Experimental Results
IV. EXPERIMENTAL RESULTS

To justify most appropriate species with highest antioxidant potential and antimicrobial properties for possible application as natural antioxidant and antimicrobial agents in fishery products and marine functional food ingredients, the pre-screening experiment was carried out using two species of common horticultural waste found in fruit processing industry. In the present investigation naturally occurring phenolic compounds from grape seeds (*Vitis vinifera*) and papaya seeds (*Carica papaya*) were extracted. Further, antioxidant activities of grape and papaya seeds were assayed for total phenolic content, total flavonoid content, DPPH free radical scavenging activity, ABTS radical scavenging activity, ferric reducing power assay, metal chelating activity, lipid peroxidation inhibition and antimicrobial activity. In this section results pertaining to antioxidant and antimicrobial properties of GSE and PSE, storage stability of the extracts at different temperature, pH and storage period and the effect of GSE and PSE on quality changes of Indian mackerel during iced and frozen storage have been presented.

4.1. Percentage of total extractable matter from grape and papaya seeds

It was observed that, the maximum extractable matter was obtained from GSE. The percentage of extractable matter of grape and papaya seeds was 6.00 and 2.75% respectively as shown in Table 3 and Fig. 14.

4.2. Total phenolic content (TPC) of GSE and PSE

Total phenolic content of GSE and PSE were expressed as gallic acid equivalent per gram of sample as presented in Table 4 and Fig. 15. Since total phenolic content contributes to overall antioxidant capacity, the total phenolic content of each extract was determined by Folin-Ciocalteu assay and calculated using a standard curve of gallic acid at a concentration of 20 - 100 mg /L as shown in Fig. 9. The grape seeds were observed to
possess higher TPC i.e. 1.23 mg GAE/g dry matter when compared to papaya seed with 0.31 mg GAE/g DW. It was found from the study that, GSE contains 4 times higher phenolic content than PSE.

4.3. Total flavonoid content (TFC) of GSE and PSE

Grape and papaya seed extracts significantly varied in total flavonoid contents i.e. 1.66 ± 0.30 and 0.28 ± 0.30 mg CE/g DW respectively. It was observed that GSE had 6 times higher total flavonoid content when compared to PSE as shown in Table 4 and Fig. 15. Total flavonoid content of GSE and PSE were calculated using the standard curve of catechin prepared at concentration of 20-100 mg/L as shown in Fig.10.

4.4. In vitro antioxidant activity of GSE and PSE

To find out the potential antioxidant having good antioxidant properties, ethanolic extracts of the GSE and PSE were subjected to 2,2'-Diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity (ABTS+•), ferric reducing antioxidant power assay (FRAP), metal chelating activity (MCA) and lipid peroxidation inhibition (LPI) in linoleic acid model system.

4.4.1. DPPH radical scavenging activity of GSE and PSE

In the present investigation the DPPH radical scavenging activity of GSE and PSE is shown in Table 5A, 5B and Fig. 16. The DPPH radical scavenging activity of GSE and PSE were seen at 100-500 mg/L and were compared with reference standard antioxidants BHA and BHT at concentration of 200 mg/L. The activity of both the extracts increased with increase in concentration (p < 0.05). At the same concentration used, the descending order of DPPH radical scavenging activity of the seed extracts was as follows: GSE > PSE. The DPPH radical scavenging activity of GSE increased upto 300 ppm and thereafter the
activity did not show much further increase (p > 0.05). Results suggest that BHA and BHT show highest radical scavenging activity of 91.05 and 89.72% respectively, whereas GSE and PSE showed an activity of 87.02 and 61.43% respectively at 500 mg/L.

4.4.2. ABTS⁺⁺ radical scavenging activity of GSE and PSE

ABTS⁺⁺ radical scavenging activity of GSE and PSE at different concentrations is shown in Table 6A, 6B and Fig. 17. The ABTS⁺⁺ radical scavenging activity of GSE and PSE was measured against the standard Trolox equivalent ranging from 50 to 600 µM as shown in Fig.11. ABTS⁺⁺ radical scavenging activity of both the extracts increased as the concentration increased (p < 0.05). However, the radical scavenging activity varied with the type of extract tested. The GSE and PSE varied in ABTS⁺⁺ radical scavenging activity as former contains higher phenolic and flavonoid content compared to latter. The dose-dependent scavenging activity was found in PSE while in case of GSE there was no significant difference at 200 - 600 mg/L (p > 0.05). The highest ABTS⁺⁺ radical scavenging activity of GSE and PSE were seen at 600 mg/L which is 622.99 ± 2.40 and 149.22 ± 2.09 µM TE/mL respectively.

4.4.3. Ferric reducing antioxidant power of GSE and PSE

The ferric reducing ability of the GSE and PSE were evaluated at different concentrations (100- 500 µg/mL) as shown in Table 7A, 7B and Fig.18. The FRAP of GSE and PSE was compared with reference standard antioxidants BHA and BHT at concentration of 200 mg/L. The ferric reducing antioxidant power of BHA and BHT was found to be 1.27 and 1.01 Abs respectively whereas, GSE and PSE showed highest ferric reducing power of 0.84 and 0.13 Abs respectively at 500 µg/mL. All the extracts were capable of reducing Fe³⁺ in a linear dose dependent manner. Among the two extracts tested, GSE showed highest ferric reducing activity at all concentrations (p < 0.05) and showed
highest reducing power at 500 µg/mL which is almost equivalent to BHT at 200 mg/L, indicating that GSE could easily donate electron to Fe$^{3+}$, thus reducing it to Fe$^{2+}$. Hence ferric reducing antioxidant activity of GSE and PSE are in agreement with DPPH and ABTS radical scavenging activity. This suggests that ferric reducing ability of GSE has strong correlation with total phenolic and flavonoid content.

4.4.4. Metal chelating activity of GSE and PSE

GSE and PSE were assayed for their metal chelating activity at different concentrations as depicted in Table 8A, 8B and Fig. 19. The activity was compared with synthetic metal chelator (EDTA) at 0.5 mM and 1.0 mM. The results of the present study showed that GSE chelated more iron than PSE (P < 0.05), although both the extracts were less efficient than commercial metal chelator (EDTA). The maximum metal chelating activity of GSE and PSE were seen at 3 mg/mL which was 76.92 and 62.13% respectively whereas EDTA at 0.5 and 1.0 mM showed 85.31 and 97.22% respectively. The metal chelating ability of both the seed extracts was very less at lower concentration but increased with concentration.

4.4.5. Linoleic acid inhibitory activity of GSE and PSE

The inhibitory activity of GSE and PSE against oxidation in linoleic acid model system at different concentration viz., (500, 750 and 1000 mg/L) is shown in Table 9A, 9B and Fig. 20. GSE was observed to have higher inhibitory activity (p < 0.5) compared to PSE. With the increase in concentration, inhibitory activity of both the extracts increased with a maximum of 81.20 and 65.04% respectively at 1000 mg/L. The lipid peroxidation inhibition at 500 and 750 mg/L for GSE was found to be 67.67 and 73.22% whereas, for PSE was found to be 46.43 and 53.13% respectively. This indicates that more the phenolic concentration, higher will be the scavenging activity and more will be the lipid
peroxidation inhibition. Hence, the lipid peroxidation inhibition percentage of GSE and PSE are in agreement with the results of radical scavenging, metal chelating and ferric reducing antioxidant power.

4.5. *In vitro* antimicrobial activity of GSE and PSE

The antimicrobial activity of GSE and PSE were checked by disc diffusion method and results are depicted in Table 10 and Fig. 21A, 21B, 21C, 21D, 21E. The antimicrobial activity of GSE and PSE were seen at 10 mg/mL with ampicillin as positive control at concentration of 1.0 mg/L. It was observed that GSE and PSE were potentially active against gm +ve bacteria viz., *Staphylococcus aureus* and *Bacillus subtilis* whereas, it showed smaller zones of inhibition against gm –ve bacteria.

4.6. Changes in antioxidant activity (AOA) of GSE and PSE by the effect of heat, pH and storage stability

4.6.1. Effect of heat treatment on AOA of GSE and PSE in linoleic acid model system

The effect of heat treatment on AOA of GSE and PSE in linoleic acid model system is depicted in Table 11A, 11B and Fig.22. The results of the present study indicated that AOA of the extracts varied with increase in temperature. The AOA of GSE and PSE at 500 mg/L was found to be 67.67 and 46.43% respectively. On heating at 100 °C for 15 min the antioxidant potency of GSE and PSE decreased to 52.16 and 32.27% respectively. This indicated that heat processing resulted in degradation of antioxidants present in the crude extracts, thereby decreasing the AOA.

4.6.2. Effect of pH on AOA of GSE and PSE in linoleic acid model system

The lipid peroxidation inhibition of GSE and PSE at 3 different pH viz., 4, 7 and 9 at concentration of 500 mg/L in linoleic acid model system is shown in Fig. 23 and Table 12A, 12B. The results of the study showed that the antioxidant activity of GSE and PSE
varied with change of pH. The lipid peroxidation inhibition of GSE and PSE at 500 mg/L was recorded as 67.67 and 46.43% respectively as shown in Table. 9A, 9B and Fig.20. The antioxidant activity at pH 4, 7 and 9 were found to be 69.01, 68.11 and 72.18% respectively for GSE whereas, 52.20, 46.02 and 54.06% respectively for PSE. The investigation showed maximum increase in antioxidant activity at pH 9 while least at pH 7.

4.6.3. Effect of storage time on AOA of GSE and PSE in linoleic acid model system

The effect of storage time at different temperatures on the antioxidant activity of the GSE and PSE at 500 mg/L is shown in Fig. 24 and Table13A, 13B. The GSE and PSE were stored in dark at 5 and 25 °C for 15 d to study the AOA in linoleic acid model system. The lipid peroxidation inhibition at temperature 5 and 25 °C was found to be 67.42 and 64.13% respectively for GSE whereas, 46.66 and 46.40% respectively for PSE. The results of the study indicated a slight change in the antioxidant activity of GSE whereas, the antioxidant activity of PSE remain unchanged.

4.7. Raw material characteristics of fresh mackerel

4.7.1. Biochemical composition of mackerel

Raw material used in the present study was the Indian mackerel, commonly called “Bangude” in Karnataka. The biochemical composition in terms of moisture content, crude protein, crude fat and ash content of fish meat was estimated on wet weight basis and results are represented in Table 14. Moisture content of the Indian mackerel was present in bulk and found to be 71.02% in fresh condition. The crude protein being second highest accounting for 21.02% and crude fat to be 6.96% along with ash content as 1.20%.
4.7.2. Biochemical, microbiological and sensory characteristics of fresh mackerel

The fresh Indian mackerel were assessed for quality using biochemical, microbiological and sensory parameters. The chemical characteristics such as TVB-N, TMA-N and pH as well as lipid quality of fresh fish such as PV, TBARS and FFA value were determined. The average values recorded are shown in Table 14.

The microbiological characteristics such as TPC, *Staphylococcus aureus* and *E.coli* were examined and the average values recorded.

The sensory characteristics such as appearance, odour, color and overall acceptability were determined from fresh Indian mackerel and the respective scores are represented.

4.7.3. Physical characteristics

The average length, standard length and weight of Indian mackerel used in the present study were 18.80 ± 1.26 cm, 13.88 ± 1.46 cm and 74.20 ± 12.22 g respectively.

4.8. Effect of GSE and PSE on lipid oxidation products of whole mackerel and steaks during iced storage

4.8.1. Changes in peroxide value (PV) during ice storage

Peroxide value is a measure of the primary degree of oxidation. The changes in peroxide value of mackerel and steaks with the effect GSE and PSE during ice storage condition are presented in Table 15 (A), 15(B) and Fig. 25(A), 25(B). The results indicated that the PV values of ice stored mackerel increased throughout the storage period. The steady increase in PV value was seen in all the treatment groups and the PV value of chilled whole control (CWC) reached limit of acceptability on 9\(^{th}\) day of ice storage whereas, PSE treated samples reached limit of acceptability on 12\(^{th}\) day of ice storage but GSE and BHT treated samples remained within acceptable limit on 15\(^{th}\) day of ice storage.
The inhibition of hydroperoxide formation due to GSE and PSE was more marked in steaks compared to mackerel. Initial peroxide value was found to be 1.60 mg hydroperoxide/kg meat sample of all the treatment groups. The effect of treatment with GSE and PSE had found limiting in hydroperoxide formation and reached maximum of 14.10, 5.73, 6.10 and 9.87 mg hydroperoxide/kg meat sample for CSC, BHT, GSE and PSE treated samples respectively. The results of the peroxide value indicated that GSE was more effective in limiting hydroperoxide formation but it was more effective in steaks compared to mackerel.

4.8.2. Changes in thiobarbituric acid-reactive substances (TBARS) during ice storage

The secondary lipid oxidation of mackerel and steaks was measured by malonaldehyde produced by the fish sample. The pattern of changes in TBARS during ice storage of mackerel and steaks treated with GSE and PSE are illustrated in Table16A, 16B and Fig. 26A, 26B. The initial TBARS value of CWC and CSC was found to be 0.32 and 0.33 mg malonaldehyde/kg sample which showed increase throughout the storage period \((p < 0.05)\) and reached maximum TBARS value of 2.25 and 2.30 mg malonaldehyde/kg meat sample after 15 d of ice storage. The increase in TBARS formation throughout the storage period indicated secondary lipid oxidation of CWC and CSC. The changes in TBARS value of mackerel for BHT, GSE and PSE treated samples in the beginning of ice storage was found to be 0.32 mg of malonaldehyde/kg meat sample which increased to maximum of 1.27, 1.36 and 1.99 mg of malonaldehyde/kg sample for BHT, GSE and PSE treated samples at the end of 15 d of storage. The effect of lowering the TBARS formation was found more effective in steaks and showed maximum of 0.73, 0.86, and 1.43 mg of malonaldehyde/kg of sample for BHT, GSE and PSE respectively at the end of storage. The present investigation revealed that GSE and PSE supplementation to mackerel and
steaks helps to inhibit the malonaldehyde formation but GSE was more effective in limiting TBARS formation in steaks compared to mackerel.

4.9. Effect of GSE and PSE on biochemical changes of whole mackerel and steaks during ice storage

4.9.1. Changes in free fatty acid (FFA) content during ice storage

The hydrolytic rancidity was evaluated by free fatty acid (FFA) value. Changes in FFA value of mackerel is shown in Table 17(A) and Fig. 27(A). The increase in free fatty acid content of mackerel and steaks were detected from the 0 d, which showed significant increase throughout storage period (p < 0.5). The FFA values of whole mackerel increased from 1.45% of oleic acid to 9.02, 7.30, 7.60 and 8.72% oleic acid respectively for CWC, BHT, GSE and PSE respectively during 15 d of ice storage. On the day of sensory rejection of CWC (9th d), FFA value was found to be 5.81, 5.58 and 6.02% oleic acid for BHT, GSE and PSE treated samples respectively indicating effectiveness of treated samples in reducing lipid deterioration. The changes in FFA of steaks were comparatively lower than mackerel, as the present result agreed with the lipid oxidation parameters. The changes in the free fatty acid (FFA) value of steaks during ice storage is represented in Table 17(B) and Fig. 27(B). The FFA value of steaks increased from 1.42% of oleic acid to 8.60, 7.09, 7.25, and 8.31% oleic acid for CSC, BHT, GSE and PSE respectively during 15 d of ice storage. These results indicate GSE inhibit enzymatic action to liberate free fatty acids whereas, PSE was not found much effective in controlling the liberation of free fatty acid.

4.9.2. Changes in trimethylamine nitrogen (TMA-N) content during ice storage

In the present investigation changes in TMA-N content of mackerel due to the effect of GSE and PSE during ice storage were investigated and the results are presented in
The initial TMA-N value of mackerel was 3.22 mg% and was equal in all four batches of samples, which steadily increased to 23.18 mg% for CWC, 15.22 mg% for BHT, 16.50 mg% for GSE and 18.26 mg% for PSE on the 15th d of ice storage. The CWC sample reached maximum limit of acceptability on 9th d of ice storage whereas, GSE and PSE treated mackerel remains in acceptable limit upto 12th d of ice storage. The pattern of changes in TMA-N during ice storage of treated steaks was lower compared to mackerel as shown in Table 18 (B) and Fig. 28 (B). The TMA-N content in steaks increased from 3.01 mg% to 19.99, 12.28, 13.72 and 14.95 mg% for CSC, BHT, GSE and PSE respectively at the end of 15 d of ice storage. The results of TMA-N content of mackerel treated with GSE and PSE depicts that values of TMA-N were in acceptable limit till 12th d of ice storage whereas, in case of steaks treated with GSE and PSE, the TMA-N content remains in acceptable limit till the end of 15 d of ice storage.

4.9.3. Changes in total volatile base- nitrogen (TVB-N) content during ice storage

The change in TVB-N content during ice storage of mackerel is shown in Table 19 (A) and Fig.29 (A). The TVB-N contents of the mackerel were 8.45 mg/100g at the beginning of the ice storage for CWC, BHT, GSE and PSE respectively, which increased drastically to 49.91, 34.01, 36.07 and 41.00 mg/100 g for CWC, BHT, GSE and PSE respectively during 15 d of ice storage. At the end of 9 d of storage in iced condition, TVB-N value of whole mackerel reached the limit of acceptance while as GSE and PSE samples were acceptable limit till 12th d of iced storage. The changes in TVB-N value of steaks were much lower as compared to treated samples of mackerel as depicted in Table 19(B) and Fig. 29(B). The initial TVB-N content was equal in all the 4 batches of samples. At any given storage period GSE and PSE treated steaks showed minimum increase in values compared to CSC. The treated samples of steaks reached a maximum to 30.32, 32.10 and
36.14 mg/100 g for BHT, GSE and PSE respectively at the end of 15 d of ice storage and all the samples were in acceptable limit.

4.9.4. Changes in pH during ice storage

The changes in pH value of mackerel during iced storage are given in Table 20(A) and Fig. 30(A). The initial pH values for CWC, BHT, GSE and PSE treated mackerel were 6.18, 6.20, 5.92, and 6.19 respectively and showed increase during storage period of 15 d in iced condition. The lowering in pH was found only in GSE treated mackerel and steak after dip treatment. At the end of ice storage study, the pH values of BHT, GSE, and PSE mackerel increased to 6.70, 6.47, and 6.73 respectively. Changes in pH of steaks in iced stored condition are presented in Table 20 (B) and Fig. 30 (B). The initial pH values for CSC, BHT, GSE and PSE treated steaks were 6.17, 6.15, 5.73, and 6.15 respectively and showed increase during storage period of 15 d in iced condition. At the end of 9 d of storage study, the pH values of BHT, GSE, and PSE steaks increased to 6.31, 6.23, and 6.40 respectively.

4.10. Effect of GSE and PSE on drip loss (%) of whole mackerel and steaks during ice storage

In the present study, changes in drip loss percentage of mackerel treated with GSE and PSE during storage in iced condition for a period of 15 d are depicted in Table 21(A) and Fig. 31(A) The drip loss was significantly higher (p < 0.05) for CWC samples as compared to treated samples. A value of 8.13% was observed for CWC samples on the day of sensory rejection. The parallel values for BHT, GSE and PSE treated samples on the day of sensory rejection were observed to be 5.89, 6.95 and 7.15% respectively. Changes in drip loss percentage in steaks is shown in Table 21(B) and 31(B). Steaks treated with GSE and PSE showed remarkably lower drip loss as compared to mackerel. The value of drip
loss in steaks at the end of 9 d of ice storage was recorded as 6.10, 2.62, 3.45 and 4.81% for CSC, BHT, GSE and PSE respectively.

4.11. Effect of GSE and PSE on microbiological characteristics of whole mackerel and steaks during ice storage

Changes in total plate count of mackerel with the effect of GSE and PSE during ice storage are presented in Table 22 (A) and Fig.32 (A). The results show that the microbial population decreased significantly (p < 0.05) with the addition of GSE and PSE. Among the different experimental groups, CWC showed the most rapid increase in the number of microorganisms, followed by PSE, GSE and BHT. The mackerel of CWC group attained a TPC value of 8.0 log CFU/g on 9th d of ice storage, which was slightly higher than microbiological acceptability and indicated that microbiological shelf life of Indian mackerel is 9 d in ice storage. A difference of around 2 log cycles was observed between the CWC and GSE treated samples at the end of 15th d of storage while difference of 1 log cycle was observed between CWC and PSE treated samples. The initial total plate count of the steaks in ice storage was in the range 3.5-4.6 log cfu/g of meat as shown in Table 22(B) and Fig.32 (B). The increase in total plate count was observed in all the four batches of samples but the least total plate count was shown by GSE treated samples. At the end of 9 d of storage, samples treated with GSE and PSE showed total plate count of 4.9 and 5.9 log cfu /g meat respectively.

4.12. Effect of GSE and PSE on organoleptic characteristics of whole mackerel and steaks during ice storage

Changes in organoleptic quality characteristics of GSE and PSE treated mackerel during ice storage are presented Table 23(A) and Fig. 33(A). The overall acceptability score is the mean of attributes such as colour, texture, appearance, flavor and taste. The
scores for CWC sample registered a value of 4.11 at the end of 9 d of ice storage. Among treated samples, the acceptability score at the end of 9 d of storage in ice storage were 7.06, 7.05, and 6.29 for BHT, GSE and PSE respectively. Sensory characteristics for steaks during ice storage are presented in Table 23 (B) and Fig. 33(B). The judgment reported the acceptability of BHT, GSE and PSE treated steaks in ice storage condition at the end of 15 d of storage. The final organoleptic score of steaks at the end of 9 d of storage in ice is 5.27, 7.82, 7.64 and 6.64 for CSC, BHT, GSE and PSE respectively.

4.13. Effect of GSE and PSE on lipid oxidation products of whole mackerel and steaks during frozen storage

4.13.1. Changes in peroxide value (PV) during frozen storage

In the present investigation changes in PV of mackerel with the effect of GSE and PSE during frozen storage period of 180 d were analyzed and the results of study are presented in Table 24(A) and Fig.34 (A). In this study, PV was significantly greater in the FWC (p < 0.05) with respect to other treatment groups but there was no significant difference between GSE and BHT treated mackerel (p > 0.05) during frozen storage period. The PV of mackerel for FWC, BHT, GSE and PSE treated samples in the beginning of frozen storage was 1.62 mg of hydroperoxide/kg of sample. After 120 d of frozen storage, the PV of FWC reached the limit of acceptability and found to be 10.47 mg of hydroperoxide/kg sample whereas, at the same period of time BHT, GSE and PSE treated mackerel were having PV of 4.67, 5.27 and 6.19 mg of hydroperoxide/kg of sample respectively. The effect of GSE and PSE with frozen storage period of 180 d was found more effective in limiting the hydroperoxide formation in steaks compared to mackerel and the result are depicted in Table 24(B) and Fig. 34(B). The initial PV of FSC was 1.62 mg of hydroperoxide /kg of sample and reached the limit of acceptability on 120th d of frozen
storage period. The PV of FSC shows significant difference among the other treated groups (p < 0.05) whereas, there is no significant difference among BHT and GSE treated steaks (p > 0.05) which clearly indicates GSE having same potency as BHT in limiting the hydroperoxide formation in steaks during frozen storage period. The PV of steaks for BHT, GSE and PSE at the start of frozen storage was 1.62 mg of hydroeroxides/kg of sample which increased to maximum of 12.20, 4.74, 5.00 and 8.13 for FSC, BHT, GSE and PSE respectively at the end of frozen storage of 150 d and thereafter, PV of steaks shows decreasing trend and reaches 12.07, 4.78, 4.93, 7.15 mg of hydroeroxides/kg of sample for FSC, BHT, GSE and PSE respectively. The minimum increase in peroxide value was seen in samples treated with GSE and BHT.

4.13.2. Changes in thiobarbituric acid-reactive substances (TBARS) during frozen storage

The pattern of changes in thiobarbituric-acid reactive substances (TBARS) during frozen storage of mackerel and steaks treated with GSE and PSE are illustrated in Table 25(A), Table 25(B) and Fig. 35(A) Fig. 35(B). The TBARS value of frozen whole control (FWC) and frozen steaks control (FSC) shows significant difference with other treatment groups (p < 0.05). Among the treated groups, BHT and GSE treated samples were not significantly different (p > 0.05) upto 90 d of frozen storage and thereafter, both the groups shows significant difference (p < 0.05). The TBARS value of mackerel for FWC, BHT, GSE and PSE in the beginning of frozen storage was 0.33 mg of malonaldehyde/kg of sample which reached to maximum of 2.34, 1.09, 1.33 and 1.70 mg of malonaldehyde/kg of sample for FWC, BHT, GSE and PSE at the end of 150 d of frozen storage and thereafter, TBARS value shows decreasing trend and reached 2.09, 0.99, 1.38 and 1.59 mg of malonaldehyde/kg of sample for FWC, BHT, GSE and PSE at the end of 180 d of
frozen storage. The TBARS value of steaks for FSC, BHT, GSE and PSE at the start of frozen storage was 0.33 mg of malonaldehyde/kg of sample and reaches it’s peak at the end frozen storage of 150 d and follows similar trend as in mackerel during frozen storage period. The TBARS value reached the maximum of 2.14, 0.81, 1.00 and 1.47 mg of malonaldehyde/kg of sample for FSC, BHT, GSE and PSE respectively and at the end of storage.

4.14. Effect of GSE and PSE on biochemical changes of whole mackerel and steaks during frozen storage

4.14.1. Changes in free fatty acid (FFA) content during frozen storage

The changes in free fatty acid (FFA) of mackerel and steaks with the effect of GSE and PSE during frozen storage period of 180 d are presented in Table 26(A), 26(B) and Fig. 36(A), 36(B). The FFA value of treated groups of mackerel and steaks show significant difference with respect to FWC and FSC (p < 0.05). The GSE and BHT treated samples helps in lowering the FFA value and do not find any significant difference in treated samples of mackerel and steaks (p > 0.05). The FFA values of mackerel increased from 1.42% of oleic acid to 9.47, 5.04, 5.19, and 7.17% oleic acid respectively for FWC, BHT, GSE and PSE respectively during 180 d of frozen storage. At the end of 180 d of frozen storage period, the treated samples of GSE and BHT shows minimum free fatty acid liberation as it showed that GSE was having positive effect on inhibition of hydrolytic rancidity. Changes in FFA value of steaks increased from 1.42% of oleic acid to 9.36, 2.94, 3.95 and 5.23% oleic acid for FSC, BHT, GSE and PSE respectively during 180 d of frozen storage.
4.14.2. Changes in trimethylamine nitrogen (TMA-N) content during frozen storage

The changes in TMA-N content of mackerel and steaks with the effect of GSE and PSE during frozen storage of 180 d are presented in Table 27(A), 27(B) and Fig. 37(A), 37(B). The TMA-N content of FWC and FSC were significantly different from other treatment groups of mackerel and steaks (p < 0.05). The initial TMA-N value of mackerel was 3.01 mg% for all the four batches, which steadily increased to 19.20 mg% for FWC, 11.14 mg% for BHT, 12.52 for GSE and 15.17 for PSE during 180 d of frozen storage. The TMA-N content of FWC reached the limit of acceptability on 120\textsuperscript{th} d of frozen storage whereas, BHT, GSE and PSE found TMA-N value of 7.60, 8.15 and 10.45 mg-N /100g respectively. The pattern of changes in TMA-N during frozen storage of treated steaks was lower compared to whole fish. The TMA-N content in steaks increased from 3.01 mg% to 18.47, 9.32, 11.41 and 14.52 mg% respectively for FSC, BHT, GSE, and PSE after 180 d of frozen storage.

4.14.3. Changes in total volatile base- nitrogen (TVB-N) content during frozen storage

The changes in TVB-N content during frozen storage of whole mackerel are shown in Table 28(A) and Fig. 38(A). The TVB-N contents of the whole mackerel were 8.51 mg /100g at the beginning of the frozen storage for FWC, BHT, GSE, and PSE, which increased drastically to 41.05, 29.57, 30.19 and 34.68 mg/100g for FWC, BHT, GSE, and PSE respectively at the end of 180 d of frozen storage. The gradual increase in TVB-N continued till end of storage period. The TVB-N content of mackerel without treatment registered higher values of TVB-N as compared to those treated with GSE, BHT and PSE at the end of frozen storage study. The TVB-N content of all the treatment groups of mackerel showed marked significant difference compared to FWC (p < 0.05). The effect of treatment in steaks shows higher inhibition in TVB-N formation compared to mackerel and
shows significant difference among the different groups of steaks. The initial TVB-N value of steaks was 8.51 mg/100g for FSC, BHT, GSE and PSE as shown in Table 28(B) and 38(B). During frozen storage, TVB-N values increased steeply in FSC to 40.57 mg/100g by the end of 180 d of frozen storage. The treated samples of steaks reached a maximum to 23.19, 24.66 and 31.43 mg/100 g for BHT, GSE and PSE respectively at the end of 180 d of frozen storage.

4.14.4. Changes in pH during frozen storage

The effect of GSE and PSE on the pH value of mackerel and steaks during frozen storage are shown in Table 29(A), 29(B) and depicted in Fig. 39(A), 39(B). The pH value of different groups of mackerel and steaks shows significant difference with the effect of treatment with GSE and PSE (p < 0.05). The GSE treated samples registered lower pH value on 0 day of storage whereas; other treatment groups did not find any change in pH value. The initial pH values for FWC, BHT, GSE, and PSE were 6.17, 6.20, 5.90 and 6.19 respectively and showed increase during storage period of 180 d. At the end of frozen storage studies, the pH values of FWC, BHT, GSE and PSE treated mackerel were registered as 7.14, 6.68, 6.53 and 6.74 respectively. The pH of steaks treated with GSE and PSE were found comparatively lower than that of mackerel. The initial pH value of steaks were found to be 6.17, 6.16, 5.73, 6.14 for FSC, BHT, GSE and PSE, which steadily increased to 7.06, 6.59, 6.50, 6.65 respectively for FSC, BHT, GSE and PSE during 180 d of frozen storage.

4.15. Effect of GSE and PSE on drip loss (%) of mackerel and steaks during frozen storage

The changes in drip loss percentage of mackerel and steaks with the treatment effect of GSE and PSE during frozen storage of 180 d are presented in Table 30(A), 30(B)
and Fig. 40(A), 40(B). The percentage of drip loss was found to be 0% in all the groups of mackerel on the initial day of storage and it increases with the increased in storage period and final drip loss were 14.21, 7.13, 6.04 and 10.12 % for FWC, BHT, GSE and PSE samples respectively after frozen storage period of 180 d. The percentage of drip loss in steaks treated with GSE and PSE showed remarkably lower values as compared to drip loss of mackerel. The value of drip loss recorded in steaks at the end of 180 d of frozen storage is 13.84, 5.05, 5.28 and 8.28% for FSC, BHT, GSE and PSE respectively.

4.16. Effect of GSE and PSE on microbiological characteristics of whole mackerel and steaks during frozen storage

The effect of GSE and PSE on lowering the total plate count (TPC) of mackerel and steaks during frozen storage studies of 180 d and the results are presented in Table 31(A), 31(B) and Fig. 41 (A), 41(B). The initial total plate count of the all groups of mackerel and steaks were recorded to be 4.5 and 4.0 log cfu/g meat. During freezing and frozen storage, the total plate count of both the products decreased over 30 d of frozen storage. The total plate count of FWC and FSC registered significant difference compared with the treatment groups of mackerel and steaks (p < 0.05). The initial total plate count of FWC was 4.3 log cfu/g meat and at the end of storage periods of 180 d total plate count reached to its maximum of 4.7, 4.1, 3.9 and 4.3 log cfu/g meat for FWC, BHT, GSE and PSE whereas, TPC of steaks reached maximum of 4.3, 3.7, 3.3 and 4.3 log cfu/g meat for FSC, BHT, GSE and PSE respectively.
4.17. Effect of GSE and PSE on organoleptic characteristics of whole mackerel and steaks during frozen storage

Changes in organoleptic quality characteristics of GSE and PSE treated mackerel during frozen storage are presented Table 32(A) and Fig. 42(B). The overall acceptability score is the mean of attributes such as colour, texture, appearance, flavor and taste. The scores for FWC sample registered a value of 5.20 at the end of 120 d of frozen storage. Among treated samples, the acceptability score at the end of 120 d of storage in frozen storage were 7.13, 6.80, and 6.30 for BHT, GSE and PSE respectively. Sensory characteristics for steaks during ice storage are presented in Table 32 (B) and Fig. 42(B). The panelists reported the acceptability of BHT, GSE and PSE treated steaks in frozen storage condition at the end of 120 d of storage. The final organoleptic score of steaks at the end of 120 d of frozen storage is 5.53, 7.50, 6.93 and 6.73 for FSC, BHT, GSE and PSE respectively.