Indian J.Sci.Res. 19(2): 56-61, 2018

ISSN: 0976-2876 (Print)
ISSN: 2250-0138(Online)

INCIDENCE OF FORMALDEHYDE RESIDUES IN FRESH SEER FISH (*Scomberomorus guttatus*) COLLECTED FROM ERNAKULAM FISH MARKET

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ABSTRACT

Seafood, an excellent source of high quality protein, essential amnio acids, fatty acids, vitamins and minerals is also considered as the highly perishable food commodity. Use of harmful chemical additives to extend the shelf- life of perishable food items including fish has been reported from India. Formaldehyde, the simplest of the aldehydes used in chemical industry is top in the list of illegal harmful food additives. In this study an attempt is made to detect the level and occurrence of formaldehyde in fresh seer fish (*Scomberomorus guttatus*) samples collected from a local fish market in Ernakulam district, Kerala,south west coast of India.Total volatile Base Nitrogen (TVB-N), Trimethyl amine (TMA-N), Thiobarbituric acid value (TBA), Peroxide vale (PV), Free fatty acid value (FFA) and Total Plate Count (TPC) analysis were done in addition to the formaldehyde estimation of collected fish samples. The results of the study revealed that the seer fish quality was not spoiled and all the biochemical & microbial results were within the limits. However, the formaldehyde content of the samples in the study ranged from 0.704 mg % to 5.83 mg %. TMA-N values obtained from the same fish samples ranged from 0.46 mg % to 3.73 mg % only, hence the chances of natural formaldehyde formation in the sample was insignificant. Continuous monitoring and quality evaluation of seafood meant for domestic consumption is essential to curtail the illegal practices and also to safeguard the health of growing fish eaters in the country.

KEYWORD: Formaldehyde, seer fish, spoilage, shelf life, chemical additives

Fish is considered as the prime nutritionally balanced diet for the growing populations around the world. Seafood includes finfish, shell fish and other edible aquatic organisms from the wild and cultured fisheries. Per capita fish consumption in the world has increased from 9.9 kg in 1960 to 19.7 kg in 2013. (FAO, 2016). About 75% of India's total fish production was consumed or marketed domestically in different forms (Zacharia and Najumudheen., 2012). There are many studies reported from India on the sensory, chemical and microbiological assessment of fresh fish landed in markets or at harbours of various parts of India (Lakshmanan et al., 1996, Jha et al., 2010., Biji et al., 2016). At ambient temperature marine fish species are highly susceptible to spoilage. One of the most effective ways used for retarding spoilage is preservation in ice (Ozogul & Gokbulut, 2006; Raatikainen *et al.,* 2015). Fish traders while transporting fishes to domestic marketing chain sometimes add or spray formalin to prevent spoilage and thereby increases the shelf life (Yeasmin *et al.,* 2010).

Formaldehyde is the simplest member of aldehyde family. It is very reactive chemical. Formalin is used as a sterilizer, as an embalm fluid and preservative in medical laboratories. Formalin is well known for using in the preservation of tissues. As it is a carcinogenic substance this chemical is endangering the public health (Kibria, 2007). Natural food like fish flesh naturally contains formaldehyde which is formed by the enzymatic breakdown of Trimethyl amine oxide. This process of breakdown not only takes place by enzymatic breakdown but also by bacteria (Sikorski *et al.,* 1982). According to the United States Environmental Protection Agency (EPA), maximum daily dose reference (RfD) for formaldehyde is 0.2 µg/g body weight per day (Wang *et al.,* 2007; Noordiana *et al.,* 2011). Spotted seer fish (*Scomberomorus guttatus*) of the genera Scomberomorus and Acanthocybium (family Scombridae), commonly called as seer fishes are esteemed table fishes in all parts of the world. In India, they are considered as one of the high value resources due to their quality meat content, high economic return and export market. In this study, an attempt is made to detect the level of formaldehyde content of fresh seer fish collected from Ernakulam fish market, located in south west coast of India. The study also analysed the quality parameters and the status of spoilage of fresh seer fish collected for the study.

MATERIALS AND METHODS

Fish Sample Collection

Spotted seer fish (*Scomberomorus guttatus*) was selected for the study. The fish samples were collected from a local fish market located in Ernakulam, south west coat of India in a time interval of one week. A total of six samples were used in this time interval and were labelled as S1, S2, S3, S4, S5 and S6. Samples were brought to the laboratory under iced condition and kept under refrigerated storage till further analysis.

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Sample Preparation and Determination of Formaldehyde

The seer fish sample collected was prepared for spectrophotometer method followed by IS 2237: 1997. In brief, the fish muscle tissue was minced using a tissue homogenizer first and 10 g of fish mince then macerated using 10 percentage trichloro acetic acid for 2 minutes using a mortar and pestle. The mix then filtered through Whatman No. 42 filter paper and made up the volume to 100 ml in a volumetric flask.5 ml trichloroacetic acid extract of the tissue was taken in a 50 ml beaker. Added 10 ml of distilled water (IS 1070) and made the solution alkaline with a few drops of 30 percentage NaOH and adjusted the pH to 6.0 with 5 percentage acetic acid. Finally the solution made up to 25 ml with water and from this 5 ml mixed with 5 ml of acetyl acetone acetone – ammonium acetate reagent. Kept the mixture still for 50 minutes at 37^0 C and read the color at a wave length of 410 nm.

Calibration Curve

The standard curve was prepared (Figure 1) by plotting absorbance of known formaldehyde concentration (*viz.* 0.5, 1.0, 1.5, 2.0 and 2.5 ppm) from a stock solution of formaldehyde followed by (IS 2237: 1997).

Biochemical and Microbial Analysis

The biochemical analysis of the fresh fish samples were conducted to assess the status of freshness. Moisture content was estimated by following AOAC, 2000. The volatile base compounds like Trimethyl amine Nitrogen and total volatile base nitrogen weredetermined by Conway micro diffusion method (Conway 1962). Lipid oxidation hydrolysis products like Thiobarbituric acid value was determined as described by Tarladgis *et al*., 1960. Peroxide value followed by AOCS, 1989 and free fatty acid value followed by AOCS, 1982. The microbial analysis of the sample was also done. The identification of total bacterial count was done by the pour plate method recommended by Bacteriological Analytical Manual, FDA, 1998.

Statistical Analysis

All data were analysed by one-way ANOVA using SPSS software (release 18 for windows). Duncan's multiple comparisons test was used to determine the differences between the sample means. Results were considered statistically significant at the level of $P<0.05$.

RESULTS AND DISCUSSION

Determination of Formaldehyde

The results of formaldehyde content of different fish samples during the study were presented in Table 1 and graphically represented in figure 2. The results revealed that highest amount of formaldehyde were reported in sample 2 (5.83 mg %) and the lowest content of formaldehyde in sample 1 with 0.70 mg % (Figure 2).

Biochemical and Microbial Analysis

The quality indices of all the samples were analyzed. The moisture content, volatile base compounds, lipid oxidation- hydrolysis products, and total bacterial count were identified in biochemical and microbial analysis. The mean moisture values and their statistical comparison of the samples are presented in Table 2 and graphical representation in Figure 3. Moisture content of the different samples was ranged from 77.13 % to 80.42 %. The TVB-N values increased according to time of storage. The TMA-N value for the sample ranged from 0.46 mg/100 g to 3.73 mg/ 100 g given in the Table 3. It is graphically represented in Figure 4. Value for TMA-N was below the suggested limit which was 12 mg/ 100 g. In this study the FFA value were observed between 0.0037 mg % oleic acid and 0.0075 mg % oleic acid for the samples given in Table 7 and graphically represented in Figure 8. In the present study, the peroxide value (PV) was employed for determining the formation of primary oxidation products of fish species. The values ranges from 0.073 milli equivalence of O_2 Kg^{-1} fat to 0.183 milli equivalence of O_2 Kg^{-1} fat represented in Table 6 and graphical presentation in Figure 7. TBA is a widely used as an indicator for the assessment of degree of secondary lipid oxidation. TBA values of the samples ranged from 0.436 mg Malonaldehyde Kg^{-1} of fish sample to 0.621mg Malonaldehyde Kg^{-1} of fish sample. The bacterial load of the samples was ranged from 1.48 log cfu g^{-1} to 2.49 log cfu g^{-1}.

The natural mechanism of formaldehyde formation in dead fish is due to the breakdown of TMAO to TMA and formaldehyde. Uddin *et al.*, 2015 described the reason for natural increase of formaldehyde concentration in fresh fish with respect to time. The acceptable daily intake of formaldehyde is 0.2 mg/kg body weight per day (Wang *et al.,* 2007; Noordiana *et al.,* 2011). Mean formaldehyde content in different fish samples collected from the Ernakulam market, in south west coast of India was estimated from the absorbance and molar concentrations of standard curve. Hoque *et al*., 2016

conducted a study at Moghbazar market and was reported that traded fish in the market is about 20% of total fish.

To prevent spoilage and extend shelf life of perishable commodities, formalin (FA) is reported to be frequently added as preservative either by dipping or spraying to the fresh fishes by the fish traders while transporting to domestic marketing chain (Yeasmin *et al.,* 2010). Paul *et al*. (2014) conducted a study in Jessore district reported that a total of 21 formalin treated fishes which accounts 4.2% of the total examined fishes (500 samples). Jaman *et al.,* 2011 studied the presence of formaldehyde in market samples of rohu, tilapia and Kachki from three markets of Mymensingh Sadar with the different ranges of 1.4 µg/g to 7.35 µg/g. The result obtained in the present study is in agreement with Noordiana *et al*., 2011. The study wasto determine the quality characteristics and formaldehyde content of fish and seafood from wet markets. Formaldehyde content was in the range of 0.38 to 15.75 µg g-1 (Noordiana *et al*., 2011).

The quality classification of fish and fish products proposed by Lang (1983) based on the TVB-N value. According to him the fish will be ‘‘high quality” if the TVB-N value is up to 25 mg/ 100 g, ‘‘good quality” up to 30 mg/100 g, ‘‘limit of acceptability” up to 35 mg/100 g, and ‘‘spoilt” above 35 mg/100 g.In the present study the TVB-N value of the samples ranged from 0.93 mg % to 6.06 mg % . As a result of enzymatic hydrolysis of esterified lipidsfree fatty acids (FFA) are formed. FFA may have been involved in reactions with myofibrillar proteins and promote protein aggregation (Pacheco-Aguilar *et al*., 2000).

Table 1: Mean value of Formaldehyde obtained from the sample.

FORMALDEHYDE			
SAMPLE	MEAN	±	SD
SAMPLE 1	0.70	±	0.03^{a}
SAMPLE 2	5.83	±	1.37^{b}
SAMPLE 3	1.17	±	0.00^{a}
SAMPLE 4	1.10	±	0.00^{a}
SAMPLE 5	5.33	±	0.16^{b}
SAMPLE 6	5.04	±	0.00^{b}

Formaldehyde values in the column showing different superscript have significant difference between the samples at 5% level.

Table 2: Mean value of Moisture content obtained from the sample.

MOISTUR CONTENT			
SAMPLE	MEAN	±	SD
SAMPLE 1	78.88	±	0.76^{b}
SAMPLE 2	80.00	±	0.10^{b}
SAMPLE 3	77.13	±	1.11^{a}
SAMPLE 4	80.42	±	0.60^{c}
SAMPLE 5	79.19	±	0.09^{b}
SAMPLE 6	77.52	±	0.37^{a}

Moisture values in the column showing different superscript have significant difference between the samples at 5% level.

Table 3: Mean value of TMA- N obtained from the sample.

TMAN			
SAMPLE	MEAN	±	SD
SAMPLE 1	3.73	±	0.80^{b}
SAMPLE 2	0.93	±	0.80^{a}
SAMPLE 3	0.46	±	0.80^{a}
SAMPLE 4	2.33	±	1.61^{ab}
SAMPLE 5	1.86	±	0.80^{a}
SAMPLE 6	0.93	±	0.80^{a}

TMA-N values in the column showing different superscript have significant difference between the samples at 5% level.

Table 4: Mean value of TVBN obtained from the sample.

TVBN			
SAMPLE	MEAN	±	SD
SAMPLE 1	3.26	±	0.80^{c}
SAMPLE 2	0.93	±	0.80^{a}
SAMPLE 3	4.66	±	0.80^{cd}
SAMPLE 4	6.06	±	0.80^{d}
SAMPLE 5	2.33	±	0.80^{ab}
SAMPLE 6	5.13	±	1.61^{d}

TBA values in the column showing different superscript have significant difference between the samples at 5% level.

Table 5: Mean value of TBA obtained from the sample

TBA			
SAMPLE	MEAN	±	SD
SAMPLE 1	0.48	±	0.004^{c}
SAMPLE 2	0.62	±	0.009^{e}
SAMPLE 3	0.44	±	0.004^{b}
SAMPLE 4	0.44	±	0.004^{b}
SAMPLE 5	0.43	±	0.000^{a}
SAMPLE 6	0.5	±	1.61^{d}

TBA values in the column showing different superscript have significant difference between the samples at 5% level

Table 6: Mean value of Peroxide value obtained from the sample

PEROXIDE VALUE			
SAMPLE	MEAN	±	SD
SAMPLE 1	0.18	±	0.005^{e}
SAMPLE 2	0.12	±	0.005^{c}
SAMPLE 3	0.07	±	0.005^{a}
SAMPLE 4	0.1	±	0.011^{b}
SAMPLE 5	0.15	±	0.017^{d}
SAMPLE 6	0.14	±	0.005^{d}

Peroxide values in the column showing different superscript have significant difference between the samples at 5% level.

Table 7: Mean value of Free fatty acid value obtained from the sample

FREE FATTY ACID VALUE			
SAMPLE	MEAN	±	SD
SAMPLE 1	0.003	±	0.001^{a}
SAMPLE 2	0.005	±	0.000^{a}
SAMPLE 3	0.004	±	0.001^{a}
SAMPLE 4	0.006	±	0.001^{a}
SAMPLE 5	0.004	±	0.003^{a}
SAMPLE 6	0.007	±	0.003^{a}

FFA values in the column showing different superscript have significant difference between the samples at 5% level.

Table 8: Mean value of TPC obtained from the sample

TPC			
SAMPLE	MEAN	±	SD
SAMPLE 1	2.18	±	0.09^{c}
SAMPLE 2	1.79	±	0.04^{b}
SAMPLE 3	2.49	±	0.10^{d}
SAMPLE 4	1.66	±	0.24^{ab}
SAMPLE 5	1.83	±	0.10^{b}
SAMPLE 6	1.48	±	0.16^{a}

TPC values in the column showing different superscript have significant difference between the samples at 5% level.

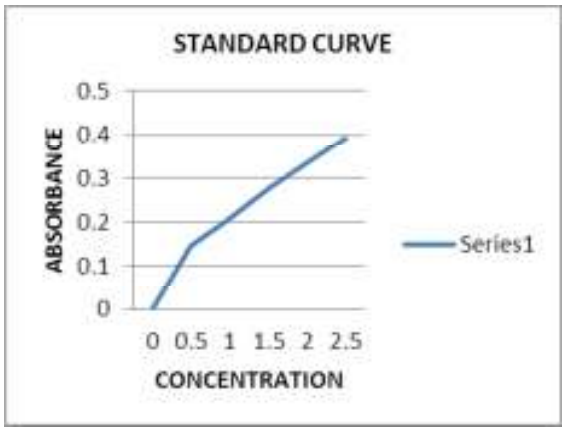

Figure 1: Standard curve of FA concentration

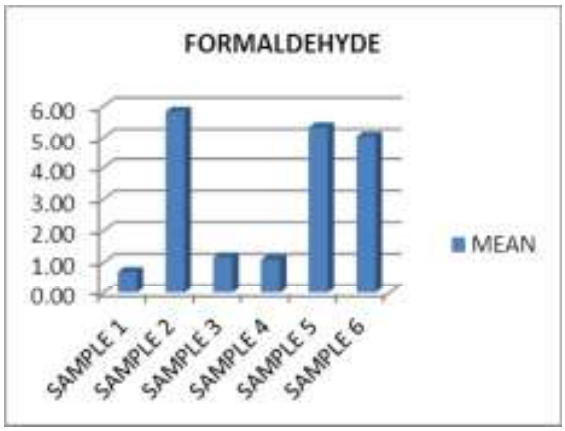

Figure 2: Graphical representation of Formaldehyde

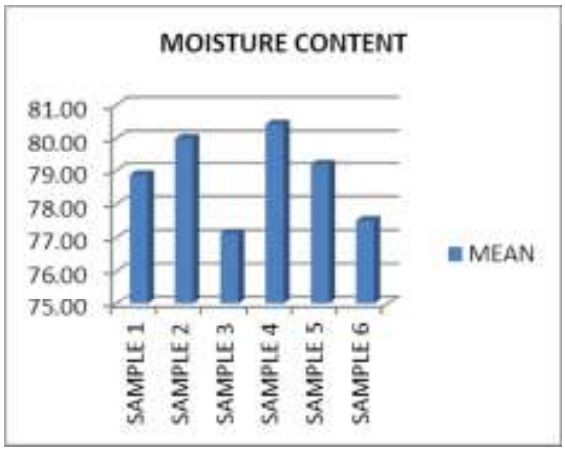

Figure 3: Graphical representation of Moisture content

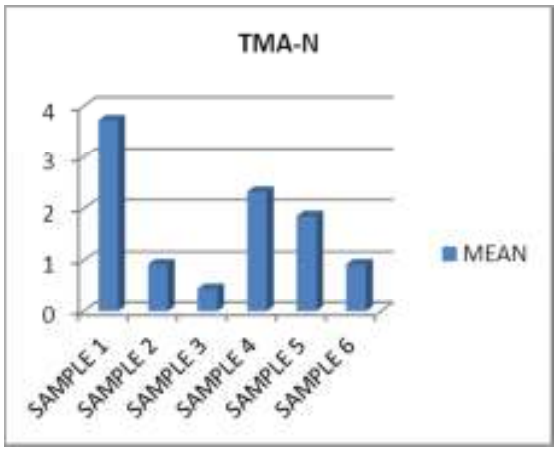

Figure 4: Graphical representation of TMA-N

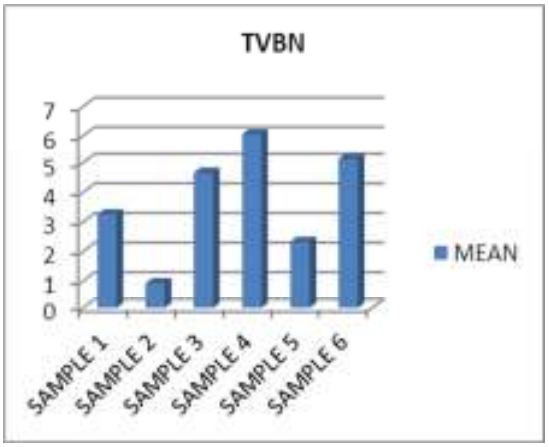

Figure 5: Graphical representation of TVBN

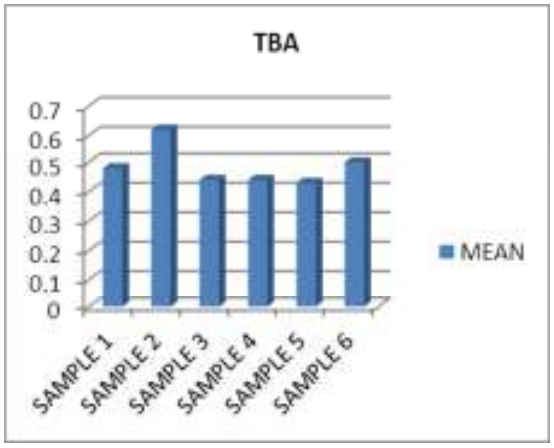

Figure 6: Graphical representation of TBA

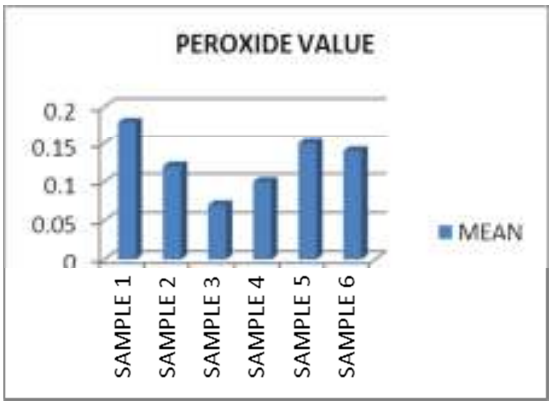

Figure 7: Graphical representation of PV

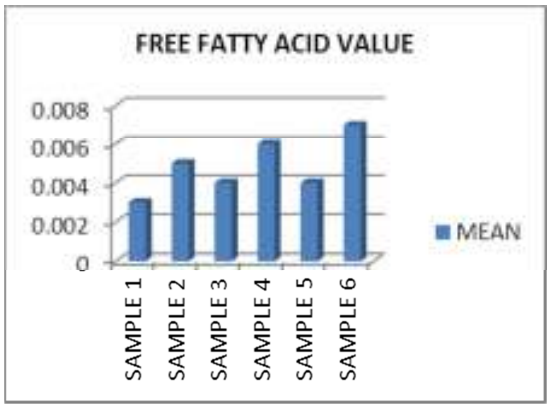

Figure 8: Graphical representation of FFA value

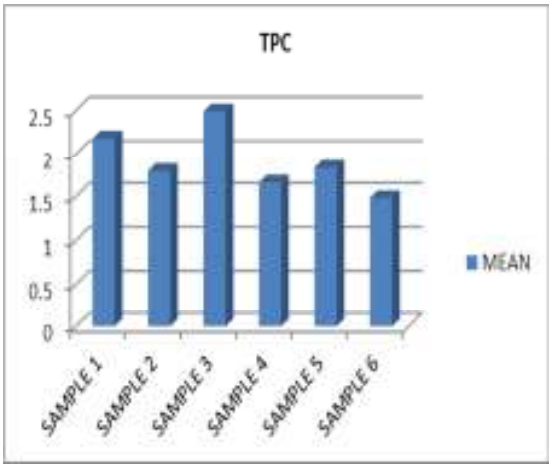

Figure 9: Graphical representation of TPC

CONCLUSION

The results of the study revealed that the quality of seer fish (*Scomberomorus guttatus*) collected from the market in Ernakulam, south west coast of India was fresh and all the biochemical & microbial results were within the acceptable limits. However, the formaldehyde content of the samples in the study ranged from 0.704 mg % to

5.83 mg % whereas the TMA-N values obtained from the same fish samples ranged from 0.46 mg % to 3.73 mg % only. It is evident from the study that chances of natural formaldehyde formation in the seer fish samples collected for the studywas insignificant. Continuous monitoring, quality evaluationof seafood and enforcing quality standards meant for domestic consumption is essential to curtail the illegal practices and also to safeguard the health of growing fish eaters in the country.

AKNOWLEDEMENT

The authors would like to thank the Director, School of Industrial Fisheries, Cochin University of Science and Technology for giving permission and necessary facilities for conducting the study. Thanks are also due to the organizers of 27th Swadeshi Science Congress for selecting the paper for presentation.

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