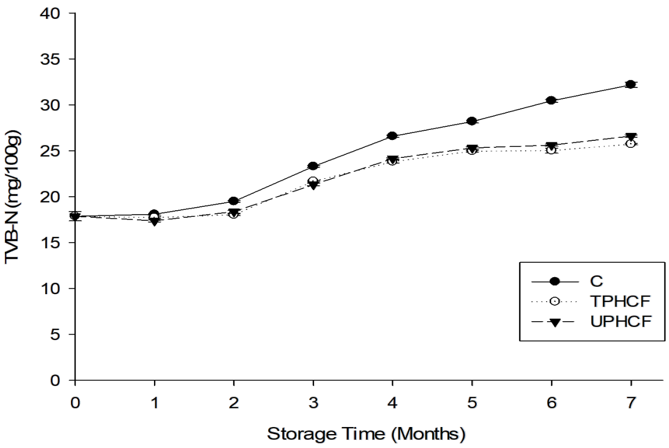

Figure 5. TVB-N values of C: Control; TPHCF: Traditional Protein Hydrolysate Coated Fillet; UPHCF: Ultrasound-treated Protein Hydrolysate Coated Fillet during the storage of bonito fillets stored at -18±1°C

Bonito fillets had 17.86 mg TVB-N/100 g at 0 day. TVB-N value of fresh bonito was calculated as 15.33±1.12 mg/100 g in research (Gargacı Kınay & Duyar, 2021). In the present study TVB-N values represented a statistically significant increase during the whole time of the storage for all three groups ($p<0.05$). They were 18.08±0.10, 17.71±0.09 and 17.36±0.12, for C, TPHCF and UPHCF, respectively at the first month of storage. The values reached to 32.18±0.29, 26.61±0.12 and 25.72±0.08 at 7th months of storage for C, TPHCF and UPHCF, respectively. TVB-N values around 25 mg/100 g in coated groups indicate that the products are in consumable condition. The results were in accordance with the results of pH. Although the more increasing values was found for both pH and TVB-N values of C than TPHCF and UPHCF, these values were not exceeded the consumable limits at the end of the storage. It can be said that the coating materials prepared with TPH and UPH may be effective in the microbial deterioration of proteins and other nitrogenous compounds, therefore, the coating material used in addition to the temperature during freeze storage may also be effective in keeping the TVB-N of bonito fillets under control.

Supporting results were collected by previous investigations. Luo et al. (2018) stored mackerel fish treated with nisin, chitosan and phytic acid at -18±1°C for 1 year and inspected the effects of coating materials on the quality of the fish. According to the results obtained, TVB-N of the coated groups were below the control group, within acceptable limits, during storage. Kulawik et al. (2019) analyzed the effects of a film (FUR/HGEL) consisting of active bilayer furcellaran/gelatin hydrolysate and Ala-Tyr peptide system on fresh Atlantic mackerel stored at -18±1°C. In the study, it was reported that the TVB-N value of the control group increased significantly after the first month and showed an unfluctuating regime in the following months, no significant increase was observed in the film-coated groups during storage. Literature shows that the low TVB-N indicates that the coating materials protect the fish fillets better than the control groups during cryopreservation. On the other hand, it can be concluded that depending on the factors such as the coating material used, the type of fish, the microbial load that may arise from contamination at the time of fishing or during processing, the storage time and temperature, it may affect the deterioration rates of fish fillets and thus the protection rates of the coating materials.

Lipid oxidation, which causes quality loss in fish during frozen storage, is higher in oily fish species than in lean fish. One of the problems that occur during the frozen storage of oily fish is rancidity due to lipid oxidation. The rancidity causes the expiration of the shelf life of the frozen product (Parvathy et al., 2018; Lee et al., 2019). Figure 6 represents the change of TBA values of bonito fillets during the storage.

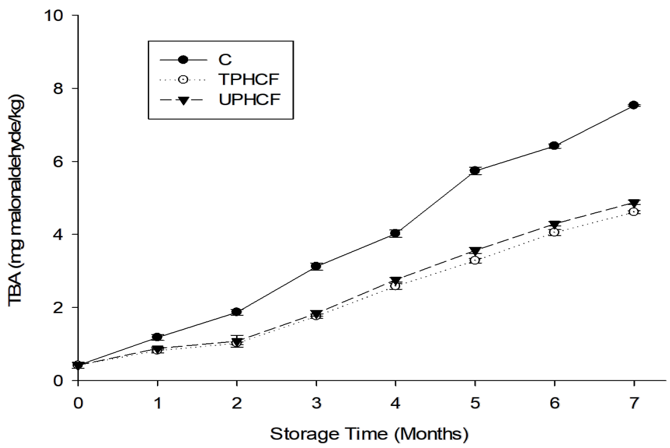

Figure 6. TBA values of C: Control; TPHCF: Traditional Protein Hydrolysate Coated Fillet; UPHCF: Ultrasound-treated

Protein Hydrolzate Coated Fillet during the storage of bonito fillets stored at -18±1°C

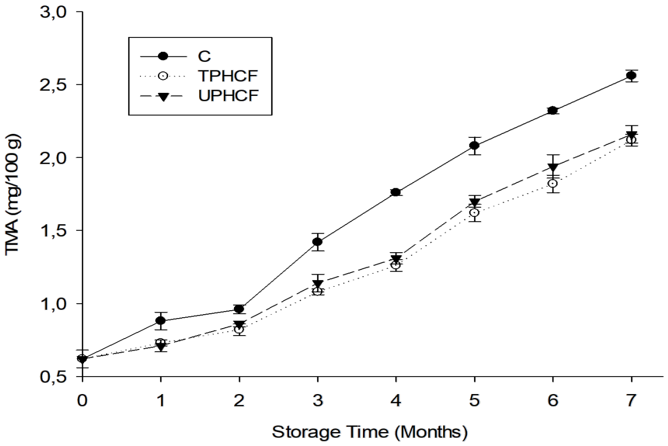

Figure 7. TMA values of C: Control; TPHCF: Traditional Protein Hydrolysate Coated Fillet; UPHCF: Ultrasound-treated Protein Hydrolysate Coated Fillet during the storage of bonito fillets stored at -18±1°C

TBA was 0.42 mg MA/kg at the beginning of the storage. Çorapçı (2018) calculated TBA value of fresh bonito meat as 0.95 mg MA/kg. Similar to TVB-N values, TBA values increased in all groups during the storage. TBA value of the K was 7.53±0.02 mg MA/kg in the 7th month, as exceeding the acceptable limit, they were 4.88±0.06 and 4.61±0.04 mg MA/kg for TPHCF and UPHCF, respectively at the same period. It is seen that TPHCF and UPHCF, are below the consumable limits in terms of TBA values until the last month of storage. This situation is compatible with the literature and can be explained as the coating materials used have good antioxidant properties and protect the products against oxidation. The differences between the TBA values of the TPHCF and UPHCF in the last 3 months of storage were assessed to be statistically significant ($p<0.05$). TBA values UPHCF were lower than the TPHCF. This situation can be interpreted as ultrasound application may be effective in providing more effective protection against oxidation in the product. Also, it could be said that ultrasound application creates a difference in the peptide sequences of protein hydrolysates, so amino acids with high antioxidant activity can come to the fore (Elias et al., 2005; Marchioni et al., 2009). It could be also emphasized that the coating materials could delay the lipid oxidation of the fish, so natural coating materials can be effective alternative materials for the preservation of the freshness of oily fish stored by freezing at -18±1°C. Rodriguez-Turienzo et al. (2012) demonstrated that ultrasound-treated protein-based coating materials preserve fish fillets better than non-ultrasound coating materials. The researchers reported that the TBA values of the ultrasound-treated groups were significantly below than the other groups. TMA analyzed monthly during storage in the study remained within consumable limits in all months (Figure 7).

In the study, the inhibition of the rise of microbial load by the effect of temperature in the freezer was effective in lowering TMA. In addition, the increases in the C group were higher than the TPHCF and UPHCF groups ($p<0.05$). Since protein hydrolysates show antimicrobial and antioxidant properties, it can be said that TMA values remain at lower levels with the inactivation of bacteria. TMA for consumption should be <10–15 mg/100 g. Varlık et al. (1993) stated that values above this value considered as deteriorated.

Vale et al. (2020) demonstrated that chitosan coating ensured low levels of TMA-N. Aref et al. (2018) investigated the role of transglutaminase enzyme, chitosan and rosemary extract and their different combinations on the quality parameters of fish fingers produced from catfish in frozen storage. Although there was an increase in TMA values during storage in all groups in the study, TMA value was lower in all coated groups compared to the control group. In addition, the rate of increase in the coated groups was also lower than control group.

Appearance, texture, odor and overall sensory scores are presented in Figure 8. Frozen storage protects foodstuffs from undesirable sensory and chemical deteriorates influenced by microorganisms, but not able to prevent degradation completely. Especially the reactions originating from proteins and lipids have an impact on sensory properties and cause bad smell, taste, and texture. Researchers have reported that sensory characteristics are the most crucial parameters that identify the quality of food, supposing a product that cannot be acceptable sensory cannot be offered for consumption (Erkan & Gökoğlu, 1999).

In this study, all three groups of bonito fillets remained within the acceptable limits over the study period, but the C group values were lower than the coated groups according to the scores given by the panelists to the sensory quality parameters. Therefore, the coating materials created a barrier against the passage of oxygen between the external environment and the fillets, and contributed to the prolonging of the storage period by affecting the stability of parameters such as odor and color, which are released as a result of rancidity due to lipid oxidation. In addition, by preventing textural deterioration due to microbial activities, coatings contributed to the tightness of the tissue and a smoother general appearance.

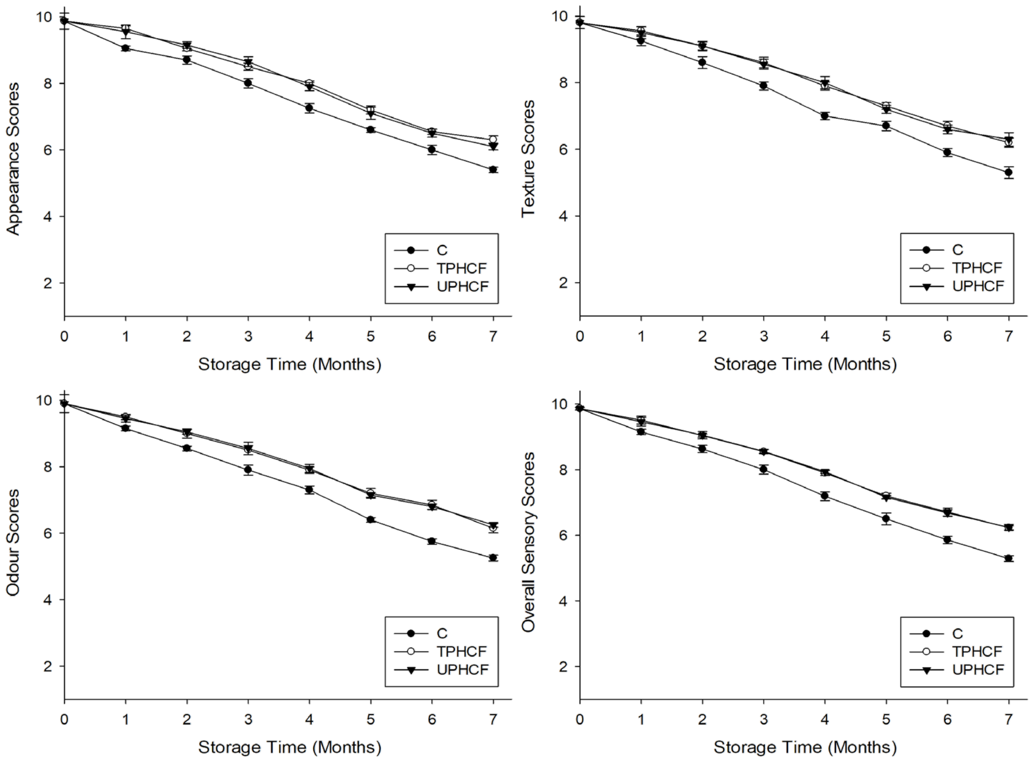

Figure 8. Appearance, texture, odor and overall sensory scores of C: Control; TPHCF: Traditional Protein Hydrolysate Coated Fillet; UPHCF: Ultrasound-treated Protein Hydrolysate Coated Fillet during the storage of bonito fillets stored at -18±1°C

Conclusion

This research evaluated that traditional and ultrasound-treated enzymatic protein hydrolysate coatings on the quality properties of the frozen bonito fillets. It is thought that using protein hydrolysates obtained from fish wastes as a coating in aquatic products, the products can be protected physically and chemically effectively. However, the expectation that UPH might have superior properties compared to TPH was not met in the study. If FPH is to be used as a coating material, it has been revealed that the ultrasound application used in the study conditions is unnecessary. However, in the functional property analyses made, it can be recommended to use UPH as an improver of the functional properties of the product in emulsion-form seafood products, especially the superior and functional properties of UPH.

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Compliance With Ethical Standards

Authors' Contributions

GBM: Conceptualization, Methodology, Software, Investigation, Resources, Writing original draft.
SK: Methodology, Software, Review & Editing, Supervision.
Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Data Availability Statements

All data generated or analysed during this study are included in this published article.

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