SUMMARY AND CONCLUSIONS
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Fish plays a very vital role in human nutrition and is the primary source of animal protein for over one billion people of developing countries. About 85-90% of fish protein are easily digestible and contain all essential amino acids. In a developing country like India, where frozen fish industry for internal marketing may not flourish in near future due to lack of facility of cold chain, the preservation of fish by icing will play a vital role in the distribution of fish. Bulk transportation of fresh fish in ice provides only a limited extension of shelf life. Since the fish are not surface protected by over wrapping or other packaging cross contamination, leaching of soluble nutrients and other forms of abuse cannot be avoided.

In today's affluent society, people prefer to buy ready-to-cook and ready-to-serve convenience products from supermarkets than buying raw fish. Quality issues are vital for every industrial sector in today's market. Especially in the food industry, quality is a primal condition in any phase within the food-processing chain. Consumers became very conscious and define and judge food quality by either visual or physical quality. These phenomena in the international food chain markets require also new requirements and demands for food packaging. In the first place, food should be able to be stored or distributed for longer periods. Packaging can differentiate the food in respect of freshness, taste or even image. One of the major developments in food packaging for fulfilling the new challenges is packaging under vacuum or
modified atmosphere conditions. Vacuum packaging suits the demands and requirements of today and tomorrow.

Vacuum packaging, a technologically viable method has been developed as a supplement to ice or mechanical refrigeration to reduce losses and to extend the storage life of fresh seafood products. In vacuum packaging, the product is contained in a package made of a material having low oxygen permeability and is sealed airtight after evacuating the air. It fits into an important area of preservation where shelf life is extended without the loss of those important and exclusive properties, which constitute freshness in consumer’s mind, and therefore move the product into a premium bracket. Preservatives such as sodium acetate and potassium sorbate are found to be effective in preventing microbial growth and improving shelf life under different storage conditions. However, collective works on various quality aspects of vacuum packaged fish of tropical region, under refrigerated storage are found to be scarce. So, the present work was carried out to study the effect of vacuum packaging on the shelf life of fresh pearlspot and black pomfret during chill storage and also the effect of chemical preservatives such as sodium acetate and potassium sorbate in extending the shelf life. Pearlspot and Black pomfret are highly cherished food fishes of India and have good export market.

Fresh pearlspot and black pomfret from Fort kochi fish market were brought to the laboratory in iced condition for further processing. Both the species were beheaded, scaled, gutted, washed in potable water and made into
steaks in case of Black pomfret. Fish samples were given a dip treatment in 2 ppm chilled chlorinated water for 10 min. and drained well. Pearl spot/black pomfret steaks were divided into 4 lots. Control Air Pack (CAP), Control Vacuum Pack (CVP), Sodium acetate (2% w/v) treated vacuum pack (SAVP) and Potassium sorbate (2% w/v) treated vacuum pack (PSVP) to study the effect of vacuum packaging with and without treatment in comparison to air packed samples.

Pouches made of 12μ polyester laminated with 300 gauge low density polyethylene were used for packing Pearlspot/black pomfret steaks. The physical properties of the packaging material studied include Tensile strength, Elongation at break and Heat seal strength which were determined in both machine direction and cross direction. Water vapour transmission rate and oxygen transmission rate of the packaging material were measured to be 3.62 g/m²/24h at 37°C and 90±2% RH and 65cc/m²/atmosphere/24hrs at room temperature respectively. The overall migration residue (water extractives) at 21.1°C for 48 hrs of the sample was found to be 3.35 mg/l, which is below the acceptable limit for food contact application. The values indicate that the packaging material meet the requirements for vacuum packed products. Pearlspot/Black pomfret samples after treatment and packing were stored in ice and kept in a chill room maintained at 0-2°C. Samples were subjected to biochemical, textural, microbiological, and sensory evaluation at regular intervals. The changes during chill storage in these samples are discussed below.
In case of pearlspot drip loss increased from an initial value of 2.63% on 4th day to 4.92% on 10th day in CAP, 3.52 to 6.43% on 12th day in CVP, 3.1 to 8.92% in SAVP and 3.66 to 9.25% in PSVP samples on 17th day of storage. In black pomfret, drip was found to increase from an initial value of 1.92% on 4th day to 2.23% on 10th day in CAP, 2.61 to 6.71% on 12th day in CVP, 2.34 to 6.70% in SAVP and 2.97 to 7.05% in PSVP samples on 18th day of storage. Drip loss was observed to be more in vacuum packed samples compared to treated samples. In both the species, air packed samples exhibited minimum drip loss.

TVB-N values increased gradually in all the samples during storage. In pearlspot, value increased from initial 5.6 mg% to 19.45, 18.3, 21.6 & 17.15 mg% in CAP, CVP, SAVP & PSVP samples on 10th, 12th and 17th day of storage respectively. The TVB-N values of treated samples were found to be comparatively lesser than those of control packs. In black pomfret, the value increased from initial 4.5 mg% to 19.6, 18.4, 21.55 & 16.65 mg% in CAP, CVP, SAVP & PSVP samples on 10th, 12th and 18th day of storage respectively. In both the species, TVB-N contents of potassium sorbate treated samples were slightly lower than sodium acetate treated samples. This might be attributed to the greater inhibition of aerobic gram -ve bacteria by potassium sorbate than sodium acetate. However, TVB-N values in all the samples were within the suggested limit throughout the storage period.

A gradual increase was observed in TMA-N values of all the samples during storage. In pearlspot, value increased from initial 1.4 mg% to 6.55, 6.0, 6.1 &
5.25 mg% in CAP, CVP, SAVP & PSVP samples on 10th, 12th and 17th day of storage respectively. The TMA-N values of treated samples are found to be comparatively lesser than those of control packs in both the species. In black pomfret, the value increased from initial 2.8 mg% to 15.8, 16.1, 16.35 & 14.25 mg% in CAP, CVP, SAVP & PSVP samples on 10th, 12th and 18th day of storage respectively. In case of pearl spot, TMA-N values were within the limit till the end of storage, whereas in case of Black pomfret the values exceeded the acceptability limit in control as well as sodium acetate treated packs on the day of sensory rejection.

An increasing trend in the TBA values was noticed in all the samples with storage time. The initial TBA value in Pearlsplot and Black pomfret was found to be 0.08 and 0.28 mg malonaldehyde/kg of fish respectively. On the day of sensory rejection, the values rose to 0.31, 0.34, 0.39 and 0.31 in CAP, CVP, SAVP and PSVP samples respectively in case of pearlsplot. In black pomfret, TBA values on the day of sensory rejection were found to be 1.85, 1.33, 1.14 and 1.13 in CAP, CVP, SAVP and PSVP samples respectively. In both the species, vacuum packed and treated samples showed lower value in comparison to air packed samples. Potassium sorbate treated samples exhibited still lower TBA values than those of sodium acetate treated samples. In all the samples, TBA values were within the limit throughout the storage period.

Pearlspot and black pomfret had an initial pH of 6.5 and 6.47 respectively. The pH values were found to increase gradually during storage in air packed
samples of both the species. In CVP, SAVP and PSVP samples of both the species, the values exhibited a slight decrease initially and then increased. Slight decrease in pH values may be attributed to the dissolution of CO₂ in the fish muscle. Increase in pH may be attributed to the production of volatile basic compounds by bacterial activity. On the day of sensory rejection, the pH values of pearlspot were found to be 6.58, 6.56, 6.56 and 6.58 in CAP, CVP, SAVP and PSVP samples respectively. In black pomfret, the values in CAP, CVP, SAVP and PSVP samples on the day of sensory rejection were found to be 6.59, 6.54, 6.55 and 6.52 respectively.

In both the species, K value was found to increase during storage. In pearlspot, K value increased from initial 4.87% to 68.7, 61.01, 68.39 and 69.4% in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. In black pomfret, the value increased from initial 7.81% to 67.13, 63.69, 63.43 and 62.17% on the day of sensory rejection. K values of all the samples have exceeded 60% on the day of sensory rejection, which indicates good correlation of K value with the sensory scores.

Textural parameters studied involve changes in hardness1 and hardness2 values, cohesiveness, springiness and chewiness of fish muscle during storage. In both the species, Hardness 1 and Hardness 2 values were found to decrease during storage. In pearlspot, hardness 1 decreased from an initial value of 1.99 kgf to 1.86, 1.60, 1.54 and 1.47 kgf in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. In black pomfret, Hardness 1 decreased from initial 1.51 kgf to 0.92, 1.05, 0.99 and 0.86 kgf in
CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection.

Hardness 2 decreased from initial 1.77 kgf to 1.63, 1.40, 1.32 and 1.25 kgf in CAP, CVP, SAVP and PSVP samples of pearlspot respectively on the day of sensory rejection. In black pomfret, hardness 2 decreased from initial 1.26 kgf to 0.88, 0.83, 0.74 and 0.72 kgf in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. Decrease in hardness 1 and hardness 2 values might be attributed to the weakening of connective tissue of fish muscle during storage.

Significant change was not observed in cohesiveness of both the species during storage. In pearlspot, cohesiveness slightly decreased from initial 0.34 to 0.28, 0.26, 0.22 and 0.22 in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. In black pomfret, also a slight decrease was observed from initial 0.32 to 0.28 and 0.27 in CAP and CVP samples respectively and to 0.26 in SAVP and PSVP samples. This indicates that there was not much change in the internal bonding of fish muscle during storage.

A decreasing trend was observed in the springiness of both the species during storage. In Pearlspot, springiness decreased from initial 1.01mm to 0.92, 0.86, 0.75 and 0.68 mm in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. In black pomfret, the values decreased from initial 2.72mm to 2.66, 2.45, 2.10 and 2.41mm in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection.
Chewiness was found to decrease in both the species during storage. In Pearlspot, chewiness decreased from initial 0.65 kgf.mm to 0.48, 0.26, 0.24 and 0.23 kgf.mm in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. In black pomfret, the values decreased from initial 1.35 kgf.mm to 0.85, 0.73, 0.64 and 0.53 kgf.mm in CAP, CVP, SAVP and PSVP samples respectively on the day of sensory rejection. Decrease in chewiness indicates that the fish muscle becomes soft during storage.

In both the species, a significant decline in sensory score was observed in control and treated packs during storage. A sensory score of 4 was taken as the borderline of acceptability. In Pearlspot, sensory scores declined from an initial 8.6 to 3.4, 3.8, 3.6 and 3.8 in CAP, CVP, SAVP and PSVP samples respectively. Thus, CAP and CVP samples were found to be acceptable up to 8 and 10 days respectively, whereas SAVP and PSVP samples remained in good and acceptable condition up to 15 days. Thus, vacuum packaging alone was not much effective in extending the shelf life of pearl spot, but vacuum packaging along with preservatives (sodium acetate / potassium sorbate) was found to extend the shelf life by 7 days compared to air packed samples.

In black pomfret, the sensory scores declined from an initial 8.8 to 3.4, 3.8, 3.6 and 3.8 in CAP, CVP, SAVP and PSVP samples respectively on the day of rejection. Thus, air packed and vacuum packed samples were acceptable up to 8 days and 10 days respectively. As in case of pearlspot, an extension of only 2 days was noticed due to packing under vacuum. SAVP and PSVP samples remained in good condition up to 16 days. Thus, an extension of 8
days in shelf life was obtained by the combination of vacuum packaging along with preservatives (sodium acetate and potassium sorbate) compared to air packed samples, which were acceptable only up to 8 days. In both the species, significant difference was not noticed between sodium acetate and potassium sorbate treated samples.

Microbiological parameters studied include, changes in total viable count (TVC) at 20°C and 37°C, H2S producing bacterial count, *Pseudomonas*, Lactic Acid Bacteria, sulphite reducing clostridial count and *Clostridium botulinum* toxin detection by mouse bioassay.

The initial total viable counts at 20°C and 37°C in black pomfret were 5.48 and 5.52 log10 cfu/g respectively. In CAP samples, TVC exceeded 10⁷ cfu/g on 10th day when they were sensorily rejected. In CVP samples, after a lag phase of 8 days significant increase in TVC was noticed and the limit count of 10⁷ cfu/g was reached on 12th day of storage. The lowest counts were noticed in SAVP and PSVP samples where the log phase was apparently extended. The initial total viable counts at 20°C and 37°C did not differ significantly. At the end of storage, counts at 20°C were significantly higher than that at 37°C and it indicates that considerable proportion of the bacteria easily got adapted to grow at low temperature. Based on TVC’s shelf life of 16 days was noticed for both SAVP and PSVP samples compared to 10 days for CVP and 8 days for CAP samples.

The initial TVC of fresh pearlspot at 20°C and 37°C were 4.98 and 4.94 log10 cfu/g respectively. In CAP samples and CVP samples, TVC rose
continuously and reached ca. $10^7$ cfu/g on 10th and 12th day of storage respectively when the fish were deemed spoiled based on sensory scores. A 1 log increase in the TVC’s of treated samples (SAVP and PSVP) was observed on 12th day and the counts increased gradually and reached $10^7$ cfu/g on 17th day of storage. Surface treatments with sodium acetate (2%) and potassium sorbate (2%) was equally effective in inhibiting microbial growth and extending storage life of Pearlspot to 15 days compared to 10 days for CVP samples and 8 days for air stored samples.

The initial concentration of H$_2$S producing bacteria in Black pomfret was $10^3$-$10^4$ cfu/g and it constituted 1% of the total viable counts at day 0. In CAP and CVP samples, H$_2$S producing bacteria proliferated and the counts reached ca. $10^7$ cfu/g respectively on 10th and 12th day when the samples were sensorily rejected. The H$_2$S producing bacterial counts reached 5.26 and 4.65 log$_{10}$cfu/g in SAVP and PSVP samples of black pomfret respectively when they were rejected based on sensory scores. Proliferation of H$_2$S producing bacteria was prevented by treatment with potassium sorbate. The number of H$_2$S producing bacteria at rejection of vacuum packed samples constituted 60-70% of the total count while that of CAP was 30-40%. _S. putrefaciens_ was identified as the main spoilage organism of black pomfret. Treatments with sodium acetate and potassium sorbate suppressed the growth of H$_2$S producing bacteria by extending the log phase and thereby extending shelf life.
The initial level of H$_2$S producing bacteria in Pearlspot was 4.0 log$_{10}$cfu/g. The counts reached 5.78 and 5.84 log$_{10}$cfu/g in CAP and CVP samples on 10$^{th}$ and 12$^{th}$ day of storage respectively, when the samples were rejected based on sensory scores. H$_2$S producing bacteria grew most quickly in Pearlspot stored in air, followed by those in CVP and SAVP samples. The lowest counts were with PSVP samples of pearlspot where the log phase was apparently extended. H$_2$S producing bacteria at the time of sensory rejection of CAP samples constituted only 5% of the total count whereas in CVP samples it constituted 9% of the total count.

The initial level of Lactic acid bacteria in black pomfret was 3.47 log$_{10}$cfu/g. The LAB count decreased or remained almost constant in CAP and CVP samples. In SAVP and PSVP samples, their count decreased in the first two weeks of storage. Their population density was always <3.8 log$_{10}$cfu/g in SAVP and PSVP samples whereas in CAP and CVP samples it was 3.29 -3.31 log$_{10}$cfu/g at the time of sensory rejection. In Pearlspot the initial LAB populations were low (3.04 log$_{10}$cfu/g) and it did not grow in CVP, SAVP and PSVP samples of pearlspot during the 17 days of storage at 0°C. In CAP samples, a slight increase was noticed and the counts reached 3.23 log$_{10}$cfu/g at day 10, i.e. at sensory rejection. The results of the present study on Pearlspot and black pomfret suggest that this flora is not likely to be responsible for spoilage of air or vacuum packed Pearlspot and Black pomfret.

In CAP and CVP samples of black pomfret, *Pseudomonas* count increased from initial 2.05 log$_{10}$cfu/g and reached 5.1 log$_{10}$cfu/g and 4.45 log$_{10}$cfu/g
respectively when they were sensorily rejected. In PSVP samples, population density gradually increased and reached $4.23 \log_{10}\text{cfu/g}$ on 18th day of storage at $0^\circ\text{C}$. Significant reduction in *Pseudomonas* count was observed in SAVP samples of black pomfret where the log phase was apparently extended.

The initial *Pseudomonas* population in fresh Pearlspot was $2.48 \log_{10}\text{cfu/g}$ and constituted <1% of total flora of fresh pearlspot. Their population density reached $5.79$ and $5.94 \log_{10}\text{cfu/g}$ in CAP and CVP samples respectively when the fish were deemed spoiled based on sensory scores. Lowest counts were noticed in SAVP samples of pearlspot. By the end of storage, 8-9% of the total flora was constituted by *Pseudomonas* in CAP and CVP samples respectively. In PSVP samples, still higher percentage (10%) was noticed. The results indicate that *Pseudomonas* together with *S. putrefaciens* constitutes the spoilers in both the species. Sodium acetate was more effective in suppressing *Pseudomonas* population compared to potassium sorbate.

In both the species sulphite reducing clostridial counts were low initially. Initial count in Pearlspot and black pomfret were $3.12 \log_{10}\text{cfu/g}$ and $3.19 \log_{10}\text{cfu/g}$ respectively. Decrease in counts was observed in both the species during storage, which indicated that the flora is sensitive to chilling. Treatment with potassium sorbate and sodium acetate had no additional inhibitory effect in reducing sulphite reducing clostridia in both the species.
Counts of *Staphylococcus aureus*, *Faecal Streptococci* and *Enterobacteriacea* were determined for fresh fish and for fish at the time of sensory rejection. The *Enterobacteriacea* population increased from an initial level of 2.9 log_{10}cfu/g to 4.35, 4.83, 3.48 and 4.67 log_{10}cfu/g respectively in CAP, CVP, SAVP and PSVP samples of black pomfret. Inhibition of growth was noticed in SAVP samples possibly due to the preservative action of sodium acetate. *Staphylococcus aureus* count was about 1.4 log_{10}cfu/g initially in fresh black pomfret and the count decreased or almost remained constant (1.06 log_{10}cfu/g) throughout the storage in CAP, CVP, SAVP and PSVP samples. *Faecal streptococci* population showed a little increase (from 3.08 to 3.1-3.2 log_{10}cfu/g) in CAP and PSVP samples while that of CVP and SAVP samples counts decreased (2.6-2.8 log_{10}cfu/g) by the end of storage. *Enterobacteriacea* counts in fresh Pearlspot were 2.81 log_{10}cfu/g. By the end of storage, a 0.2 log increase was noticed in CVP, SAVP and PSVP samples whereas in CAP samples of pearlspot 0.4 log increase was observed. *Staphylococcus aureus* count was 1.2 log_{10}cfu/g in fresh pearlspot. On the day of sensory rejection, the counts decreased (0.2 log reduction) in all the samples. *Faecal streptococci* population was 2.58 log_{10}cfu/g in fresh Pearlspot. A 0.2-0.25 log reduction in *Faecal streptococcal* counts were noticed in CAP, CVP and SAVP samples. In PSVP pearlspot, 0.3 log reduction was found. The results of the study indicate good microbiological quality of fresh Pearlspot and Pearlspot samples stored at 0-2°C.
Clostridium botulinum toxin was not detected in any of the samples throughout the storage period. This indicates that there was no abuse of temperature during storage.

The microbiological data are in agreement with sensory data and TMA-N values. The low TMA-N values may be attributed to the low levels of S. putrefaciens by the end of storage of Pearlspot (<9%). In contrast, higher percentage of S. putrefaciens is found at the end of storage in Black pomfret contributing to high TMA-N values.

The treatments with sodium acetate and potassium sorbate influenced the microbial association of Black pomfret/Pearlspot stored under vacuum, but no pronounced inhibition was evident in aerobically developed microbiota. The significant changes in microbial attributes (compared to the air packed samples) due to preservative treatments can be summarized in three primary effects: (a) reduction of microbial load, (b) reduction of growth rates of fish spoilage organisms; and (c) in some cases increase in lag phases.

Research findings can be summarized as follows:

- 12µ polyester laminated with 300 gauge low density polyethylene was found to be suitable for vacuum packaging of fresh fish.
- Vacuum packaging alone, without preservatives is not of much use in extending the shelf life of Pearl spot and Black pomfret.
- 2% sodium acetate and 2% potassium sorbate treatment in conjunction with vacuum packaging can be successfully used for
preserving fish and thereby extending the shelf life of Pearlspot and Black pomfret with preservation of quality.

- The treatment with sodium acetate and potassium sorbate increased the shelf life of pearlspot stored under vacuum from 10 days to 15 days whereas the shelf life of air stored samples was only 8 days.

- The treatment with sodium acetate and potassium sorbate increased the shelf life of black pomfret stored under vacuum from 10 days to 16 days whereas the shelf life of air stored samples was only 8 days.

- *Pseudomonas* together with *S. putrefaciens* constituted major spoilers in both the species.

- Inhibitory effect of potassium sorbate on H₂S producing bacteria was found to be more significant than sodium acetate in both the species.

- Vacuum packaging can be safely applied to extend the shelf life of fresh fish, provided that proper refrigeration temperature (<38°F or 3.3°C) is absolutely maintained during distribution, storage, and retailing and in the home.

Today's opportunities and differences in the global market have also had their impact on the food processing, production, and consumption markets. International consumers demand the same quality, features, and products wherever they go. Vacuum packaging has the opportunities to establish a position in today's and tomorrow's dynamic markets of food processing, production and consumption. Vacuum technology can be applied to extend
storage life, lengthen distribution channels, decrease purchasing costs, preserve product quality, or even improve the product presentation.