Asian Fisheries Science **13**(2000): 49-55
Asian Fisheries Society, Manila, Philippines

https://doi.org/10.33997/j.afs.2000.13.1.006

Changes in the Functional Properties of Dressed Thread Fin Bream (*Nemipterus japonicus)* Proteins During Frozen Storage

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Abstract

Frozen storage of thread fin bream for 12 weeks at -18 ºC resulted in a significant ($P < 0.05$) decrease in salt soluble proteins (SSP), protein solubility (PS), emulsifying capacity (EC), water binding potential (WBP) and relative viscosity (RV) of the SSP extracts. Functional properties correlated significantly ($P < 0.05$) with SSP and PS suggesting that myofibrillar proteins are the main proteins that determine these properties. Significant correlations ($P < 0.05$) also existed among EC, RV and WBP. Marked variations in the functional properties investigated suggest that these functional properties may be considered as valuable indicators in determining the quality of fish proteins.

Introduction

Freezing and frozen storage are important methods of preserving fish and fishery products. Although undesirable changes such as microbial growth and other biochemical changes are largely controlled by frozen storage, changes in the functional properties do occur (Sikorski et al. 1976; Shenouda 1980). There are many reports on the biochemical changes in the

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muscle of different Indian marine fishes during frozen storage. However, little work has been reported on the functional properties of fish proteins during processing and storage (Srikar and Vidya Sagar Reddy 1991). These studies demand more interest, as changes undergone by proteins may influence subsequent processing. Also, such investigations could lead to better utilization of low-priced fishes *viz.,* thread fin bream, in the preparation of paste products, especially emulsion type of products, where functionality of proteins is of great significance.

The objective of this paper, therefore is aimed at investigating the effect of frozen storage on certain functional properties of thread fin bream muscle proteins, which is a major by-catch of shrimp fishery in the Indian coastal waters, contributing approximately 3.5% of the total marine landings in India.

Materials and Methods

Processing

Fresh thread fin bream *(Nemipterus japonicus)* with a model length of 162mm and weighing 64 grams, caught off Mangalore coast by a local trawler were iced at the fishing ground in a ratio of 1:1 (Fish : Ice) and transported to the processing laboratory. The fish were immediately dressed and washed in chilled water (4 ºC). Samples of 1.5kg dressed fish were packed in low-density polythylene (LDPE) covers (25 x 30cm. and 200 gauge), stacked in galvanised trays, and immediately frozen in a coil freezer at -28 ºC for 48 hours. The frozen samples were glazed (dipped for 30 secs.) in chilled water and stored at -18 ºC in a master carton. Samples (frozen block) were drawn at random; immediately after freezing and at regular intervals of frozen storage for analysis of functional changes. The analysis was carried out on the homogenised meat obtained from the hand picked meat of thawed fish.

Analysis

Proximate composition was determined following the procedures described in AOAC (1975). Protein solubility (PS) was calculated as the total of water soluble proteins (WSP) and salt soluble proteins (SSP) expressed as percentage of total protein. WSP was measured according to Srikar and Vidya Sagar Reddy (1991) and SSP according to Dyer et al. (1950). Protein content in the respective extracts were determined using the Biuret method of Gornall et al. (1949). All extractions were carried out at 4 ºC. The total nigtrogen (TN) was determined using the method of Srikar and Chandru (1983) following the Kjeldhal procedure using hydrogen peroxide (Total Protein = TN x 6.25). Emulsifying capacity (EC) measurements were performed on soluble protein extracts according to Swift et al. (1961) using refined groundnut oil and results were expressed as millilitres of oil emulsified per

1.25g meat. Water-binding potential of muscle proteins (WBP) in terms of absorbed moisture in water (AM_w) was determined using the method of Li-Chan et al. (1986) and the results were expressed as percentage weight gain over original meat weight. Relative viscosity (RV) of the SSP extracts (constant volumes) were determined using Ostwald U - tube viscometer according to the method of Spinelli et al. (1973).

Statistical analysis

The mean values of the parameters analysed were subjected to ANOVA and students 't' test to determine significant differences between the experimental periods of storage.

Results and Discussion

The average content of moisture, fat and protein of freshly caught thread fin bream was 80.15%, 2.15% and 17.07% respectively.

Alterations in protein functional properties of dressed thread fin bream during storage at -18 ºC is presented in Table 1. The protein solubility (PS) decreased significantly ($P < 0.05$) at the end of the 12 week storage. The decrease in PS may be attributed to the protein denaturation and protein aggregation induced by frozen storage (Grabowska and Sikorski 1974). According to Srikar et al. (1989) the oxidised products of lipids and free fatty acids (FFA) formed during storage of fishery products are known to influence the solubility of proteins. Moreover, FFA is said to react hydrophilically or hydrophobically to the appropriate sites on protein surfaces creating a hydrophobic environment which results in lower protein extractability (Sikorski et al. 1976). On the other hand, oxidised lipids interact with amino acids in fish proteins, such as cysteine - SH, the ε-NH_2 group of lysine, and the N-terminal groups of aspartic acid, tyrosine, methionine and arginine; these interactions increase the hydrophobicity of proteins, thus increasing aggregation (Kussi et al. 1975).

Emulsifying capacity (EC) of soluble protein extracts decreased by 13% by the end of 12 weeks storage (Table 1). Usually two factors affect the EC of protein solution and these are the amount of soluble protein available and the efficiency of the protein to emulsify fat (Saffle 1960). The decline in EC values observed in the present study is attributed to the decrease in muscle protein solubility, as a positive linear correlation existed between the two.

During frozen storage, salt soluble protein (SSP) decreased significantly ($P < 0.05$) from 8.87 to 7.25% at the end 12 weeks storage (Fig. 1). This decrease amounting to 18% is attributed to protein denaturation induced by frozen storage (Kussi et al. 1975; Shenouda 1980). It is generally acknowledged that salt - soluble proteins (SSP) rather than the sarcoplasmic proteins (WSP) are the primary emulsifiers in the muscle systems (Saffle 1960; Srikar and Vidya Sagar Reddy 1991). Further, as the EC technique is accomplished by sodium chloride (1M) extraction, it is postulated that only

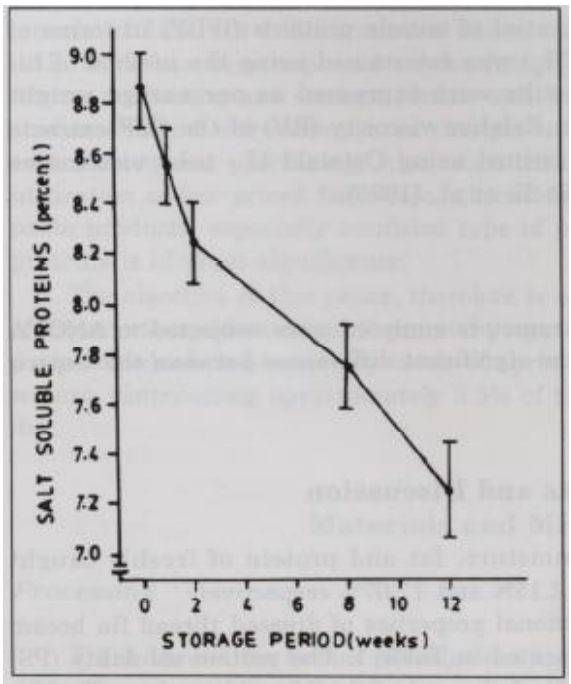

Fig. 1. Changes in salt - soluble proteins (SSP) in dressed threadfin bream during frozen storage (Φ : standard deviation).

Table 1. Changes in protein solubility (PS), emulsifying capacity (EC), water - binding potential (WBP) and relative viscosity (RV) in dressed thread fin bream during frozen storage.

Storage time (Weeks)	PS * (%)	EC* (ml oil/1.25g meat)	WBP* (%)	RV* (Centipoise)
0	67.77[a]	83.79[a]	89.52[a]	137.72[a]
	(2.05)	(1.04)	(0.02)	(0.53)
1	66.25[ab]	80.02[a]	73.90[b]	122.41[b]
	(1.38)	(1.44)	(0.02)	(0.85)
3	64.73[b]	76.87[b]	47.60[c]	105.28[c]
	(1.12)	(3.07)	(0.01)	(0.70)
8	59.70[c]	74.60[c]	40.29[d]	101.49[d]
	(0.29)	(1.49)	(0.05)	(1.58)
12	59.09[d]	73.06[d]	37.76[d]	96.60[e]
	(1.00)	(2.75)	(0.01)	(0.28)

*Values are means of minimum three estimates (Standard deviations are given in parenthesis). [a,b,c,d,e]Means followed by the same superscript within a column do not differ significantly ($P > 0.05$).

SSP were effective in the emulsification of oil. Hence, the higher the level of SSP in the meat is, the higher is the EC of muscle proteins. In the present study, EC correlated significantly with SSP ($P < 0.01$) and PS ($P < 0.05$) indicating that surfactant properties such as EC are mainly influenced by levels of soluble myofibrillar proteins. Srikar and Vidya Sagar Reddy (1991) observed a similar relationship in pink perch mince reporting that EC and PS; which were significantly correlated, decreased due to denaturation and protein aggregation induced by frozen storage. Additional reports

demonstrating the existence of linear relationships between the two parameters are those of Grabowska and Sikorski (1974), Colmenero and Borderias (1983) and Colmenero et al. (1988).

The water binding properties of meat determines its degree of interaction with water. Water-binding potential (WBP) refers to the ability of a raw meat system to bind extra water, in the presence or absence of salt, when subjected to an external force (Regenstein et al. 1979). Water-binding potential in terms of absorbed moisture in water (AM_w) showed a significant ($P < 0.05$) decrease over 12 weeks of storage (Table 1). The present findings corroborate the experimental findings of Hsien and Regenstein (1989) in cod and ocean perch.

There was a close association between the decrease in WBP and decrease in SSP and PS, in that when there was a relatively low value of soluble proteins, the WBP was also low. In the present study, there was a significant positive correlation between WBP and SSP ($P < 0.05$) and between WBP and PS ($P < 0.05$) (Table 2). Existence of a similar association between the two parameters have been reported by Kim and Heldman (1984) in cod muscle.

Relative viscosity (RV) of the SSP extracts decreased significantly ($P < 0.05$) from 137.72 to 96.60 centipoise at the end of 12 weeks storage (Table 1). Relative viscosity of a protein solution is a function of molecular size, shape, flexibility, degree of hydration and intermolecular interactions (Kinsella 1979). Among the various factors influencing viscosity such as pH, temperature, ionic strength; the chief factor affecting it is the protein concentration (Colmenero and Borderias, 1983). In the present study, RV correlated significantly with both SSP ($P < 0.05$) and PS ($P < 0.05$) indicating that viscosity is influenced by the myofibrillar protein concentration (Table 2). Significant linear relationships have been established in other fish species (Kinsella 1976; Colmenero and Borderias 1983 and Colmenero et al. 1988).

Results shown in Table 2 demonstrate significant ($P < 0.01$) positive correlation between EC and RV. Existence of these associations seem logical as alterations in these properties are to a great extent determined by the same set of factors. A direct linear relationship between EC and RV has also been detected in fish muscle (Colmenero and Borderias 1983; Colmenero et al. 1988). Further, WBP also correlated significantly with EC ($P < 0.01$) and RV ($P < 0.001$). Among the different factors conditioning EC, RV and WBP, the main one is soluble protein concentration, which decreased throughout the frozen storage period as a result of protein denaturation and aggregation.

Table 2. Correlation coefficients (r) of salt-soluble proteins (SSP) and protein solubility (PS) with other functional properties and also between the functional properties evaluated.

Parameters	EC	RV	WBP
SSP	0.9607*	0.9176*	0.9109*
PS	0.9399*	0.8865*	0.8919*
EC	1.0000	0.9896**	0.9830**
RV	0.9896**	1.0000	0.9948***

***Significant at 0.001 level.
**Significant at 0.01 level.
*Significant at 0.05 level.

In conclusion, it can generally be said

that functional properties of proteins decreased during frozen storage. All the functional properties *viz.*, PS, EC, WSP & RV were affected by protein solubility which decreased due to denaturation induced by frozen storage.

Functional properties in addition to being controlled by the composition and structure of proteins are also controlled or influenced by their interaction with one another and with other substances. Research therefore, needs to be directed towards a deeper understanding of the changes in the functional properties during processing and storage, with reference to quality of protein in the muscle foods.

Acknowledgments

The authors express their gratitude to Shri Kamal Sarma for helping in the statistical analysis and Shri Nagesh Bolar for helping in the procurement of fish for this work. The authors also thank Shri N.S.Sudhakar, Associate Professor of Fish Processing for his keen interest in this investigation.

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Manuscript received 06 January 1998; Accepted 15 November 1999.