Fishery Technology 49 (2012) : 147 - 152

Changes in Muscle Protein During Fat Oxidation in Indian Mackerel *Rastrelliger kanagurta* (Cuvier, 1817) and Threadfin Bream *Nemipterus japonicus* (Bloch, 1791) Under Accelerated Conditions

I. P. Lakshmisha[1], T.V. Sankar*, R. Anandan, V. Geethalakshmi and J. Joseph
Central Institute of Fisheries Technology, Matsyapuri, P. O., Cochin - 682 029, India

Abstract

Changes in proteins during exposure conditions which favour deterioration (40 ± 0.50°C) of mackerel (*Rastrelliger kanagurta*) and Japanese threadfin bream (*Nemipterus japonicus*) were investigated. Samples were drawn at regular intervals for quantifying protein solubility *viz*., sarcoplasmic proteins (SPP) and myofibrillar proteins (MFP). During the study period, both mackerel and threadfin bream showed a decreasing trend in solubility *viz*., 91 and 88% respectively whereas SPP did not show much change. Results showed change in solubility of MFP, in mackerel was highly significant ($p<0.05$) than in threadfin bream, indicating the overall loss of protein solubility with exposure.

Key Words: Solubility, myofibrillar protein, sarcoplasmic protein, temperature exposure

Received 20 September 2011; Revised 25 April 2012; Accepted 11 June 2012

* *E-mail: sankartv@sify.com*

[1] *Present address: College of Fisheries, Hoige Bazar, Mangalore - 575 001, India*

Introduction

Fish protein is considered to be of high quality due to its easy digestibility and high content of sulphur containing amino acids. The protein content in the fish muscle falls in the range of 16-24%. The essential amino acid content of fish protein is about 30% higher than that of plant source (Sankar & Ramachandran, 2001). The characteristics of different food formulations are closely related to the ability of proteins to interact with other components like lipids. Fish proteins are unique in nature and exhibit high degree of physico-chemical properties - functional properties, which affect the processing and behaviour of proteins in food systems (Kinsella, 1981). There are three types of proteins in fish-myofibrillar or contractile proteins (65-75%), sarcoplasmic proteins (20-30%) and stroma or connective tissue proteins (1-3%).

Lipid oxidation is a major cause of deterioration of quality of muscle foods leading to changes in colour, odour, texture and nutritive value and formation of potentially toxic compounds (Hettiarachchy et al., 1996). Temperature abuse causes alterations in the nature of lipids which in turn affect fish quality. For every 10°C increase in temperature the rate of lipid oxidation doubles (Pokorny, 1987). Marine species generally show highly unsaturated lipid composition and the presence of pro-oxidant molecules facilitate development of rancidity (Mohri et al, 1992; Richards & Hultin, 2000). Furthermore, lipid oxidation is more pronounced in fatty fish due to its high degree of unsaturated lipid content (Brannan & Erickson, 1996). Also in food, due to strong interactions between proteins and lipids the oxidative reactions can easily transfer from lipids to proteins (Viljanen, 2005). Hence the present study aims to investigate the solubility of protein in fishes with varying lipid content namely mackerel and threadfin bream during exposure to conditions favorable for lipid oxidation.

Materials and Methods

Indian mackerel (*Rastrelliger kanagurta*) and threadfin breams (*Nemipterus japonicus*) were procured from a commercial fish-landing centre in Cochin,

India. Average length and weight of *R. kanagurta* and *N. japonicus* were 20 ± 1 cm; 160 ± 5 g and 15 ± 1 cm; 100 ± 1 g respectively. Fish were immediately iced (1:1 ratio of fish: ice) and transported in an insulated container to laboratory. They were de-iced and thoroughly washed to remove blood, slime and dirt and washed with chilled potable water (0-2°C) containing 2-ppm chlorine. Fish were filleted carefully without contaminating with the entrails and the meat separated from the skin was minced in a mixer grinder (PHILIPS Super silent, HL1643) for 1–2 min. Care was taken to keep the temperature of the meat below 5°C during sample preparation. The homogenised meat sample (400 g portion) was divided into four different groups in glass dishes and was exposed to a temperature of 40 ± 0.5°C in an incubator (BIOCHEM Incubator, Universal Biochemicals, Madurai-625003). The sample of minced meat was drawn at 4h intervals up to 12 h. Samples were taken in triplicates for each analysis.

Composition of fishes

Moisture content crude fat and crude protein were estimated by (AOAC, 2000; 2005; 1990). Results were expressed as percentage.

Determination of sarcoplasmic and myofibrillar protein

Sarcoplasmic (SPP) and myofibrillar protein (MFP) were extracted at every 4h interval by maintaining a meat buffer ratio of 1:6 in a homogenizer (Polytron PT 3000, Kinematica, Switzerland) at 10000 rpm for 1 min. The mince was homogenized with 0.05 M sodium-bi-carbonate and the homogenate was centrifuged using a refrigerated centrifuge (REMI R24, India) at 5°C. Supernatant was collected and the residue was re-extracted as above and the pooled supernatant was collected as SPP. Pellet obtained was extracted twice in chilled Dyer's buffer and the pooled supernatant was collected as MFP according to the method of Sankar & Ramachandran (2001). Care was taken to keep the temperature of the meat below 5°C and the solubility of extracted protein was quantified by Biuret method of Gornall et al. (1949).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoresis of SPP and MFP was carried out; the migration of protein in the electric field as indicated by bromophenol blue was followed by using the method of Laemmeli (1970).

Determination of peroxide value, TBARS value and free fatty acids

Peroxide value (AOCS, 1999), thiobarbituric acid reacting substances (TBARS) (Tarladgis et al., 1960) and free fatty acids (Link, 1959) were determined in the control and heat exposed samples by following standard methods.

Statistical Analysis

Results are expressed as mean ± standard deviation (n = 3). One-way analysis of variance (ANOVA) was carried out between groups, using statistical package programme (SPSS 10.0 for Windows).

Results and Discussion

Biochemical composition of Fishes

Biochemical composition of Indian mackerel and threadfin bream maintained at accelerated conditions is presented in Tab. 1 & 2 respectively. Mackerel showed a fat content of 5.98% and fat content of threadfin bream was 2.85% indicating fatty nature of mackerel (Zuraini et al., 2006). With duration of exposure, moisture content decreased with a concomitant increase in fat content. Osman et al. (2001) reported that lean fish have higher moisture content, as observed in this study, threadfin bream showed higher moisture content. The crude protein content, calculated on equal moisture basis showed a loss of 21% in mackerel during the exposure of the meat at 40±0.5°C for a period of 12 h. For the same conditions, the decrease in crude protein noticed in threadfin bream was only 12%. Ash content of both mackerel and threadfin bream did not change much during the study period.

Changes in peroxide value, TBARS value and free fatty acids

Peroxide value in mackerel was more pronounced probably due to its lipid content (Fig. 1). PV was very low for fresh (0 h sample) mackerel and threadfin bream i.e., 2.8 and 0.0 meq O_2 kg^{-1} of lipid respectively indicating good quality of the raw materials. PV showed a gradual increase as time increased but a sharp increase was observed at the end of 12 h *viz*., 25.5 and 12.5 meq O_2 kg^{-1} of lipid respectively. In threadfin bream, a slower development of primary oxidation was noticed. Smith (1995)

studied initial lipid oxidation in dried salted fish and showed that lipid oxidation increased with the increase of storage temperature up to 40°C. Widjaja et al (2009) reported that a faster development of primary oxidation was noticed at ambient temperature (28± 2°C) in the first 24h of experiment when compared to chill and ice stored bagridae catfish (*Mystus nemurus*). Lipid peroxidation in marine food, even if moderate, can influence the nutritional value of the diet and consequently health (Alves Martins et al., 2007; Zhong et al., 2007). Lipid oxidation products formed during storage of fishery products are known to adversely affect soluble proteins (Sarma et al., 2000).

The levels of TBARS in fresh (0 h sample) mackerel and threadfin bream were very insignificant *viz*., 0.45 and 0.07 mg malonaldehyde kg^{-1} of tissue respectively (Fig. 2). The content of TBARS was found to be as high as 6.99 mg malonaldehyde kg^{-1} of tissue at end of 12 h showing a significant difference ($P< 0.05$) when compared to threadfin bream *viz*., 0.971 mg malonaldehyde kg^{-1} of tissue. Peroxides are unstable compounds and they break down to aldehydes, ketones and alcohols. These breakdown products which react with TBA are volatile compounds causing off-flavor in fishery products and hence content of TBARS is considered as one of the major chemical indices of oxidative rancidity (Rahman, et al., 2009). A significant increase in TBARS from an initial value of 0.45 to 6.99 and 0.07 to 0.97 mg malonaldehyde kg^{-1} of tissue was observed in mackerel and threadfin bream respectively indicating oxidative deterioration (Fig. 4). These results compare with that of bagridae catfish stored at 28 ± 2°C (Widjaja et al., 2009) and sardines (Lubis and Buckle, 1990). Grigorakis et al (2010) reported pronounced increase in TBARS in sardine oil during thermal treatment substantiating lipid oxidation.

FFAs are derived mainly from enzymatic or non-enzymatic hydrolysis of lipids, particularly the phospholipids. In fresh (0 h sample) mackerel and threadfin bream, the FFA contents were found to be 1.33 and 1.07 oleic acid % respectively (Fig. 3) which showed significant difference ($P< 0.05$) in both fishes at 8h of storage at 40 ± 0.5°C indicating extensive hydrolysis of lipids. The FFA content increased further up to 12 hours but indicated no significant difference between both fish. Widjaja et al (2009) reported a progressive increase of lipid hydrolysis with storage time and temperature. Shah et al (2009) reported that there was a significant increase in acid value of migaki-nishin to various extents during the drying period and suggested that free fatty acids might be released by partial hydrolysis of lipids during drying.

Changes in Solubility of Proteins

SPP for fresh (0 h sample) mackerel and threadfin bream were 44.01 and 36.81 mg g^{-1} tissue respectively indicating high SPP in mackerel compared to threadfin bream (Fig. 4). SPP in both fish did not show much variation during the study period. At the end of 12 h, SPP were 42.62 and 34.95 mg g^{-1} tissue, a decrease of 3% and 5% respectively. In mackerel, the SPP content was found to be more when compared to threadfin bream. *R. kanagurta* being pelagic fish, is reported to have higher content of SPP (Haard *et al*., 1994).

The MFP in both mackerel and threadfin bream showed a decreasing trend from the initial value of 127.68 and 125.59 mg g^{-1} respectively for the two fishes (Fig. 5). After 4 h of exposure, insolubilisation of MFP was around 46% and 39% respectively for mackerel and threadfin bream. At 8^{th} h the denaturation of MFP increased to 70% and 67% and at the end of 12 h mackerel and threadfin bream showed significant decrease ($P< 0.05$) *viz.,* 91% and 88% respectively. Yongsawatdigul & Park (2003) reported similar results in threadfin bream at temperatures above 40 ± 0.5°C indicating decreased solubility of actomyosin due to aggregation at higher temperatures.

Myosin rod, which is responsible for the salt solubility of myosin, is denatured very rapidly during heat treatment (Azuma & Konno, 1998). The myofibrillar proteins, particularly myosin of many fish species gets altered by their interaction with different types of lipids on lipid oxidation products (Saeed et al., 1999; Saeed & Howell 1999). This interaction causes considerable changes in some functional properties and in the texture of fish muscle (Howell, 1999).

There is good correlation between fat oxidation as indicated by the formation of hydroperoxides, secondary oxidation product like, malonaldehyde and free fatty acids and insolubilisation of MFP. This could be due to the interaction of the free fatty acids formed and/or products of lipid oxidation with the proteins as reported earlier (Srikar et al., 1989). The FFA is said to react hydrophilically or

hydrophobically with appropriate sites on protein surfaces creating a hydrophobic environment which results in lower protein extractability (Sikorski et al., 1976). Also secondary interactions have been identified as the main cause of the lower extractability and reduced functionality of MFP during storage (Tejada et al., 2003; Parthiban et al., 2005). Decrease in solubility due to aggregation of myosin molecules is reported during ice storage (Mohan et al., 2006; Devadasan & Nair, 1971; Crupkin et al., 1979; Reddy and Srikar, 1993). In the present study the percentage loss due to denaturation of protein was pronounced in mackerel compared to threadfin bream, which could be implicated to its high fat content and its susceptibility to oxidation.

Changes in SDS-PAGE Pattern of Protein during Oxidation under Accelerated Conditions

The composition of MFP of both mackerel and threadfin bream are shown by SDS-PAGE in Fig. 6 & 7. The myofibrillar protein from mackerel showed 10 bands including bands at 200 kDa (Myosin), 45 kDa (Actin) and 35 kDa (Tropomyosin). The initial result (0 h) showed the presence of 14 bands for both the fishes and compares with results of Nagai et al. (1999) and Thorarinsdottir et al. (2002). The SDS-PAGE pattern of MFP during storage under conditions of experiment revealed significant changes in mackerel in comparison with threadfin bream. The intensity of myosin component started decreasing significantly after 4 h and by the end of 12 h of exposure a complete loss of myosin band was observed. In the case of threadfin bream, myosin bands were still present up to 8 h, which could be attributed to its lower fat content and hence lower rate of fat oxidation and interaction of lipids with proteins but the bands disappeared by the end of 12 h signifying the complete insolubilisation of fish myosin. The decrease in solubility occurs due to the formation of aggregates between the different chains of myosin (Tironi et al., 2004). Alongside the loss of high molecular weight proteins, the intensity of certain low molecular weight proteins below 14.2 kd were noticed in the electrophoretic profile of *N. Japonicus* (Fig. 9), which compares well with similar findings in sea salmon during chilled storage (Tironi et al., 2007). In both fishes in the 0 h samples, certain very high molecular weight proteins or dimers are seen at the origin which are not present in subsequent stages indicating protein interaction with lipids leading to the loss of extractability in buffer. It was further stated that the high molecular weight polymers formed during oxidation were produced via disulfide linkages derived from both myosin and actin (Decker et al., 1993). However, certain studies indicate the formation of stronger non disulfide bonds during frozen storage leading to insolubilisation of protein (Careche & Li-chan 1997; Careche & Tajeda 1994).

Conclusion

Results indicate changes in solubility of MFP, in mackerel are highly significant ($p<0.05$) than in threadfin bream, indicating overall loss of protein solubility with exposure period. This could be attributed to faster rate of lipid oxidation. Thus, lipid oxidation should be controlled to minimize the loss of valuable nutritive compounds, specially proteins during the preparation of value added products.

Acknowledgements

The authors wish to express their sincere thanks to the Director, Central Institute of Fisheries Technology, Cochin, for providing facilities to conduct this work. Authors also thank the Head, Biochemistry & Nutrition Division for extending facilities and support to conduct this work. The financial assistance from Indian Council of Agricultural Research, New Delhi, India, in carrying out this study is gratefully acknowledged.

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