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RESEARCH PAPER

Sprat Protein Hydrolysate as a Natural Cryoprotectant: A Sustainable Strategy to Preserve Freeze-thawed Fish Mince Quality

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Abstract

Cryoprotective effects of fish protein hydrolysates (FPHs) on freeze-thawed fish mince were determined. Produced from sprat (*Sprattus sprattus* L., 1758) by conventional and ultrasound-assisted methods, the hydrolysates were used as natural alternatives to traditional cryoprotectants, specifically to prevent quality deterioration during freeze-thaw (F-T) cycles of rainbow trout (*Oncorhynchus mykiss*) mince (hereafter called fish mince). Four groups were prepared from fish mince for analysis: a control group without FPH addition (C); a group with 4% sucrose and 4% sorbitol (SSM); a group with traditional FPH (THM); and a group with ultrasound-assisted FPH (UHM). 250 g samples for each group, packaged in polyethylene bags, stored at -18°C. Physicochemical properties and microbiological changes were analyzed at 15-day intervals over six F-T cycles. Inhibited lipid oxidation, nitrogenous compounds, and microbial degradation were observed in FPH-added fish minces. Compared to the control (C) and sucrose-sorbitol mixture (SSM), both FPHs exhibited excellent water absorption capacity, significantly preventing water loss in the THM and UHM groups; the lowest loss was observed in THM during the cooking process. In conclusion, sprat hydrolysates represent a promising natural cryoprotectant that can effectively preserve the quality and significantly extend the shelf life of fish mince during multiple freeze-thaw cycles.

Introduction

Fish and seafood represent a crucial component of global food systems and contribute significantly to national economies (FAO, 2024). The global consumption of fish and seafood has been increasing rapidly due to their high nutritional value with high-quality proteins, unsaturated fatty acid, vitamins and minerals. Nevertheless, the high water and unsaturated fatty acids content make them highly susceptible to microbial proliferation and enzymatic degradation, thereby limiting their shelf life. To effectively preserve seafood, freezing stands out as one of the best methods available. This technique not only maintains the nutritional and sensory qualities but also significantly extends the shelf life of your favorite seafood products (Rabiepouret al., 2024). Cellular integrity is compromised by ice crystal formation during repeated freeze-thaw cycles, often due to inconsistent storage or distribution. This disruption can lead to critical physicochemical changes. These alterations are primarily driven by protein denaturation, which reduces protein solubility, water-holding capacity, and overall product quality (Tan and Xie, 2021; Zhang et al., 2023). Wu et al. (2022) highlighted substantial structural changes in crayfish arising from repeated F-T cycles, underscoring the importance of maintaining proper temperature control in food storage.

The food industry frequently employs synthetic cryoprotectants to improve the preservation and quality of frozen products. These substances play a crucial role in maintaining the texture and flavor during the freezing

process. By preventing ice crystal formation and reducing water-protein separation, they help preserve protein structure and functionality during storage (Carpenter and Crowe, 1988). Sucrose and sorbitol are utilized generally; however, their sweet flavor can have adverse effects on sensory perseption of the final output, especially in seafood (Cheung et al., 2009). Additionally, increasing consumer concerns about food additives, along with trends toward clean labeling and environmental sustainability, are fueling the demand for natural and biodegradable alternatives (Balasubramaniam et al., 2023). In this regard, Protein hydrolysates derived from the byproducts of seafood processing have gained considerable interest as natural cryoprotectants, owing to their valuable functional and bioactive properties (Patil et al., 2024).

Researchers have suggested that FPH's and peptides can serve as functional additives in refrigerated and frozen foods, helping to prevent quality loss during storage (Jenkelunas and Li-Chan, 2018; Karnjanapratum and Benjakul, 2015; Shen et al., 2022). FPHs contain valuable levels of hydrophobic amino acids, which may help reduce the ice crystal development from water owing to their water-holding properties (Nikoo et al., 2015). Additionally, peptides derived from fish skin can effectively slow down the degradation of myosin and actin during repeated freeze–thaw cycles. (Nikoo et al., 2014). Shen et al. (2022) explored the protective effects of FPH on surimi subjected to F-T treatments, at -18°C and -60°C. They found that these hydrolysates can help moderate carbonyl formation, prevent reductions in free sulfhydryl, and maintain the stability of protein bands compared to control and SuSo groups. Furthermore, lipids that undergo chemical oxidation can negatively impact the quality of frozen seafood, leading to rancidity. High levels of polyunsaturated fatty acids can make fish mince particularly susceptible to oxidation during long-term storage (Jacobsen et al., 2008). To combat this degradation, antioxidants are widely employed. Many protein hydrolysates exhibit antioxidant activity. Oprea et al. (2024) evaluate the potential of the fish protein hydrolysate for protein enrichment in confectionary. The findings of this study revealed that antioxidant activity was higher about 50-times in FPH flour than wheat flour. Nikoo et al. (2015) also investigated the antioxidant and cryoprotective properties of gelatin hydrolysate in unwashed fish minced meat. The researchers observed that gelatin hydrolysate prevents the displacement activity of water molecules in various parts, thereby maintaining the water during F-T processes in unwashed fish minced meat. They concluded that protein hydrolysates, which are effective at inhibiting lipid oxidation, can help maintain the quality of fishery products as functional additives. Ultimately, the researchers emphasized the need for further studies to explore the cryoprotective effects of fish protein hydrolysates.

Sprat, a small pelagic species, is frequently discarded or processed only into fishmeal (Urbanet al., 2024). Valorization of such low-value species through enzymatic hydrolysis can generate protein hydrolysates rich in bioactive peptides, contributing to sustainable resource utilization and circular economy approaches in fisheries (Naseem et al., 2023). These hydrolysates have been reported to exhibit antioxidant, antimicrobial, and protein-stabilizing properties, making them promising functional ingredients for various food applications (Tang et al., 2023; Mardani et al., 2023).

Although the growing interest in fish protein hydrolysates, their cryoprotective potential—particularly in preserving muscle-based products during F-T cycles—remains underexplored. F-T processes are known to deteriorate the structure, water-holding properties, and protein integrity of seafoods, posing a challenge to protect and ensuring the quality of frozen seafood. FPH could serve as natural cryoprotectants, reducing protein denaturation and oxidative damage during freezing (Zhou et al., 2019).

This research evaluate the efficacy of both traditional and ultrasound-assisted fish protein hydrolysates derived from sprat in preserving the quality of fish mince during six F-T cycles. The findings are expected to provide new perspectives on the potential utilization of FPHs as cryoprotectant in seafood processing.

Materials and Methods

Materials

Sprat (*Sprattus sprattus* L., 1758) was used to produce fish protein hydrolysate (Balçık Mısır et al., 2023). Sprat protein hydrolysate was employed as a cryoprotectant in fish mince obtained from rainbow trout. Traditionally and ultrasound-assisted sprat protein hydrolysates (FPH) were produced as given in a previous study (Balçık Mısır et al., 2023). Briefly, whole sprat was homogenized and hydrolysed enzymatically by Alcalase (E:S; 0.5%, w/w). The hydrolysis process was conducted at 60°C for 1 hour stirring. After enzymatic treatment, the enzyme was inactivated at 90°C for 10 minutes. The hydrolysate was then centrifuged (e.g., 10,000 × g, 15 min, 4°C) to remove insoluble fractions, and the supernatant was collected and freeze-dried for further analysis. Ultrasound pretreatment was performed using a probe-type ultrasound device set to 40% amplitude with a pulse mode of 10 s on/10 s off. The protein content of the traditional FPH was 75.88%, while the ultrasound-assisted FPH showed a protein content of 74.45%. Antioxidant activities- determined by the DPPH assay- at concentrations of 10%, 15%, and 20%, were 11.83, 21.52, and 31.54 for traditional FPH and 23.96, 27.90, and 30.96 for ultrasound-assisted FPH, respectively. Fresh rainbow trout was purchased from a fish market and transported to the laboratory in a container equipped with ice packs to maintain a low temperature. Figure 1 presents the sprat protein hydrolysate and the rainbow trout used in the study.

Methods

Preparation of Fish Mince

Cryoprotectant-added fish mince was prepared according to Cheung et al. (2009). Fish were filleted without skin rinsed drained and minced. Four groups of fish mince were prepared: a control group with no additives (C); a group containing 8% sucrose-sorbitol mixture (4% sucrose and 4% sorbitol) (SSM); a group with 8% traditional fish protein hydrolysate (THM); and a group with 8% ultrasound-assisted fish protein hydrolysate (UHM). Each group was thoroughly homogenized using a KitchenAid stand mixer set to speed 2 for 1 minute. The homogenates were portioned into 250 g samples and packaged in zip-lock polyethylene bags (Figure 2). Unfrozen samples were used for initial analysis (day 0). Other samples were shocked at –25°C for 12 hours and then stored at –18°C. For each freeze–thaw (F-T) cycle, fish minces were stored at-18C for 18 h and followed thawing at +4°C for 6 hours. A total of six F-T cycles were applied at 15-day intervals. After each thawing cycle physical and functional properties were determined on fish minces.

Figure 1. Sprat protein hydrolysate and rainbow trout (*Oncorhynchus mykiss*) (Balçık Mısır, 2016).

Figure 2. Preparation of FPH added fish mince groups; C, SSM, THM and UHM.

Determination of pH

Homogenized samples were diluted in a 1:1 ratio with distilled water, and measurements were done at room temperature by immersing the electrode (Mettler Toledo, FiveGo F2, Greifensee, Switzerland) directly into the diluted samples, (Ludorff and Meyer, 1973).

Determination of Color

The L* (lightness), a* (red-green), and b* (yellow-blue) values were recorded (with Konica Minolta CR-10 colorimeter) according to Amanatidou et al. (2000) and Gobantes et al. (1998).

Determination of Nitrogenous Compounds

The content of total volatile basic nitrogen (TVB-N), an indicator of nitrogenous compounds linked to protein degradation, is determined in the samples using the distillation method. (Antonacopoulos and Vyncke, 1989), and modified by Varlık et al. (1993). A homogenized sample was treated with magnesium oxide and subjected to distillation. Released volatile bases were collected in a boric acid solution and titrated with standardized hydrochloric acid to quantify the TVB-N value, and expressed as mg N/100 g of sample.

Determination of lipid oxidation

Lipid oxidation in fish mince samples was assessed by measuring malonaldehyde (MDA) concentration using the thiobarbituric acid (TBA) assay described by Tarladgis et al. (1960). This assay is based on the formation of a pink chromogen through the reaction of MDA with TBA.

Approximately 10 g of fish mince was homogenized with 47.5 mL distilled water and 2.5 mL 4% acetic acid solution for 1–2 min. Subsequently, 1 mL of 1% TBA solution was added, and the mixture was incubated in tightly sealed tubes in a water bath at 100°C for 35 min. After incubation, samples were cooled to room temperature, and the absorbance of the colored complex was read at 532 nm,, spectrophotometrically. Calculated MDA were given as mg MDA/kg fish mince.

Reagents were freshly prepared, and analyses were performed in triplicate.

Determination of Cooking Loss

Referring to method by Cheung et al. (2009), cooking loss of the fish minces was determined with slight modifications. Approximately 20 g sample was put into polyethylene bags, heated at 90°C for 15 min. Following incubation, samples were removed from the water bath, carefully blotted with paper towels to remove surface moisture, and cooled to room temperature (Figure 3).

The cooking loss was given according to the formula:

Cooking loss %= (Uncooked sample weight - Cooked sample weight)/ (Uncooked sample weight) x 100

Determination of Expressible Moisture

Expressible moisture was determined the method described by Gómez-Guillén et al. (1996). One and a half g of fish mince was introduced into centrifuge tubes with pre-weighed cellulose acetate filter papers and allowed to cool for 10 minutes, after that centrifuged at 4000 × g for 10 minutes at 4°C. The expressible moisture was calculated by the weight difference of the filter paper before and after centrifugation.

Determination of Natural Actomyosin

Natural actomyosin (NAM) was measure by modified procedures reported by Sultanbawa and Li-Chan (2001) and Wang et al. (2003). Twenty grams of fish minces was mixed with 100 mL of 0.02 M sodium phosphate buffer (pH 7) containing 0.05 M NaCl (Buffer A) and homogenized for 1 minute using a Waring blender. The homogenate was centrifuged at 10,000 × g for 5 minutes at 4°C, and the supernatant was separated. The pellet was re-homogenized with 100 mL of Buffer A and centrifuged under the same conditions. The resulting pellet was then homogenized in 200 mL of 0.6 M NaCl prepared in 0.02 M sodium phosphate buffer (pH 7) (Buffer B) and centrifuged at 10,000 × *g* for 10

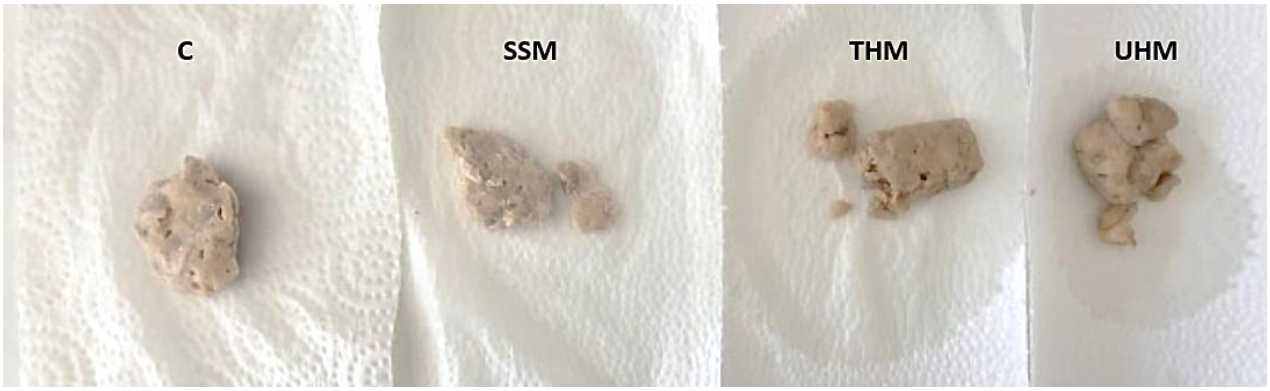

Figure 3. Cooking loss experiment of C, SSM, THM and UHM.

minutes at 4°C. The supernatant was filtered through double-layered cotton gauze (grade 40) into a beaker containing 1200 mL of Buffer A and incubated with constant stirring at 4°C for 30 minutes. After incubation, the mixture was centrifuged at 10,000 × *g* for 10 minutes at 4°C. The pellet obtained was resuspended in 100 mL of Buffer A and homogenized. The dense pellet recovered after centrifugation was considered as the extracted natural actomyosin (NAM). Protein concentration of the extracted NAM was analysed by the method described by Lowry et al., 1951. using bovine serum albumin (BSA) as the standard.

Determination of Microbiological Counts

Ten g of fish mince from each treatment group. Samples were diluted appropriately with sterile 0.85% NaCl solution, and serial dilutions were arranged. All analyses were conducted using 3M™ Petrifilm™ plates according to the respective AOAC Official Methods. Total aerobic mesophilic bacteria were enumerated following the AOAC Official Method 990.12 (AOAC, 1995). Appropriate dilutions were plated on 3M™ Petrifilm™ Aerobic Count Plates and incubated at 35±1°C for 48±3 hours under aerobic conditions. Colonies appearing on the films were counted and expressed as log CFU/g. Total coliform bacteria were determined using AOAC Official Method 991.14 (AOAC, 1995). Prepared dilutions were plated on 3M™ Petrifilm™ Coliform Count Plates and incubated at 35±1°C for 24±2 hours and results were expressed as log CFU/g. Yeasts and molds were enumerated according to AOAC Official Method 997.02 (AOAC, 1995). Diluted samples were plated on 3M™ Petrifilm™ Yeast and Mold Count Plates and incubated at 20-25°C for 3-5 days. Colonies were differentiated morphologically and counted separately for yeasts and molds. Results were given as log CFU/g.

Statistical Analysis

Analysis of variance (ANOVA) was used for the difference values in days and groups. Differences among measurement times within each group, as well as differences between groups at each measurement time, were calculated by one-way ANOVA. Duncan's multiple comparison test was employed to determine significant differences between means. Significance level was $P<0.05$. Statistical analyses were performed using the MedCalc software package (version 17.9).

Results and Discussion

Figure 4 presents FPH added fish mince groups on 0. day and 6. F-T cycle, for the C, SSM, THM, and UHM groups.

The pH, L*, a*, b* and TVB-N and TBA values of fish minces at 0. day and 6. F-T cycle are shown in Table 1.

pH is an effective and reliable indicator for determining food stability, it is affected by the chemical reactions and microbial activities during food deterioration (Wang et al., 2017). The pH increased in the FPH supplemented samples and showed similar values ($P>0.05$), and significantly higher than those of C and SSM on day 0 ($P<0.05$). The increasing trend in pH continued after the 6. F–T cycle, with the pH of UHM

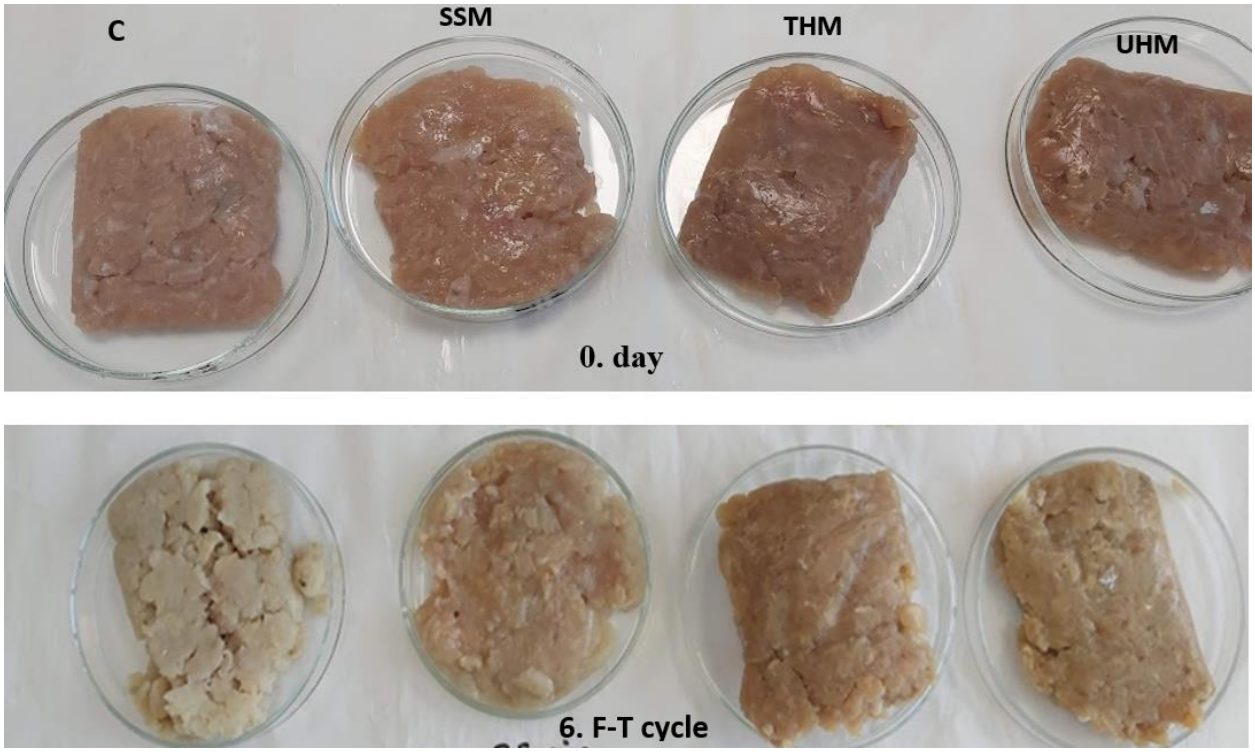

Figure 4. FPH added fish mince groups on 0th day and 6. F-T cycle.

Table 1. pH, L*, a*, b*, TVB-N and TBA values of FPH added fish mince

Analysis	F-T cycle	Fish mince groups			
		C	SSM	THM	UHM
pH	0	6.09±0.01^{aA}	5.99±0.00^{aA}	7.23±0.00^{aC}	7.23±0.00^{aC}
	6	6.44±0.01^{bB}	6.29±0.01^{bA}	7.26±0.01^{aC}	7.41±0.05^{aD}
L*	0	40.19±2.36^{aB}	40.23±0.32^{aB}	32.91±0.71^{aA}	34.77±0.71^{aA}
	6	41.17±0.32^{aC}	36.99±0.37^{bB}	32.95±0.27^{aA}	32.88±0.55^{aA}
a*	0	7.13±0.38^{aAB}	7.84±0.14^{aB}	6.57±0.20^{aA}	7.10±0.22^{aAB}
	6	2.18±0.05^{bA}	3.90±0.10^{bB}	3.42±0.25^{bB}	3.66±0.11^{bB}
b*	0	12.09 ±0.84^{aA}	12.31±0.24^{aA}	11.18±0.46^{aA}	10.50±0.46^{aA}
	6	9.95±0.22^{aA}	9.38±0.28^{bA}	7.91±0.74^{bA}	8.27±0.33^{bA}
TVB-N	0	11.67±0.46^{aA}	12.61±0.80^{aA}	15.41±0.80^{aB}	17.27±0.46^{aB}
	6	36.42±0.80^{bC}	27.08±0.46^{bA}	29.88±0.46^{bB}	28.95±0.93^{bAB}
TBA	0	0.20±0.00^{aA}	0.20±0.00^{aA}	0.20±0.00^{aA}	0.18±0.00^{aA}
	6	1.37±0.11^{bB}	0.93±0.08^{bA}	1.07±0.00^{bA}	1.17±0.05^{bAB}

± represents the standard error (n=3). Lowercase letters (a, b, c..) in the same column represent differences in a parameter between the 0th and 6th days of the same group (P<0.05), and uppercase letters (A, B, C…) in the same row represent the differences of different groups on the same days.

being significantly higher than that of THM (P<0.05). The highest pH value was recorded in UHM at the 6. F–T cycle. Due to their inherently high pH, FPHs can contribute to an increase in the pH of the medium. This finding is in agreement with the results of Cheung et al. (2009). The researchers reported that the pH of unfrozen Pacific hake ranged between 7.20 and 7.32, while significant changes occurred after six F–T cycles. The pH decreased to 6.97 in the control group, whereas SUSO, FPH-A, and FPH-F reached 7.55, 7.48, and 7.72, respectively.

As shown in Table 1, on day 0, C and SSM exhibited similar L* values (P>0.05), and demonstrated similarities for THM and UHM on the same day. A significant reduction in L* was recorded in SSM from day 0 to the 6. F–T cycle, whereas the differences in the other groups were not significant. Significant decreases were seen in the a* values of C, SSM, THM and UHM from the beginning to the finalization of the research. The maximum a* value was recorded in SSM on day 0, while the minimum was measured in C after the 6. F–T cycle. A significant decreasing trend (P<0.05) was also detected in the b* values of all groups from day 0 to the 6. F–T cycle; however, no significant differences were found among the groups on the same sampling days. The maximum b* value was readed in SSM on day 0, and the minimum in THM after the 6. F–T cycle. These results are consistent with those found by Hamzah et al. (2021).

The analysis of Total Volatile Base Nitrogen (TVB-N) revealed that the lowest value was recorded in the SSM group, it shows similarity with C but higher then THM and UHM groups (P>0.05), on day 0. According to Stanković et al. (2015), when the TVB-N value reaches 30.0 mg/100 g fish products are not permitted to be consumed. In present research, TVB-N levels increased significantly in all groups, exceeding the maximum acceptable limit in the C group after the sixth F-T cycles, indicating spoilage of the fish mince, during storage. In contrast, the TVB-N values for the other groups at the same period remained just below the acceptable limit. Mousakhani-Ganjeh et al. (2015) noted that the autolysis of fish mince and microbial activity lead to protein degradation and the formation of nitrogenous compounds, which contribute to the rise in TVB-N levels. Yeganeh et al. (2022) searched the effect rainbow trout viscera hydrolyzed protein (HP) used for fish diets. They reported lower TVB-N values in HP-fed samples than C, attributing antioxidant activity of HP. This finding was further supported by the microbiological load results of the samples. The addition of HP to the diet had a positive effect in reducing oxidative and microbial spoilage, thereby decreasing the spoilage rate and extending the shelf life, and protecting the chemical quality of the product compared to the control.

Application of F–T cycles to fish mince promotes lipid oxidation and the formation of oxidation products. At the initial stage, the TBA values of all groups were similar (P>0.05). However, an increase in TBA values was calculated in all groups from day 0 to the 6. F–T cycle, with the highest value recorded in C after the 6. cycle. Incorporation of FPH into the fish mince before freezing significantly delayed lipid oxidation, showing a similar effect to SSM (P>0.05) throughout the 6 F–T cycles. The present results are consistent with those of Mueller & Liceaga, (2016). In that study, researchers added 6% FPH fish mince and performed 6 F-T cycle to investigate the improvement effect of the additive.

The present results are in agreement with Zhang et al. (2021) for F-T fish minced meat added silver carp skin gelatin hydrolysates as a natural and high-performance antioxidant and cryoprotectant. Researchers reported that the TBA content increased significantly with increasing F-T cycles. They also explained that after 6 rounds of F-T cycle, addition of hydrolysate lower the TBA value. This phenomenon explained as the hydrolysates could effectively prevent TBA accumulation. Kittiphattanabawon et al. (2012) examined proteins of shark meat, skin, and revealed that the proteins showed superior antioxidative and anti-TBA effects under appropriate hydrolysis

parameters. Additionally, comparing to sucrose and sorbitol, protein hydrolysates have been stated to be more effective in reducing the TBA content in seafood, making them advantageous as new alternatives (Karnjanapratum and Benjakul, 2015).

Changes in cooking loss of C, SSM, THM and UHM from day 0 to 6 F-T cycle are shown in Figure 5.

In determining the yield of cooked products and the amount and loss of water they contain cooking loss is a crucial quality criterion that can be calculated by calculating weight loss after cooking.

Cooking loss values showed a significant increasing trend during the F-T cycles. Both on 0. day and 6. F-T cycle, there was a remarkable increase determined in C, SSM, THM and UHM, between days and the groups ($P<0.05$). Accordingly, the lowest was detected in THM on 0. day; the highest cooking loss is in SKK on 6. F-T cycle. Comparing to C and SSM, FPH's good water absorption capacity effectively inhibited the water loss during the cooking process in both THM and UHM.

Cheung et al. (2009) determined that the cooking losses of all groups did not show a significant difference before freezing; however, after 6 F-T cycles, no significant change was recorded in FPH-Alkalase, while significant changes occurred in the control, SUSO and FPH-Flovurenzyme groups. While the cooking losses of the SUSO and FPH-Flovurenzyme groups doubled, they increased from 3% at the beginning to 16% at the end of the study in the control group. The difference between cooking losses may have occurred due to composition differences of cryoprotectants; it has been reported that the difference between FPH-Alkalase and FPH-Flovurenzyme may be due to the use of different enzymes having a significant effect even though they are obtained from the same fish. Chang et al. (2023) investigate the chitosan ice coating's preventive effect on the quality losses of frozen fish products during repeated F-F-T cycles. The researchers found out that chitosan ice coating dramatically limited the destruction of the structure by reducing water loss, the forming of ice crystals and recrystallization of ice in the samples during the process.

Variation in expressible moisture of C, SSM, THM and UHM from day 0 to 6 F-T cycle are represented in Figure 6.

A comparable increasing tendency was seen in the expressible moisture contents with cooking loss. At 0. day, the difference between the expressible moisture contents of C and SSM was insignificant, while these two values were higher than those of THM and UHM. A serious increase was observed in C and SSM in 6. F-T cycle, compared to THM and UHM. The highest expressible moisture was obtained in the SSM group on 6. F-T cycle, and statistically differed from all other groups ($P<0.05$).

Zhang et al. (2020)" investigated the potential antioxidant-cryoprotective and gelation-enhancing effects of SPB hydrolysates on silver carp (*Hypophthalmichthys molitrix*) surimi based on protein oxidation, protein degradation, and gel-forming ability analysis". After 3 months frozen storage the control group had showed the highest expressible water.

Sucrose-sorbitol is among the common cryoprotectants used in seafood products such as surimi and fish minced meat, with its ability to stabilize proteins and retain high water. When the study results were compared, it was observed that TFPH and UFPH had superior protection effects compared to sucrose-sorbitol in terms of cooking loss and expressible moisture content, both at the beginning and at the end of the 6. F-T cycle.

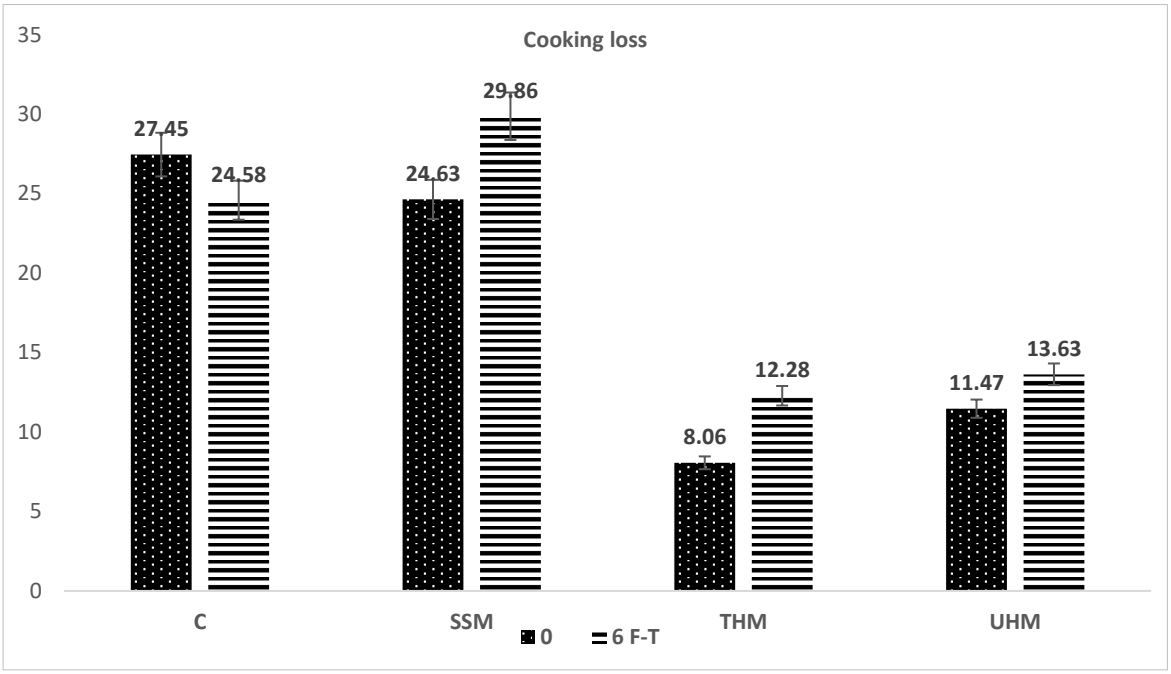

Figure 5. Changes in cooking loss of C, SSM, THM and UHM from 0th day to 6th F-T cycle.

During frozen storage, actin and myosin form protein structures with larger molecular weights and show lower solubility in salt solutions. Changes in NAM of C, SSM, THM, and UHM from 0. day to 6. F-T cycle is presented in Figure 7.

The maximum NAM value was obtained in UHM on 0. day with 0.87 g/g protein, and this value differed statistically from all groups ($P<0.05$). The minimum NAM value was reported in SSM in the 6. F-T cycle with 0.26 g/g protein and showed a statistical difference from all groups ($P<0.05$).

Cheung et al. (2009) stated that after 6 F-T cycles, more than fifty percentage of loss in NAM extractability and more than fifty percentage increase in surface hydrophobicity were determined in control cod mince. Although samples containing FPH-alkalase FPH-flovurenzyme represented a measurable decrease in extractable NAM after F-T cycle, this decrease was significantly below control samples. Additionally, researchers reported that specimens containing sucrose-sorbitol and FPH-flovurenzyme showed a preventive effect on muscle proteins, returns in higher extractable NAM than C after F-T cycle.

Table 2 demonstrates the changes in TAMB numbers of C, SSM, THM and UHM during 6 F-T cycles. Accordingly, TAMB numbers, which was 2.13 log cfu/g on 0. day, significant changes occurred in all groups after

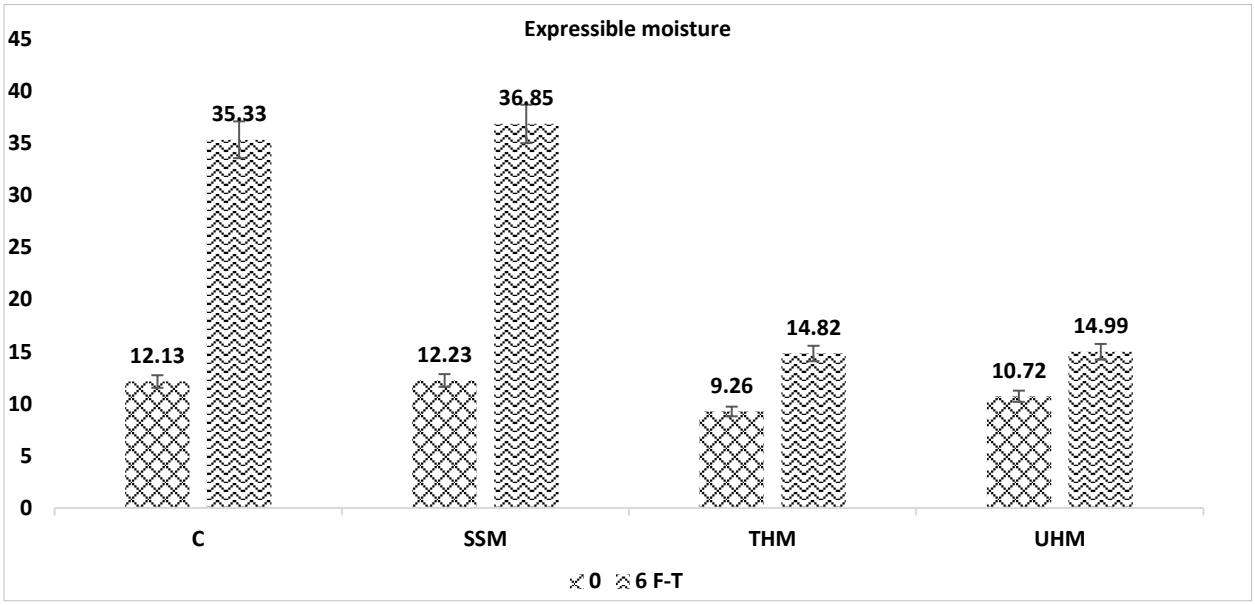

Figure 6. Variations in expressible moisture of C, SSM, THM and UHM from 0th day to 6th F-T cycle.

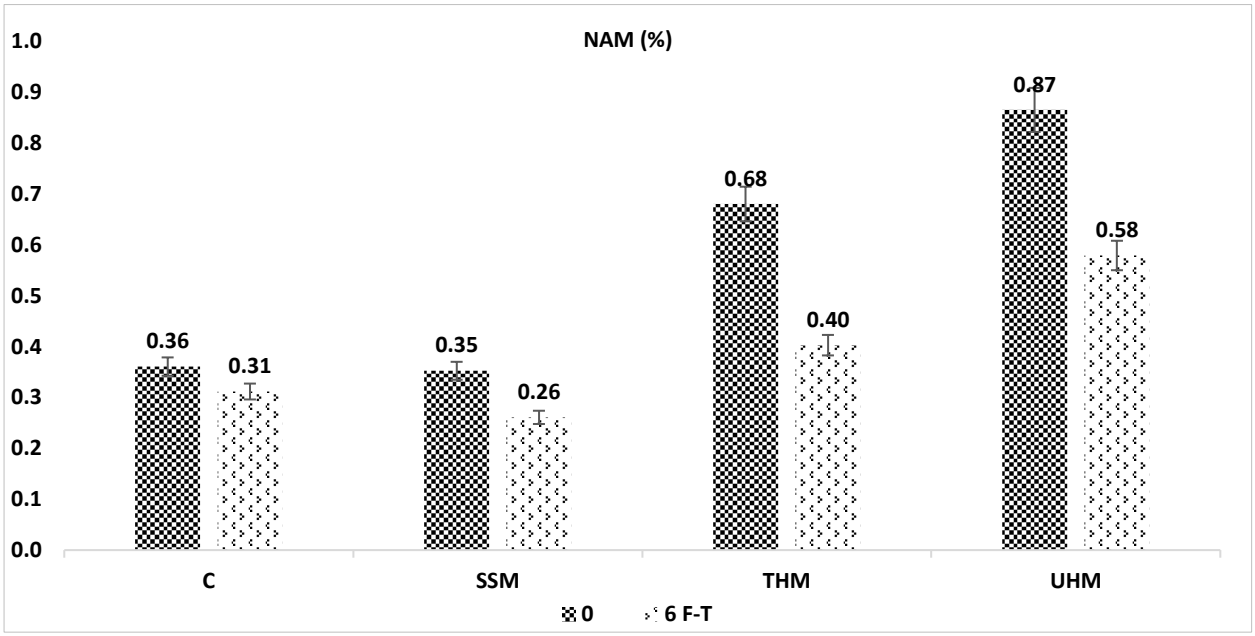

Figure 7. Changes in NAM of C, SSM, THM and UHM from 0th day to 6th F-T cycle.

Table 2. Changes in TAMB numbers of C, SSM, THM and UHM from 0. day to 6. F-T cycle

Groups	F-T	TAMB
C	0	2.13 ± 0.01^{aA}
	6	3.72 ± 0.01^{bA}
SSM	0	2.13 ± 0.01^{aA}
	6	2.49 ± 0.01^{bB}
THM	0	2.13 ± 0.01^{aA}
	6	2.50 ± 0.01^{bB}
UHK	0	2.13 ± 0.01^{aA}
	6	2.50 ± 0.01^{bB}

± represents the standard error (n=3). Lowercase letters (a, b, c..) in the same column represent differences in a parameter between the 0th and 6th 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.

6. F-T cycle (P<0.05). During storage, coliform bacteria and yeast-mold counts were well below the limit values.

Kuepethkaew et al. (2022) used sea bream skin gelatin hydrolysates produced with lizardfish stomach pepsin against commercial cryoprotectants in frozen surimi. Total bacteria, coliforms and yeast-molds were not detected in the protein hydrolysate and surimi samples produced in this study. According to these findings, researchers reported that both protein hydrolysate and surimi preserved with the addition of this hydrolysate might be suitable for consumption. Volpe et al. (2015) determined the" total bacterial count of fresh trout fillets as 4.02±0.05 log cfu/g", while they did not detect yeast-mold in minced trout during storage, and the coliform count was 1.76±0.01 log cfu/g. Similarly, Yerlikaya et al. (2015) used "protein-structuring enzyme transglutaminase (TGase) to extend the shelf life of rainbow trout minced meat during refrigerated storage". In the study, the initial coliform count of the samples was calculated as 1.8 log cfu/g, and it was reported that no yeast or mold growth occurred during storage.

Conclusion

This research evaluated the possibility of usage of FPH in fish mince as cryoprotectant during 6 F-T cycles. It was demonstrated that FPHs produced from sprat were applicable in fish mince as a cryoprotectant, which can extend the storage period effectively by protecting the fish mince physically and chemically. All samples showed an increasing in cooking loss and expressible moisture contents. But the increasing ratios of THM and UHM were significantly lower than C and SSM. Also, the increase in TBA formation was lower in FPH added groups. Although, mince color of THM and UHM become darker in comparison with C and SSM,

Different results can be obtained with studies using different application parameters such as ultrasound treatment parameters, different FPH ratio usage in fish mince.

Ethical Statement

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

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Author Contribution

GBM: Fiction, Literature, Performing the experiment, Manuscript writing,

BBD: Performing the experiment (Quality and Statistical Analysis)

SK: Performing the experiment (Quality Analysis)

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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