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Biochemical Studies on Oxidative Deterioration of Lipid Profile in Indian Mackerel (*Rastrelliger kanagurta*)

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ABSTRACT

The present work was aimed to study the oxidative deterioration of lipid profile in Indian mackerel (*Rastrelliger kanagurta*) at an accelerated storage temperature (40±0.5^0 C). Fatty acid (FA) composition and oxidative changes were analyzed at regular intervals. Initially, the total polyunsaturated fatty acid (PUFA) content was predominant followed by saturated (SFA) and monounsaturated fatty acids (MUFA). With increase in storage period, the decrease in PUFA content was significantly higher ($P < 0.05$) than SFA and MUFA. Among the essential FAs, ù-3 FAs were more predominant and with the storage period they showed a decreasing trend whereas the lipid oxidation products viz., peroxides, malonaldehyde and free fatty acids (FFA) increased significantly. The decrease ($P < 0.05$) in PUFA compounds (82.5%) has been as follows: ù-3 (91%), ù-3/ù-6 ratio (66.65%), PUFA/SFA ratio (66.3%) and Polyene index (PI) (82.24%) at the end of storage period. In addition, the peroxides and malonaldehyde contents showed a negative correlation (r^2 = 0.98 and 0.96 respectively) with PUFA and FFA showed a negative correlation (r^2 = 0.98) with phospholipids indicating the overall loss of valuable lipid fractions with the storage period. The study provides strong evidence that, oxidation of lipid affects nutritional quality of mackerel.

Key words *Lipid Profile, ù-3 fatty acids, oxidation, Indian mackerel, accelerated temperature*

Seafood is recognized as a prominent source of healthy lipids in the world. The most beneficial part of these lipids are mainly the polyunsaturated fatty acids (PUFA), in particular, Eicosapentaenoic (EPA, C20:5ù-3) and Docosahexaenoic acids (DHA, C22:6ù-3). There is strong evidence that consumption of food containing high levels of fish and these fatty acids (FAs) are favorable for human health and the PUFA is suggested as a means of improving health. It seems that PUFA composition may vary among species of fish and a little attention has been paid to this fact when selecting fish for the diet (Bayir, *et al.,* 2006). Reports show that the ù-PUFA content in mackerel especially EPA, C20:5ù-3 and DHA, C22:6 ù-3 were 0.65 and 1.10 g/100g of fillet respectively (Belitz, 1999).

Studies show that spoilage starts as soon as a fish dies because of a complex series of chemical, physical, bacteriological and histological changes that occur in the muscle tissue. Though these quality changes influenced by many factors, the most important of which is temperature. Exposure of fresh fish to temperature abuse can cause serious deterioration in fish quality; one of such problems is lipid oxidation. Model results show that lipid oxidation is extremely dependent upon temperature. For each 10 °C increase in temperature the rate of lipid oxidation doubles. A reduction in temperature from 25 to 5 °C reduces the oxidation rate by a factor of four and might increase the shelf life with the same factor. Besides, Lipid oxidation is more pronounced in fatty fish due to its high degree of unsaturation in lipids and due to high concentration of transition metals, particularly those that contain two or more valency states (Brannan and Erickson, 1996; Dragoev, *et al.,* 1998). It has a detrimental effect on the quality of fish, as it leads to the development of off-odors and off-flavors (Brannan & Erickson, 1996).

Moreover, the free radical reaction of peroxidation is often a serious problem for food manufacturers whose interests include maintenance of nutritional qualities and shelf life of lipid containing foods (Hettiarachchy *et al.,* 1996). This problem can be over come by using one of the most common preservation techniques known as freezing and frozen storage with a proper packaging method. It has been reported that, fatty fishes are highly prone to oxidative spoilage even during freezing and frozen storage, limiting the shelf life considerably (Santos-Yap, 1996). Also, there has been suggestions that lipid hydrolysis and oxidation contributes to protein denaturation, texture changes and functionality loss during processing and frozen storage of fish (Davies and Reece, 1982; Mackie, 1993; Sotelo *et al.,* 1995)

However, most of these suggestions were based on fish species restricted to temperate waters. Studies have been carried out extensively on the area highlighting lipid oxidation in fish meat during pre-processing and processing conditions. However, works specifying the studies on oxidative deterioration and lipid profiling of mackerels from Indian waters are relatively scanty. The study envisages investigating the

Table 1. Changes in biochemical parameters* of Indian mackerel (*Rastrelliger kanagurta*) stored at 40 ± 0.5 °C

Parameters	Storage period (h)			
	0	4	8	12
Moisture (%)	76.63±0.1[a]	74.46±0.06[b]	73.17±0.17[c]	71.31±0.1[d]
Crude fat (%)	5.98±0.1[c]	6.41±0.1[c]	6.78±0.1[b]	7.25±0.25[a]
Cold fat (g lipid/100g muscle)	6.45±0.2[c]	7.05±0.05[c]	7.25±0.25[b]	7.8±0.34[a]
PV (meq o_2/kg of Fat)	2.8±0.1[d]	12.5±0.9[c]	19.8±0.8[b]	25.5±0.4[a]
TBA (mg malonaldehyde/ Kg of flesh)	0.45±0.05[d]	1.44±0.22[c]	5.01±0.01[b]	6.99±0.1[a]
FFA (% as oleic acid)	1.33±0.2[d]	2.84±0.03[c]	6.26±0.06[b]	7.94±0.1[a]
Phospholipids (g/ml)	31.12±0.1[a]	27.25±0.25[b]	18.62±0.21[c]	12.12±0.1[d]

* mean ± std deviation, n = 3

[a, b, c, d] Mean values in a row with same superscript are not significantly different ($p<0.05$)

oxidative deterioration of lipids under conditions maintained to accelerate the rate of lipid oxidation focusing Indian mackerel (*Rastrelliger kanagurta*, one of the commercially important fish species belonging to family Scombridae) and its influence on the healthy lipid profile. Based on the present study more appropriate preservation methods have to be evolved to reduce the loss of the valuable lipid fractions.

MATERIALS AND METHODS

Indian mackerel (*Rastrelliger kanagurta*) were procured from the commercial fish-landing centre, Cochin, India. The average length and weight of the fish were 20 ± 1 cm and 160 ± 5 g respectively. The fishes were immediately iced (1:1 ratio of fish: ice) and transported in an insulated container to laboratory. The fishes were de-iced and thoroughly washed to remove blood, slime and dirt washed with chilled potable water (0-2 °C) containing 2-ppm chlorine. Then the fish were filleted carefully without contaminating with the entrails and the meat was separated from the skin. The meat was minced in a mixer grinder at a temperature below 5 °C for 1–2 min. The minced meat sample was divided into four different groups and was exposed to a temperature of 40±0.5 °C in an incubator. The sample of minced meat was drawn at every 4 h of interval for up to 12 h of storage at ambient temperature. Samples were taken in triplicates for each analysis. All analyses were carried out at the Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Cochin, India.

Moisture and lipid contents:

Moisture content was determined by weight difference between the homogenized muscle before and after oven-drying for 16 h at 100±1 °C. Results were expressed as percentage (AOAC, 2000) and crude fat was also estimated by using AOAC (2000) method. The total lipid content was extracted by a method of Folch *et al.,* 1957, briefly, minced meat was homogenized in a 2:1 (v/v) mixture of chloroform-methanol and filtered. Then, 20% water was added to this mixture and the layers were allowed to separate. The aqueous layer was discarded and the solvent was completely evaporated to obtain the lipid content. Results were calculated as g lipids/ 100g wet muscle.

Lipid Deterioration Methods:

Determination of peroxide value (PV), thiobarbituric (TBA) and free fatty acid (FFA)

Peroxide value (PV) of each sample was analysed at an interval every 4 h by iodometric method according to the guidlines of AOCS (1999). The thiobarbituric acid (TBA) value was determined spectrophotometrically to evaluate oxidation stability and the results were expressed as TBA value, mg malonaldehyde (MA) per kilogram of fish (Tarladgis *et al.,* 1960) and the percentage of free fatty acid (FFA) value was determined by the method of Link, 1959.

Estimation of Phospholipids:

Phospholipids content of muscle was estimated by the method of Fiske and Subbarow, 1925 as inorganic phosphorus liberated after digesting with perchloric acid as outlined by Bartlette, 1959.

Fatty acid composition:

Aliquots of the ether were methylated using BF_3-methanol and the resulting FA methyl esters (FAME) (Bakes and Nichols, 1995) were subjected to gas chromatography equipped with Perkin Elmer Elite 225 50% cyanopropyl phenyl – 50% methyl capillary column (30 m X 0.25 mm inner diameter X 0.25ìm film thickness) FID and a split/splitless injector. Nitrogen was the carrier gas. Samples were injected in splitless mode at an oven temperature of 110 °C. After 4 min, the oven temperature was raised to 240 °C at 2.7 °C/min. Peaks were quantified with Chromcard software by comparing retention time data with those obtained for authentic standards. The Polyene Index (PI) was calculated as the following fatty acid ratio: (C20:5 + C22:6)/C16:0 or EPA+DHA/C16 ratio (Lubis and Buckle, 1990).

Table 2. Changes in fatty acid composition* of Indian mackerel (*Rastrelliger kanagurta*) stored at 40 ± 0.5 °C

Fatty acid (g/100g of tissue)	Storage period (h)			
	0	4	8	12
C14	0.108±0.07[a]	0.104±.0.002[a]	0.051±0.005[b]	0.156±0.06[b]
C14:1	0.102±0.03[a]	0.091±0.010[b]	0.001±0.000[c]	0.003±0.01[c]
C15	0.017±0.02[b]	0.016±0.002[b]	0.019±0.001[b]	0.028±0.03[a]
C15:1	0.089±0.01[b]	0.098±0.001[a]	0.090±0.001[b]	0.001±0.00[c]
C16	0.550±0.03[a]	0.668±0.001[a]	0.940±0.008[b]	0.963±0.02[c]
C16:1	0.121±0.01[a]	0.075±0.003[b]	0.042±0.020[c]	0.028±0.00[c]
C17	0.037±0.01[b]	0.034±0.003[b]	0.036±0.002[b]	0.056±0.00[a]
C17:1	0.005±0.02[a]	0.082±0.001[b]	0.003±0.002[b]	0.004±0.03[b]
C18	0.357±0.02[a]	0.354±0.003[a]	0.257±0.007[b]	0.406±0.05[c]
C20	0.002±0.01[d]	0.008±0.001[c]	0.094±0.003[a]	0.021±0.01[b]
C20:1	0.007±0.01[a]	0.094±0.02[b]	0.012±0.01[c]	0.014±0.01[c]
C22	0.113±0.03[a]	0.092±0.05[a]	0.002±0.03[b]	0.005±0.01[b]
C18:1n-9c	0.147±0.07[a]	0.144±0.09[a]	0.098±0.01[b]	0.013±0.01[c]
C18:2n-6t	0.002±0.00[b]	0.001±0.00[b]	0.085±0.01[a]	0.002±0.00[b]
C18:2n6c	0.361±0.01[a]	0.107±0.01[b]	0.020±0.00[c]	0.005±0.00[c]
C18:3n-6	0.010±0.00[b]	0.089±0.01[a]	0.093±0.02[a]	0.005±0.00[b]
C18:3n-3	0.069±0.01[a]	0.023±0.10[a]	0.019±0.00[a]	0.017±0.00[a]
C20:2	0.006±0.00[b]	0.088±0.01[a]	0.095±0.05[a]	0.003±0.00[b]
C20:3n-6	0.007±0.00[c]	0.002±0.00[c]	0.085±0.02[a]	0.090±0.00[b]
C20:5n-3	0.184±0.05[a]	0.149±0.06[a]	0.103±0.01[a]	0.096±0.40[a]
C22:2	0.066±0.01[b]	0.036±0.03[c]	0.056±0.01[b]	0.084±0.01[a]
C22:6n-3	1.657±0.11[a]	1.098±0.07[b]	0.622±0.11[c]	0.058±0.39[d]
ΣSFA	1.184±0.02[a]	1.287±0.03[a]	1.599±0.01[b]	1.635±0.01[c]
ΣMUFA	0.471±0.01[b]	0.574±0.01[a]	0.246±0.00[c]	0.063±0.01[d]
ΣPUFA	2.062±0.01[a]	1.021±0.01[c]	1.178±0.01[b]	0.360±0.01[d]
PUFA/SFA	0.878±0.01[a]	0.793±0.01[b]	0.736±0.01[c]	0.220±0.01[d]
Σ ω-3	1.910±0.01[a]	1.346±0.01[b]	0.744±0.01[c]	0.171±0.01[d]
Σ ω-6	0.380±0.01[a]	0.230±0.01[c]	0.283±0.01[b]	0.102±0.01[d]
ω-3/ω-6	5.026±0.01[a]	5.852±0.01[b]	2.628±0.01[c]	1.676±0.01[d]
EPA+DHA/C16 (or) PI**	1.973 ± 0.01[a]	1.326±0.01[b]	1.085±0.01[c]	0.280±0.01[d]

* mean ± std deviation, n = 3

[a, b, c, d] Mean values in a row with same superscript are not significantly different ($p<0.05$)

** The polyene index

Statistical analysis :

The Results are expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was carried out, and the statistical comparisons among the groups were performed with Duncan's multiple range test using statistical package program (SPSS 10.0 for Windows). Correlation coefficient (r^2) was established between different parameters. The significant differences were expresses as $p< 0.05$.

RESULTS AND DISCUSSION

Moisture and lipid content:

The moisture content in the fish samples ranged from 76.63% to 71.31% as shown in Table 1. It started declining significantly ($p<0.05$) from 4th hour onwards. The declining trend in moisture may be attributed to dehydration caused by the temperature of storage (40±0.5 °C). The lipid content was observed to be in the range of 5.90% to 7.25% (Table 1). The significant increase ($p<0.05$) in lipid content was observed from 8th hour onwards, which could be due to loss of moisture as discussed earlier.

Lipid oxidation:

Shelf life of fatty fish species is limited due to the oxidation of lipid. The primary oxidation products such as hydroperoxides were measured and presented as PV. During storage period hydroperoxide production was observed in samples, the PV was in insignificant amount during 0 h. A sudden development of primary oxidation was observed at 4 h; the changes observed in this study is comparable to results reported by Widjaja *et al.,* 2009. The increase in peroxide value (PV) from an initial value of 2.8±0.1 to a final value of 25.5±0.4 meq of O_2/Kg of lipid in mackerel was significant ($p<0.05$) during storage, and indicated oxidative deterioration (Table 1). Increase of PV in frozen fish in comparison with fresh fish showed development of rancidity during frozen storage (Ben-Gigirey *et al.,*1999). Development of off-flavor is one of the

major effects of lipid oxidation (Fagan *et al.,* 2003) and at the later stage of lipid peroxidation, changes in color and nutritional value were observed (Dragoev *et al.,*1998). Moreover, oxidized products of lipids formed during storage of fishery products are known to influence the soluble proteins (Sarcoplasmic and myofibrillar proteins) (Sarma, *et al.,* 2000).

The secondary lipid oxidation was studied by the thiobarbituric acid test (TBA). The TBA number is a measure of malonaldehyde (MA), a byproduct of lipid oxidation. Huss, 1995 reported that, in later stages of oxidation, secondary oxidation products will be formed and indicates the history of autooxidaton. The significant difference ($p<0.05$) in TBA in the mackerel samples from an initial value of 0.45±0.05 to a final value of 6.99±0.1 mg of malonaldehyde/kg of fish flesh revealed the severity of oxidative deterioration during the 12 h of storage (Table 1). The increase in malonaldehyde content could be attributed to the oxidation of polyunsaturated fatty acids with at least three double bonds Gordon, 2001 and the data also revealed that higher the duration of storage at higher temperature, higher the final TBA values. This result was consistent with that obtained by Lubis and Buckle, 1990.

Lipid hydrolysis:

The free fatty acid formation due to lipid (TAG) hydrolysis has provided a suitable means for assessment of fish quality deterioration during storage and can be used as a quality index for fish and other food products (De Koning and Mol, 1991; Hui and Tung, 1997). During the storage of mackerel samples a development of progressive lipid hydrolysis was observed. FFA (as oleic acid) increased significantly ($p<0.05$) from 1.33±0.2 to 7.94±0.1 indicating extensive hydrolysis of lipids, especially phospholipids as shown in Table 1. Hydrolysis developed gradually in the samples exposed to 40±0.5 °C from 0 to 12 h, a very good correlation value with time was obtained associated to the effects of FFA (r^2 = 0.9859) on phospholipids (Fig. 1). The values obtained in this study are similar to the results reported by Auburg *et al.,* 2004. The FFA formed in frozen fish during cold storage as a result of enzymatic breakdown of their lipids. Hardy *et al.,* 1979, suggests that at -10 °C all the FFA in cod

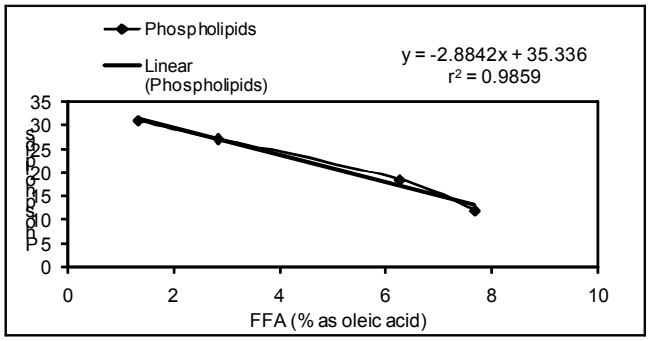

Fig. 1. Correlation of FFA with phospholipids during storage at 40±0.5°C

were formed from the phospholipids while the neutral lipids remained unchanged. While the formation of FFA itself does not lead to nutritional losses, its assessment is deemed important when the effect of FFA on lipid matter is proposed, and explained on the basis of a catalytic effect of the carboxyl group on the hydro peroxides. Moreover, being relatively small sized molecules, FFA have shown to undergo a faster oxidation rate than bigger lipid classes viz., triglycerides and phospholipids, thus significantly affecting the dietary quality of aquatic food products (Losada *et al.,* 2007). Moreover, as quality specifications for crude fish oil, Bimbo, 1998 suggested a maximum acceptable value of 5%. This study showed that the free fatty acid levels reached 5% limits in between 4 to 8 h of storage.

Fatty acid composition:

The fatty acid composition of mackerel is summarized in Table 2. Except for hours 8 and 12 no significant differences were observed among the saturated fatty acid (SFA) content. Palmitic acid (C16:0) was the major fatty acid among the SFAs, it started showing an increase from 8 h onwards and remained majority during the whole period when compared to other SFAs. This result holds good in comparison with *Channa* spp (Zuraini *et al.,* 2006). It was observed that the amount of stearic acid (C18:0) was higher in mackerel when compared to behenic (C22:0), myristic (C14:0), and margaric acids (C17:0).

Oleic acid (C18:1 ù) was observed to be the major monounsaturated fatty acid (MUFA) content in mackerel followed by palmitoleic acid (C16:1). The results obtained in this study were similar to that of Horse mackerel (*Megalapsis cordyla*) (Gopakumar. 1997) and sea bass (*Dicentrarchus labrax*) (Saglik *et al.,* 2003). Both fatty acids started showing a decreasing trend ($p<0.05$) when they reached 8th h. Other MUFAs like Myristoleic (C14:1), cis-10-heptadecenoic (C17:1) and cis-11-eicosenoic acids were present in very insignificant amount. This study reveals that a fair correlation values were obtained with time for peroxides (r^2 = 0.6765), MA (r^2 = 0.8984) and FFA (r^2 = 0.8464) with total MUFAs (Fig. 2).

In mackerel unsaturated fatty acids were significantly higher than saturated fatty acids (SFA<PUFA+MUFA), however, unsaturated fatty acids were decreased in contrast with saturated fatty acids during storage. It was observed that PUFA decreased significantly ($P< 0.05$) at the end of 12 h of storage. Furthermore, this study reveals that a high correlation values were obtained with time for peroxides (r^2 = 0.9819), MA (r^2 = 0.9599) and FFA (r^2 = 0.9669) with total PUFAs (Fig. 3). Thus the correlation analysis interprets very well that the products of lipid oxidation were highly reactive with PUFAs and degraded them gradually during the period of storage. Cho and others (1989) reported that, EPA and DHA esters were rapidly oxidized at 5°C and polymers were found to be the major secondary products of these polyenoic esters. It was observed that DHA was the main component affected,

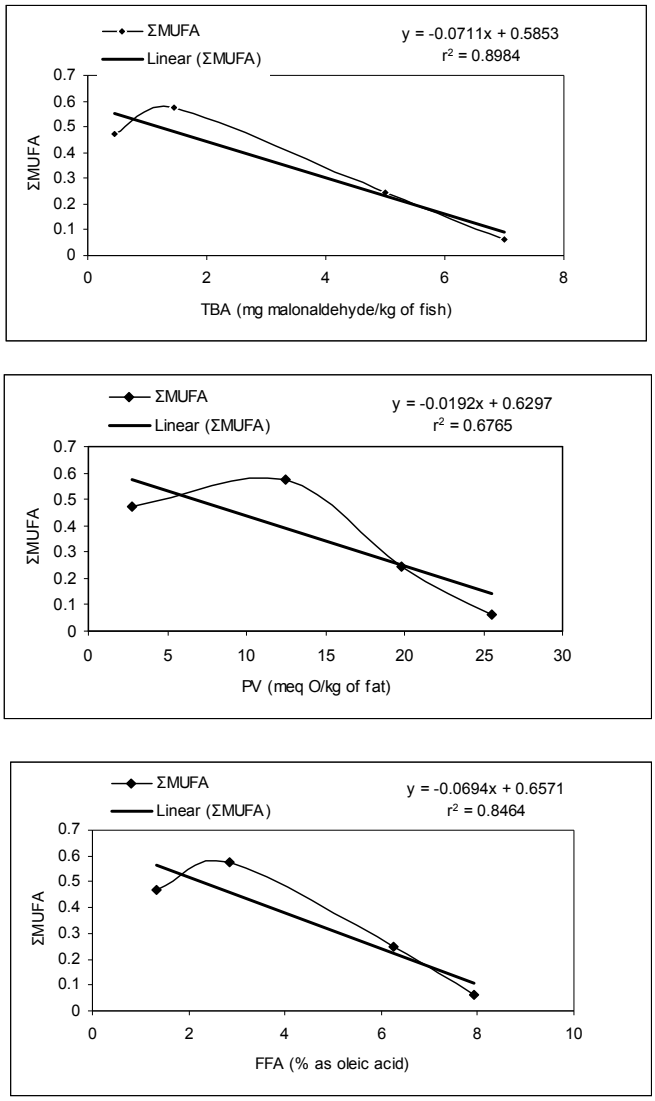

Fig. 2. Correlation of total mono unsaturated fatty acids (Ó MUFA) with TBA (a), PV (b) and FFA (c) during storage at 40±0.5 °C

while ù-6 PUFAs showed the presence of slightly higher level at the end of storage. This is in corroboration with findings linked to minced sea salmon (*Pseudopercis semifasciata*) during chill storage (Tironi *et al.,* 2007). The amount of PUFA in mackerel was significantly higher during 0 h in comparison to the later stages of storage. It is noteworthy that the highest quantity of PUFA was associated with ù-3 compounds. Eicosapentaenoic (EPA, C20:5ù-3) and docosahexaenoic acids (DHA, C22:6ù-3) were majority in total ù-3 polyunsaturated fatty acids in mackerel.

Statistical results showed that during storage, changes of PUFA, decreasing by 82.5% especially, ù-3 (91%) in mackerel. The difference in the fatty acid composition of the fish lipids had a decisive function in the formation of hydroperoxides. The oxidative changes in fish lipids may be caused by the occurrence of free radicals (Dragoev *et al.,* 1998). Free radicals could be like trace metals, particularly those that contain 2 or more valency states, are effective pro-oxidants. Trace metals are naturally occurring components of all food tissues and they are found in free and bound forms. Some metals carry out this reaction include iron (Fe^{2+}) and copper (Cu^{2+}). If present, even at low levels (0.1ppm), they can decrease the induction period and increase the rate of oxidation.

The ù-3:ù-6 ratio has been suggested to be a useful indicator for comparing relative nutritional values of fish oils. It has been suggested that the ratio of 1:1 – 1:5 would constitute a healthy human diet (Zuraini *et al.,* 2006). This study revealed that marine fish are richer in ù-3 than ù-6 PUFAs. There were significant difference ($p<0.05$) in this ratio. The ù-3: ù-6 ratio in 0 h sample was 5.026±0.01 and at the end of storage it was 1.676 ± 0.01, which were in the recommended ratio, but a decrease of 66.65% was observed, showing that nutritional value of this fish sample has not been lost completely during accelerated temperature storage.

The PUFA/SFA (P/S) ratio reveals that the marine fish are a good source of PUFA compared to saturated fatty acids. In the present study, the ratio obtained was more than one initially, indicating that the fish used in the study was a good source of PUFA. Towards the end of storage period (12 h), the ratio decreased to 0.22, signifying the oxidation of PUFA. Decrease of PUFA in contrast with SFA leads to significant decrease of this ratio (74.93%) in samples.

PI (C20:5+C22:6/C16:0) might provide a meaningful tool to measure oxidative rancidity in fishery product because it includes only two major polyenoic fatty acids that the level of which, can be measured with reasonable accuracy as they are the larger fatty acids among the large gas chromatography peaks. It is very much interesting to note that, a negative relationship was observed between EPA+DHA/C16 ratio and storage time. At 12 h the ratio was around 82.24%, which shows that, oxidation mechanisms were active during storage period. In the case of the *Sardinops sardines*, Perez-Matoes *et al.,* 2004 found that the ratio of total unsaturated to total saturated fatty acids decline during 90 days storage at -22.3 °C.

Lipid oxidation followed by lipid hydrolysis in Indian mackerel, with the resulting primary and secondary products, increased significantly beyond 8 h of storage. However, PI decreased sharply at the end of storage temperature studied. Therefore lipid oxidation and lipid hydrolysis studies proved to be a useful tool to assess the quality and safety of *Rastrelliger Kanagurta* during storage, specially because mackerel is a fatty fish and storage conditions affects largely the quality and safety of its products. Thus, the lipid oxidation should be controlled to minimize the loss of valuable nutritive compounds, specially the essential fatty acids.

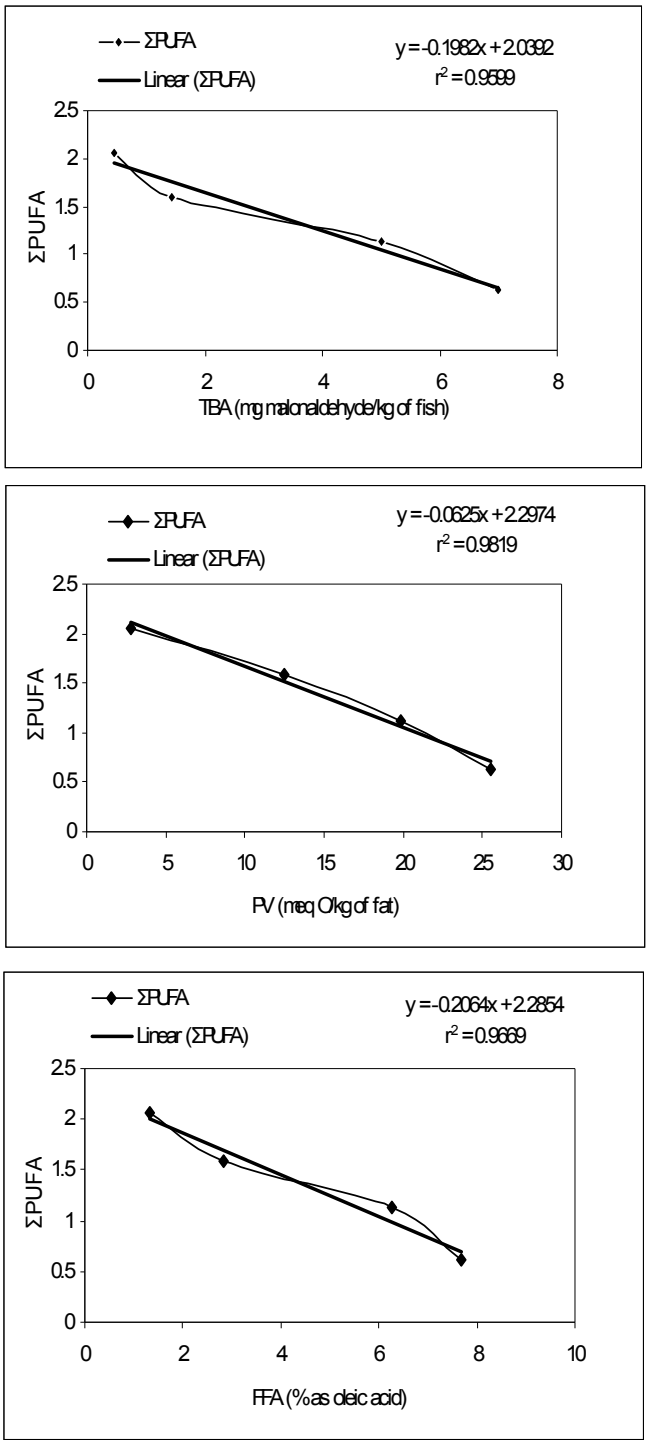

Fig. 3. Correlation of total poly unsaturated fatty acids (Ó PUFA) with TBA (a), PV (b) and FFA (c) during storage at 40±0.5 °C

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