4. RESULTS

4.1. Quality assessment and processing of the experimental fish (S. capistratus)

The quality assessment was made for raw experimental fishes (S. capistratus) immediately after collection. Based on the EEC Freshness Grades (ISO 4120 – 1983E) as well as the Quality Index Method (QIM), the fishes fell in the grade ‘E’ category. The parts such as skin, outer slime, eyes, gills, peritoneum and odour in gills and internal organs were found to be in a very fresh and good condition (Table 4.1).

The ultimate score of Quality Index Method of S. capistratus was zero, which revealed that the fishes were very fresh (Table 4.2).

The weight of S. capistratus was measured before and after the different processing stages like deskinning (after peeling) and deboning (flesh) (Table 4.3).

4.2. Microbiological analyses of the experimental fish fillets

The experimental fish fillets were subjected for microbiological examinations like Total Plate Count (TPC) and for the different microorganisms (E. coli, Staphylococcus aureus, Salmonella sp., Shigella sp., Vibrio cholerae and Vibrio parahaemolyticus). The result showed that the TPC was only $2 \times 10^2$ CFU/g, but none of the other microbes could be found (Table 4.4.).
4.3. Biochemical analyses of the experimental fish fillets

The protein content of the fresh fillet sample of *S. capistratus* was 47.63 ± 2.50%. Similarly the carbohydrate and lipid contents were 12.46 ± 0.84 and 8.6 ± 0.42%, respectively. The TMA, TVB-N and FFA contents were also analysed and were found to be 4.2 ± 0.15 mg/100g, 6.3 ± 0.20 mg/100g and 3.2 ± 0.10% (Table 4.5).

4.4. Biophysical analyses of the experimental fish fillets

The biophysical constituents of *S. capistratus* fillet samples like moisture and pH were examined and were found to be 77.70 ± 4.60% and 6.43 ± 0.22 respectively (Table 4.6).

4.5. Microbiological analyses of value added fish products

The indicator organisms like coliforms (*E. coli*), human pathogen’s like *Staphylococcus aureus, Salmonella sp., Shigella sp.* and *Vibrio cholerae* and the fish pathogen *Vibrio parahaemolyticus* were absent completely in the fresh keropok and fish wafer samples as well as the products stored and analysed for different days intervals of 15, 30, 45, 60 and 75. It was evident from the failure of colonies formation on specific culture media at different intervals of time.

The TPC colonies persisted throughout the study period i.e., during the first day of preparation of keropok, $1 \times 10^2$ CFU/g of bacterial load was found and in the case of fish wafers, it was $2 \times 10^2$ CFU/g. After 15 days storage condition, the bacterial load (TPC) of keropok and fish wafers were $2 \times 10^2$ and $3 \times 10^2$ CFU/g, respectively.
Similarly, in the subsequent days of storage condition, the microbial load was also increased from $3 \times 10^2$ to $8 \times 10^2$ CFU/g in keropok and $5 \times 10^2$ to $10 \times 10^2$ CFU/g in fish wafers from 30 – 75 days intervals, respectively (Table 4.7; Fig. 1 and 2). The data obtained for increased in TPC with advancement of storage days were positively regressed in both products. It was observed that there was a significant ($P < 0.05$) correlation between bacterial load (TPC) and days intervals ($R^2 = 0.9847$ for keropok and $R^2 = 0.9346$ for fish wafers (Fig. 1a and 2a).

4.6. Biochemical analyses of the fishery products

4.6.1. Protein

There was no variation found in the protein content of keropok, for instance protein content at the initial day was 36.44 ± 0.53%, whereas it was in between 36.55 ± 0.35 and 37.03 ± 1.11% in the subsequent days (15 – 75 days) intervals of storage (Table 4.8 and Fig. 3). One-way analysis of variance carried out for protein content revealed that the variation between days intervals was statistically non significant ($F = 0.4511 ; P > 0.05$) (Table 4.8a).

Likewise, the protein content of fish wafers was 38.28 ± 0.06% in the initial day, whereas it was slightly increased from 39.36 ± 0.26 to 46.23 ± 1.97% in the respective days intervals between 15 and 75 days (Table 4.9; Fig. 4).

The one way ANOVA test revealed that the variation between the protein content of fish wafers recorded in different days of storage conditions was statistically non significant ($F = 0.22454 ; P > 0.05$) (Table 4.9a).
The result on protein content of keropok and fish wafers kept at storage conditions were analysed for regression statistics. It reveals that the protein content of keropok was positively (moderately) regressed ($R^2 = 0.681$), whereas fish wafers were positively regressed ($R^2 = 0.847$) (Fig. 3a and 4a).

The comparison between the protein content of both value added products (36.44 to 37.03% in keropok and 38.28 to 40.23% in fish wafers) in all the tested days intervals of storage condition showed a significant variations (Students ‘t’ test) among the products ($t = 3.9528$ to $58.8897$; $P < 0.05$ to $P < 0.0001$) (Table 4.13).

**4.6.2. Carbohydrate**

The carbohydrate content of both value added products did not show any variations. For instance, the initial carbohydrate content of keropok was $46.86 \pm 0.48\%$, whereas for fish wafers, it was $45.79 \pm 0.87\%$. Subsequently this level was fluctuated from $46.89 \pm 0.35$ to $47.83 \pm 0.75\%$ and $44.87 \pm 0.03$ to $46.25 \pm 2.14\%$ respectively for keropok and fish wafers which were stored in different days (15 to 75 days) intervals (Tables 4.8 and 4.9; Fig. 5 and 6).

The regression analysis revealed that the carbohydrate content of keropok at different days intervals of storage condition was not regressed. It was observed that there was no significance ($P > 0.05$) between carbohydrate content and days of storage condition ($R^2 = 0.0079$). At the same time, the regression analysis of fish wafers revealed that the variation among carbohydrate content in different storage intervals was moderately (positive) regressed ($R^2 = 0.4884$) (Fig. 5a and 6a).
The statistical interpretation by one way analysis of variance between the carbohydrate content of both value added products revealed that the variation among the storage days intervals was statistically non-significant (F = 1.2174 (keropok) and 0.8628 (fish wafers); P > 0.05) (Tables 4.8b and 4.9b).

Comparison of carbohydrate content of keropok (46.76 to 47.83%) and fish wafers (44.84 to 46.25%) in initial day was varied significantly (t = 4.7520; P < 0.05), but it was not varied in the storage days intervals of 15 (t = 1.6678; P > 0.05) and 45 (1.2118; P > 0.05) days. Whereas in the remaining storage days intervals such as 30, 60 and 75 days, it showed significant variation (t = 3.3028 to 9.7796; P < 0.05 to P < 0.01) (Table 4.13).

4.6.3. Lipid

Lipid content of both products exhibited slight variation. In the initial day, the lipid content of keropok was 14.42 ± 0.49% and fish wafers was 13.27 ± 0.46%. Further this value was recorded in between 14.15 ± 0.14 and 15.10 ± 0.25% for keropok and 12.91 ± 0.01 and 13.91 ± 0.12% for fish wafers on the observed days intervals of 15 to 75 days of storage condition (Tables 4.8 and 4.9; Fig. 7 and 8).

The data on lipid content of both value added products when stored at different days intervals were moderately (positively) regressed. It was observed that there was a significant (P < 0.05) correlation between lipid content and different days of storage intervals of keropok (R² = 0.4118) and fish wafers (R² = 0.6048) (Fig. 7a and 8a).
One way ANOVA test carried out for the lipid content of both products through the variations between the days intervals was statistically significant (F = 5.1971; P < 0.01 for keropok and F = 3.85215; P < 0.05) (Tables 4.8c and 4.9c).

The differences between lipid content of both fishery products was varied much (14.15 to 15.10% in keropok and 12.91 to 13.91% in fish wafers) during the different days of storage condition. The statistical comparison by students 't' test showed a significant variation in lipid content among the products during different days of storage condition (t = 2.6702 to 17.00; P < 0.05 to P < 0.001) (Table 4.13).

4.6.4. Trimethyl amine (TMA)

The initial TMA content of the prepared value added products was 1.46 ± 0.05 and 0.71 ± 0.01 mg/100g for keropok and fish wafers respectively. At the same time, this level was increased gradually for the successive days intervals of storage conditions i.e. for keropok, on 15th day it was increased to 2.10 ± 0.20%, likewise it was increased many fold times to 5.46 ± 0.05, 10.29 ± 0.06%, 20.72 ± 0.17 and 38.96 ± 0.02 mg/100g in the storage days intervals of 30, 45, 60 and 75 days respectively. Similarly for fish wafers, it was 1.80 ± 0.12 mg/100g on 15th day, whereas it was increased to 2.8 ± 0.01, 5.46 ± 0.13, 11.00 ± 0.25 and 19.84 ± 0.03 mg/100g in the respective days intervals from 30 to 75 days (Tables 4.8 and 4.9; Fig. 9 and 10).

The data observed for increasing rate of TMA content of keropok and fish wafers with advancement of storage days were positively regressed. It was observed that there was a significant (P < 0.05) correlation between
TMA content and different days of storage condition in both products like keropok ($R^2 = 0.8384$) and fish wafers ($R^2 = 0.8468$) respectively (Fig. 9a and 10a).

The statistical two-way analysis of variance revealed that the variation among the TMA content of both products in different days of storage condition was statistically more significant ($F = 47916.3$ ; $P < 0.0001$ for keropok and $F = 9870.39$ ; $P < 0.0001$ for fish wafers) (Tables 4.8d and 4.9d).

The variation between TMA content of keropok (1.46 to 38.96 mg/100g) and fish wafers (0.71 to 19.84 mg/100g) was varied much in all the tested days of storage intervals. The statistical interpretation by students ‘t’ test revealed that the differences between TMA content of both products in different days of storage condition was highly significant ($t = 6.4951$ to $2869.0$ ; $P < 0.05$ to $P < 0.0001$) (Table 4.13).

4.6.5. Total Volatile Base Nitrogen (TVB-N)

The TVB-N content of keropok was 3.57 ± 0.02% on the initial day, whereas it was 2.33 ± 0.02 mg/100g for fish wafers on the same day. When the storage days increase, the TVB-N rate was also increased positively. For instance it was 5.84 ± 0.05 mg/100g for keropok and 3.70 ± 0.06 mg/100g for fish wafers on 15th day. Likewise on 30th day, the TVB-N level was increased to 10.79 ± 0.07 mg/100g for keropok and 8.13 ± 0.02 mg/100g for fish wafers. Subsequently on 30th day, it was 10.79 ± 0.07 and 8.13 ± 0.02 mg/100g for keropok and fish wafers respectively. On 45th and 60th days intervals, the TVB-N content of keropok was 21.18 ± 0.06 and 41.80 ± 0.09 mg/100g respectively. Similarly on these days for fish wafers, it was 15.98 ±
0.08 and 30.70 ± 0.04 mg/100g respectively. On 75th day, the TVB-N content of both products was highly increased i.e. 60.97 ± 0.09 mg/100g (keropok) and 51.10 ± 0.24 mg/100g (fish wafers) (Tables 4.8 and 4.9; Fig. 11 and 12).

The increase in rate of TVB-N during different days intervals of storage condition of both products were positively regressed and there was significant (P < 0.05) correlation between TVB-N content of keropok (R²= 0.8979) and fish wafers (R² = 0.8756) under different days intervals of storage condition (Fig. 11a and 12a).

The statistical one-way ANOVA revealed that the variation between TVB-N content of both products in different days intervals of storage condition was statistically more significant (keropok : F = 367487.5 ; P < 0.0001 and Fish wafers : F = 92111.76 ; P < 0.0001) (Tables 4.8e and 4.9e).

In keropok (3.57 to 60.97 mg/100g) and fish wafers (2.33 to 51.10 mg/100g), the TVB-N content was varied much in different days of storage condition. The data were subjected for statistical interpretation by students ‘t’ test. The results showed that the variation among the TVB-N content of both products was varied much and it was statistically more significant (t = 60.7706 to 384.515 ; P < 0.0001) (Table 4.13).

4.6.6. Free fatty acids (FFA)

The FFA content of fresh value added products was 7.0 ± 0.03 and 7.73 ± 0.02% respectively for the keropok and fish wafers. During the subsequent storage condition, the FFA level was increased tremendously to 9.12 ± 0.02 and 12.60 ± 0.06% on 15th day; 10.87 ± 0.07 and 16.33 ± 0.03
on 30\textsuperscript{th} day; 12.30 ± 0.16 and 18.97 ± 0.08 on 45\textsuperscript{th} day; 13.50 ± 0.17 and 20.15 ± 0.03 on 60\textsuperscript{th} day and finally 14.20 ± 0.04 and 21.55 ± 0.04\% on 75\textsuperscript{th} day respectively for the fishery products like keropok and fish wafers (Table 4.8 and 4.9; Fig. 13 and 14).

The result on FFA content of keropok and fish wafers under storage conditions were subjected for regression analysis. When the storage period increased, the FFA rate was also increased and observed a significant (P < 0.05) positive regression (R\textsuperscript{2} = 0.9729 – keropok and R\textsuperscript{2} = 0.9317 – fish wafers) (Fig. 13a and 14a).

The two-way ANOVA result revealed that the differences between the FFA content of both products at successive days of storage intervals was statistically more significant (F\textsubscript{2164} = 2164.654; P < 0.0001 for keropok and F\textsubscript{34538} = 34538.83; P < 0.0001 for fish wafers) (Table 4.8f and 4.9f).

At different days of storage condition, the FFA content of keropok (7.0 to 14.20\%) and fish wafers (7.73 to 21.55\%) was varied much. The statistical students ‘t’ test revealed that the differences between the FFA content of both products at different days of storage condition were varied much more significantly (t\textsubscript{30} = 30.508 to 238.45; P < 0.01 to P < 0.0001) (Table 4.13).

\textbf{Biophysical analysis of the fishery products}

The biophysical constituents such as moisture content as well as the pH level of the fishery products are given in the table (4.10). The moisture content of both the products was 0.60 and 0.50\% respectively for keropok and fish wafers on the initial day of observation. When the storage days
prolonged, the moisture content was decreased further and reached 0.40% for keropok and 0.30% for fish wafers respectively on 75th day (Fig. 15 and 16).

The pH level of both products was not varied much. On the initial day, the pH level of keropok was 6.44, whereas it was 6.50 for fish wafers. In every intervals of storage condition upto 75 days, the pH value was fluctuated between 6.55 to 6.83 for keropok and 6.71 to 6.84 for fish wafers. The results prevailed that a slight acidic condition throughout the period of study in both products (Fig. 17 and 18).

4.7. Sensory evaluation study

The keropok and fish wafers were yellow in colour with a score of +50 (Grade = +B) and they were firm and dry (texture properties) with a score of 10 for each property throughout the period of study, without any change. The keropok samples were oily and slightly fishy in taste when examined during the days intervals from 0 – 45 days of storage (score = 14). After 60 and 75 days of storage, it has slight fishy taste and rancid with a score of 6. The fish wafers remained fresh, oily and slightly fishy taste from initial day to 45 days of storage (Score = 16), but, it obtained a score of 6 due to their slight changes in fishy and rancid taste characters during 60 to 75 days of storage (Tables 4.11 and 4.12).

The odour of fishery product keropok was as same as the characteristic of the fish species (Score = 10 ; Grade = I) and as boiled meat which obtained a score of 9 when they were examined during the initial day and upto 30 days of storage conditions. Meanwhile, they were with slight ‘off’ odours (score = 5 ; Grade = II) and with boiled meat odours (Score = 9) after 45 days and upto 75 days of storage. Similarly, the odour of fish wafers was as that of the species (Score = 10 ; Grade = I) and as boiled meat during
the initial day and upto 45 days of storage, but they were with slight ‘off’ odours (Score = 5; Grade = II) and with boiled meat odours (Score = 9) only after 60 days and upto 75 days of storage (Tables 4.11 and 4.12).

The keropok samples were meaty (flavour characteristics) when examined during the initial day to 30 days of storage, while the fish wafers exhibited the same flavour character during the initial day to 45th days of storage with a score of 9 for each product. Trace of ‘off’ flavours evolved from the keropok samples after 45 days and it remained upto 75 days of storage, but it was found only after 60th day and remained upto 75 days of storage condition in the case of fish wafers (score for each sample = 5). The keropok samples were extremely crisp (score = 5) when examined during the initial day and showed a characteristic feature of above average crispness till the end of the storage period (Score = 4). The fish wafers were also extremely crisp (score = 5) only during the initial day, after storage condition upto 75th day they were with above average crispness character (score = 4) (Tables 4.11 and 4.12).