Summary
VI. SUMMARY

Seafood is one of the largest export commodities for many economically developing nations. As a healthy alternative to other animal protein, the sea foods are on the increasing demand all over the world. Fish and fish products are now transported between nations and hence the freshness or quality of these products is becoming more and more important, as it reflects on the returns. Spoilage prevention and shelf-life extension of products will always remain an important goal to the fish processing industry. While the greatest emphasis is placed on the prevention of chemical deterioration, microbial spoilage and specifically oxidative spoilage, is an important consideration for fresh fish and manufactured fish products as well.

Indian mackerel as a fatty fish has all necessary proteins, vitamins and minerals in the desired proportion, along with the high content of PUFA like EPA and DHA. Like any other fatty fishes, the commercial use of mackerel has been limited by the susceptibility of the fish to oxidative reactions and microbial spoilage. To retard such a quality loss, synthetic compounds had been used to decrease lipid oxidation and microbial spoilage during the processing and storage of Indian mackerel and its products. However, the use of synthetic compounds had raised questions regarding food safety and toxicity. The use of natural antioxidants and antimicrobial compounds is emerging as an effective methodology for controlling rancidity and microbial spoilage. Therefore, the present study is focused on natural antioxidant and antimicrobial compounds from horticulture waste viz., grape and papaya seeds to enhance the shelf life characteristics of Indian mackerel and its products.

With this background, the objectives of the present investigation are

(i) To extract natural antioxidants and antimicrobial substances from grape and papaya seeds.
(ii) To study antioxidant and antimicrobial properties of both the extracts.

(iii) To study the stability and shelf life characteristics of grape and papaya seed extract.

(iv) To study the efficacy of grape and papaya seed extract on quality of Indian mackerel steaks during different storage conditions.

(v) To study the shelf life characteristics of Indian mackerel in whole form treated with grape and papaya seed extract under different storage conditions.

The salient features of the investigation are summarized as follows:

Two varieties of seed extracts viz. grape (GSE) and papaya (PSE) were used for the study. The seeds were collected from fruit processing center at Mangalore, India. The seeds were dried and ground into powder. The powder of each extract was defatted and extracted with petroleum ether and 90% ethanol respectively. The combined filtrate of each extract was concentrated in rotavapor to remove residual ethanol. The obtained aqueous extracts were frozen overnight and freeze-dried at -60 °C. The yield percentage of freeze-dried extracts of GSE and PSE was found to be 6.00 and 2.75% respectively and were stored in air-tight containers at 5 °C until further use.

The total phenolic content (TPC) of GSE and PSE were expressed as gallic acid and catechin equivalent per gram of sample. The GSE possessed higher TPC of 1.23 compared to PSE 0.32 mg GAE /g DW. The former contained four times higher phenolic content than the latter. The total flavonoid content (TFC) of GSE and PSE were found to be 1.66 ± 0.30 and 0.28 ± 0.30 mg CE /g DW respectively. The TFC of GSE was found to be 6 times higher than PSE.

The antioxidant activity of GSE and PSE was investigated by using different in-vitro models viz., DPPH radical activity, ABTS scavenging activity, ferric reducing antioxidant
power and metal chelating activity. The DPPH radical scavenging activity of GSE and PSE showed concentration dependence. The descending order of DPPH and ABTS radical scavenging activity of the seed extracts tested were as follows: GSE > PSE. The DPPH radical activity of GSE increased up to 300 ppm and thereafter, the activity did not show any further increasing trend. The ABTS radical scavenging activity of PSE showed dose-dependent scavenging activity while, in case of GSE, there was no significant difference at 200-600 mg /L and showed a similar trend with the results of the DPPH assay. The ferric reducing antioxidant power of GSE and PSE were capable of reducing Fe³⁺ and did so in a linear dose dependent manner. GSE showed highest ferric reducing activity at all concentrations and exhibited highest reducing power at 500 µg /mL, which is almost equivalent to BHA at 200 mg/L. GSE chelated more iron than PSE, although both extracts were less efficient than commercial chelator EDTA. The linoleic acid model system with 5 d of incubation showed that, the addition of GSE and PSE to the reaction mixture inhibited peroxidation by 81.20 and 65.04% respectively.

The antibacterial activity of grape and papaya seed extracts at a concentration of 10 mg /mL was seen against gram positive and gram negative bacteria viz., *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2688), *Bacillus subtilis* (NCIM 2063), *Salmonella typhium* (NCIM 2501) and *Pseudomonas fluroscens* (NCIM 2099), which were procured from National Chemical Laboratory, Pune, India. Among the two extracts tested, GSE showed higher antimicrobial activity compared with PSE. GSE was more effective against *Staphylococcus aureus* and *Bacillus subtilis* compared to gram negative bacteria.

The effect of heat, pH and storage period on antioxidant capacity of GSE and PSE were assessed in linoleic acid model system. The two extracts were subjected to heat treatment at 100 °C (15 min), which resulted in significant decrease in antioxidant activity
(AOA) of both the extracts. The antioxidant activity of GSE with the influence of wide range of pH showed increase in its antioxidant activity. The lipid peroxidation inhibition at pH 4, 7 and 9 of GSE was found to be 69.01, 68.11 and 72.18% respectively, whereas, for PSE it was found to be 52.20, 46.02 and 54.06% respectively.

The GSE and PSE were stored in the dark at 5 and 25 °C and the lipid peroxidation inhibition of GSE and PSE were seen after 15 d. The results of the study indicated that, there was slight change in AOA of GSE with the change in storage time while antioxidant activity of PSE remains unchanged during entire storage period.

**Ice storage studies**

The effect of dip treatment with GSE and PSE at 500 and 1000 mg/L respectively on the quality changes of whole mackerel and steaks in iced condition was assessed. The salient features of ice storage studies are as follows

The lipid oxidation products as estimated by PV and TBARS content showed an increase in value for whole mackerel and steaks during ice storage. The GSE treated mackerel and steaks were comparable to that of BHT treated samples whereas, PSE treated samples were not found much effective in retarding lipid oxidation. The reduction in hydroperoxide formation of mackerel treated with GSE and PSE was found to be less by 56.09 and 16.00% respectively whereas, for steaks hydroperoxide formation was found to be less by 57.75 and 33.44% respectively with respect to untreated samples. The malonaldehyde formation of mackerel treated with GSE and PSE was reduced by 56.47 and 22.35% respectively. The percentage inhibition of malonaldehyde formation in steaks treated with GSE and PSE was recorded to be lower by 59.66 and 20.99% respectively, with reference to untreated samples on the 9th d of ice storage.
The effect of GSE and PSE on the keeping quality index of mackerel and steaks was assessed. The ice storage studies showed that, the nitrogenous substances such as TMA-N and TVB-N increased throughout the storage period. The effect of GSE and PSE reduced the formation of volatile amines and the percentage of reduction of TMA-N was found to be 32.27 and 19.01% for mackerel and 25.39 and 19.63% for steaks respectively when compared to untreated samples. The percentage of reduction in TVB-N with the treatment of GSE and PSE on the day of sensory rejection with respect to untreated mackerel (CWC) was found to be 31.85 and 24.70% respectively whereas, the effect of GSE and PSE on reduction of TVB-N formation with respect to untreated steaks (CSC) was found to be 22.17 and 11.45% respectively on the day of sensory rejection.

The efficacy of GSE and PSE as antimicrobial compounds on total plate count of whole mackerel and steaks were assessed. The untreated sample reached a maximum limit of acceptability on the 9th d of ice storage, beyond which it was observed to be spoiled, while GSE and PSE treated mackerel were in acceptable limit on 12th d of ice storage. The steaks treated with GSE and PSE were stable up to 15th d of ice storage in acceptable condition.

The sensory quality of the raw fish decreased during ice storage. The observed shelf life was 9 d in untreated mackerel, 12 d for PSE treated samples and 15 d for GSE treated samples, whereas GSE and PSE treated steaks were in acceptable condition at the end of 15 d of ice storage.

**Frozen storage studies**

The combination of GSE and PSE with frozen storage studies of whole mackerel and steaks on lipid oxidation were assessed. The primary lipid oxidation products of mackerel treated with GSE and PSE with respect to untreated groups on the corresponding
day was found to be lowered by 49.66 and 40.87% respectively whereas, in steaks it was found to be 61.63 and 32.32% respectively. The malonaldehyde formation of mackerel treated with GSE and PSE with respect to untreated groups on the 120th day of frozen storage was found to be less by 50.47 and 30.95% respectively whereas, steaks treated with GSE and PSE the values were found to be reduced by 45.16 and 33.33% respectively.

On the day of sensory rejection for untreated samples (FWC and FSC), the percentage of reduction of TMA-N content of mackerel with the effect of treatment with GSE and PSE was found to be 47.92 and 33.22% respectively whereas, for steaks treated with GSE and PSE the values were 44.18 and 35.36% respectively. The percentage of reduction of TVB-N content of mackerel with the effect of treatment with GSE and PSE was less by 42.10 and 25.00% respectively whereas, for steaks treated with GSE and PSE it was less by 53.71 and 32.63% respectively.

During freezing and frozen storage at -18 ± 2 °C, the bacterial counts were reduced in all the samples and thereafter, total plate count slightly increased with the increase in storage days, but growth of microorganisms did not exceed the limit throughout storage period for all groups. The GSE and PSE treated samples showed slightly lower microbial count than the control. The samples treated with GSE were preferred by the panelist when compared to mackerel and steaks treated with PSE according to sensory analysis.