

# Chilled Storage Stability of Spotfin Flathead, *Grammoplites suppositus* (Troschel, 1840)

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## Abstract

The study investigates the shelf life of the spotfin flathead *Grammoplites suppositus* (Platycephalidae) under chilled storage (2–4 °C) conditions using two distinct packaging techniques – air packaging (AP) and vacuum packaging (VP). The quality was evaluated using physio-chemical (pH), trimethylamine (TMA), total volatile base nitrogen (TVBN), free fatty acids (FFA), peroxide value (PV), thiobarbituric acid (TBA), colour and texture, microbiological (total viable count, TVC), psychrotrophic count (PC), lactic acid bacteria, *Pseudomonas*, hydrogen sulphide producing bacteria, *Brochothrix thermosphacta* and sensory analysis. A heterogeneous quality pattern was observed during the chilled storage. A significant correlation was observed between the TVBN and TVC values. *Brochothrix* was identified as a significant spoilage bacterium in VP samples. The AP samples remained fresh for up to 15 days and VP samples extended the shelf life for up to 18 days, supported by TVC and sensory assessments, indicating the superior effectiveness of vacuum packaging in preserving *G. suppositus*. This study contributes to the utilisation of *G. suppositus*, an underutilised fish species to facilitate the development of new products and enhance market opportunities, ultimately promoting its commercial viability and reducing wastage.

**Keywords:** shelf life, vacuum packaging, underutilised, preservation, air packaging

## Introduction

Seafood is a healthier choice for good - quality nutrition. However, despite the nutritional potential of numerous fish species, their utilisation remains incomplete. One such species, *Grammoplites suppositus*, a flathead belonging to the family Platycephalidae, has been observed as a bycatch of shrimp bottom trawls in the Bay of Bengal (Kapoor et al., 2002) and Iranian coastal region (Izadifar, 2019). While flatheads, in general, may not significantly contribute to the commercial fishery of India, they do represent a considerable proportion of the species that are commercially harvested (Vikas et al., 2018). The popularity of flatheads in East Asia and Australia is attributed to their taste, nutritional value, and medicinal properties (Chen et al., 2020). However, regardless of their significance, no structured studies

have been conducted to investigate the preservation and shelf life of spotfin flatheads.

Fish spoilage is a complex process that commences shortly after the death of the fish, due to a combination of intrinsic and extrinsic factors (Amaral et al., 2021). This quality degradation begins with autolytic spoilage, followed by the colonisation of surface microorganisms, which invades the fish flesh resulting in tissue breakdown and overall product deterioration (Garthwaite, 1997). The factors including the way of fish processing and preservation coupled with the storage temperature, highly influence the type and extent of spoilage (Sivertsvik et al., 2002). In addition to gutting and icing, effective packaging plays a significant role in safeguarding seafood quality. Innovative methods in food packaging have contributed to improving the storage life of fish. Vacuum packaging establishes an

oxygen free environment that hampers the production of aerobic bacteria, suppresses enzymatic reactions, and preserves the sensory characteristics of fish, thereby extending the shelf life. It prevents the detrimental effects of oxygen exposure on food quality while being cost effective and requiring minimal packaging space (Maqbool et al., 2023). Furthermore, the lower oxygen tension in the vacuum packs aid in decelerating lipid oxidation (Kumar and Ganguly, 2014). Several authors have conducted studies on the storage duration of fish using vacuum packaging, along with other packaging methods (Alice et al., 2020; Esteves et al., 2021). Hence, the current study is designed to evaluate the physiochemical, microbiological, and sensory attributes of *G. suppositus* and to explore the potential extension of its shelf life using gutting and vacuum packing techniques under chilled conditions.

## Materials and Methods

### Ethical approval

The spotfin flathead specimens were obtained already dead from local fishermen; hence, ethical approval was not required for this study.

### Raw material processing

*Grammoplites suppositus* caught in multi-day trawlers from Indian waters was collected from Munambam Harbour (10.1667°N 76.1833°E), Kochi, India. Fish were iced and taken to the laboratory within an hour, maintaining a temperature of 2–4 °C. The fishes were beheaded, eviscerated, and definned manually in the laboratory, maintaining chilled condition. The samples were separated into two lots labelled air pack (AP) and vacuum pack (VP) and packed using 12-micron polyester and 75-micron low density polythene (LDPE) pouches of dimensions measuring 31 × 24 cm. The AP samples were sealed using an impulse sealer (300 HS, NI Seal, India) and the VP samples were sealed using a vacuum sealer (QS 500, Sevana, India). Both the lots were immediately arranged in separate insulated polystyrene boxes of 25 L capacity with fish to ice at a ratio of 1:2 by weight. Flake ice was used for the experiment. The ice melt water was removed and replaced with fresh ice every 24 h. The samples from both treatments were analysed for physiochemical, microbiological, and sensory parameters every three days until the sample underwent spoilage (18 days).

### Physiochemical analysis

A 10 g sample from the anterior dorsal portion of fish tissue was homogenised with 50 mL distilled water and the pH was measured using a digital pH meter (Eutech CyberScan EI, Singapore). The total volatile base nitrogen (TVBN) and trimethylamine (TMA) compounds in the AP and VP samples were estimated according to Jinadasa (2014). The 10% trichloroacetic acid (TCA) extract of the fish muscle and saturated  $K_2CO_3$  was

added to the outer chamber of the Conway's unit. For the selective measurement of TMA, neutralised formaldehyde was added prior to the addition of potassium carbonate ( $K_2CO_3$ ). The volatile bases trapped by sulphuric acid ( $H_2SO_4$ ) in the inner chamber of Conway's unit were quantified by titration with sodium hydroxide (NaOH). The free fatty acids (FFA) analysis was carried out using the chloroform extract prepared from AP and VP samples (AOCS, 2000). The fat obtained was solubilised in neutralised alcohol and the free fatty acids in the samples were measured by titration against NaOH. The peroxide value (PV) was estimated according to AOCS (2005). The chloroform extract of fish samples was incubated with glacial acetic acid and potassium iodide in the dark for 30 min. The iodine liberated was titrated against sodium thiosulphate ( $Na_2S_2O_3$ ) using starch as an indicator. The thiobarbituric acid (TBA) value was analysed according to AOAC (2005) using TBA distillation apparatus. The filtrate collected was mixed with TBA reagent and heated in a water bath. The optical density of the samples was measured using a spectrophotometer (Specord 200 Plus, Analytik Jena, Germany) and expressed as mg malonaldehyde  $kg^{-1}$  of the fish sample. The colorimetric analysis was carried out using colorimeter (Hunter Lab Miniscan® XE plus, USA) and the  $L^*$  (lightness),  $a^*$  (redness to green), and  $b^*$  (yellow to blue) values were recorded. The texture profile analysis (TPA) was done using a texture analyser (Lloyd Instruments, UK).

### Microbiological analysis

The quantification of the total viable plate count in the samples was conducted following the ISO 4833-1:2013 (ISO, 2013) method. One mL of sample from the respective dilutions was plated in triplicate using plate count agar (PCA) (M091, Himedia, India) employing the pour plate method. The culture plates were incubated at 48 °C for a duration of 72 h in a bacteriological incubator, and colonies were enumerated using a colony counter. The psychrotrophic count was estimated according to APHA (2015). Samples from the respective serial dilutions were aseptically transferred into pre-set sterile PCA plates and incubated at 7 °C for 10 days. Samples were plated in triplicate, and the average count was taken and expressed as colony forming units per gram ( $cfu.g^{-1}$ ). Lactic acid bacteria (LAB) (ISO 15214:1998), *Pseudomonas* (ISO 13720:2010),  $H_2S$  producing bacteria (Gram et al., 1987), and *Brochothrix thermosphacta* (ISO, 1996) were also analysed.

### Sensory evaluation

The sensory assessment was done by a panel of trained evaluators according to the method described by Meilgaard (1999). The parameters analysed were general appearance, colour, texture, odour, and overall acceptability using a 9-point hedonic scale. Higher scores for each parameter indicated the freshness of the sample.

## Statistical analyses

The data gathered, based on triplicate analyses, were represented as mean  $\pm$  standard deviation. The statistical analyses included the application of the T-test, least significance difference (LSD) test, and analysis of variance (ANOVA) with a significance level of 0.05 using SPSS v. 25.

## Results

### Physiochemical analysis

#### Changes in pH values

On the initial day of storage, the pH of the fish flesh was  $6.23 \pm 0.05$  for both samples (Fig. 1). Significant differences in pH were not detected between AP and VP samples on the initial and 3<sup>rd</sup> day of storage. However, from the 6<sup>th</sup> day of storage, significant differences ( $P < 0.05$ ) emerged. By the 6<sup>th</sup> day, VP samples exhibited a gradual decrease in pH followed by a subsequent increase compared to AP samples. Starting from the 9<sup>th</sup> day, both AP and VP samples showed a gradual pH increase. At the time of sensory rejection, the pH values for AP and VP samples rose to  $7.2 \pm 0.03$  and  $7.0 \pm 0.05$  ( $P < 0.05$ ), respectively.

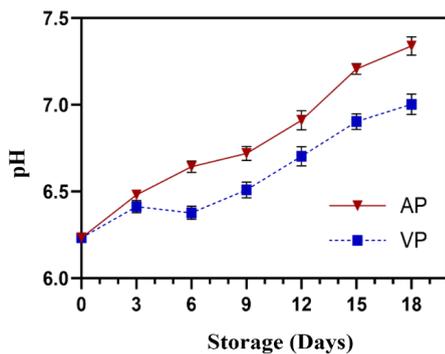
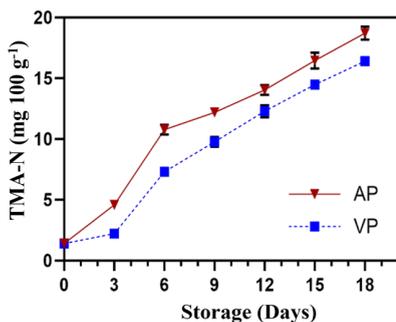


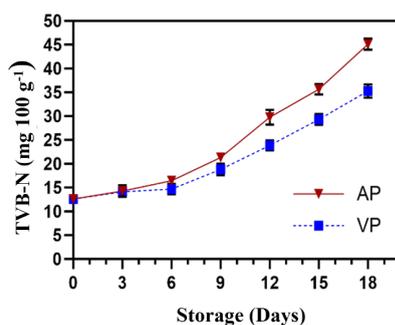
Fig. 1. Changes in mean pH values ( $\pm$  SD) of air packed (AP) and vacuum packed (VP) spotfin flathead *Grammoplites suppositus* during chilled storage over 18 days ( $n = 3$ ).

#### Changes in trimethylamine (TMA) values

Trimethylamine (TMA) formation exhibited a gradual



(a)



(b)

Fig. 2. (a) Changes in mean trimethylamine (TMA) and (b) mean total volatile base nitrogen (TVB-N) values ( $\pm$  SD) of air packed (AP) and vacuum packed (VP) spotfin flathead *Grammoplites suppositus* during chilled storage over 18 days ( $n = 3$ ).

progression during the initial stages, followed by an increase over time for both AP and VP samples. On the initial day, the TMA measured was  $1.14 \pm 0.005$  mg 100 g<sup>-1</sup> (Fig. 2a). A statistically significant difference in TMA content between the AP and VP samples was observed from the 3<sup>rd</sup> day until the end of the iced storage ( $P < 0.05$ ). The VP samples consistently maintained lower TMA levels than AP samples throughout the storage. A sudden increase in TMA values in both AP and VP samples occurred between the 3<sup>rd</sup> and the 6<sup>th</sup> day of storage. The AP samples exceeded the established TMA limit on the 15<sup>th</sup> day, while VP samples reached this limit on the 18<sup>th</sup> day of storage, which corresponded to the sensory rejection point. During the 18<sup>th</sup> day of storage, the TMA values for AP and VP samples were noted as  $18.71 \pm 0.54$  mg 100 g<sup>-1</sup> and  $16.40 \pm 0.38$  mg 100 g<sup>-1</sup> of the sample, respectively ( $P < 0.05$ ).

#### Changes in total volatile base nitrogen (TVBN) values

On the initial day, the total volatile base nitrogen (TVBN) value for the samples was  $12.63 \pm 0.01$  mg 100 g<sup>-1</sup> (Fig. 2b). A marked difference between AP and VP samples persisted from the 9<sup>th</sup> to the 18<sup>th</sup> day ( $P < 0.05$ ). On the 15<sup>th</sup> day, AP samples exceeded the threshold of 30–35 mg 100 g<sup>-1</sup> (Gopakumar, 2002), while VP samples surpassed this limit on the 18<sup>th</sup> day, potentially due to the absence of aerobic bacteria in VP samples, which correlated well with the sensory rejection point. The rise in TVBN levels was in proportion to the TVC counts ( $r = 0.97$ ).

#### Changes in free fatty acids (FFA) values

The free fatty acids (FFA) assessment on the initial day of storage recorded a value of  $0.34 \pm 0.005$  (expressed as % of oleic acid) (Fig. 3a). However, notable distinctions ( $P < 0.05$ ) between the samples commenced from the 9<sup>th</sup> day. During the time of sensory rejection, the FFA values increased up to  $7.84 \pm 0.03$  for AP samples and  $5.43 \pm 0.19$  for VP samples ( $P < 0.05$ ). Throughout the storage duration, it was noted that the FFA levels in both AP and VP samples did not surpass the established limits of acceptability. In this study, FFA values did not serve as a reliable indicator of spoilage, however, they correlated well with the TVBN values ( $r = 0.97$ ).

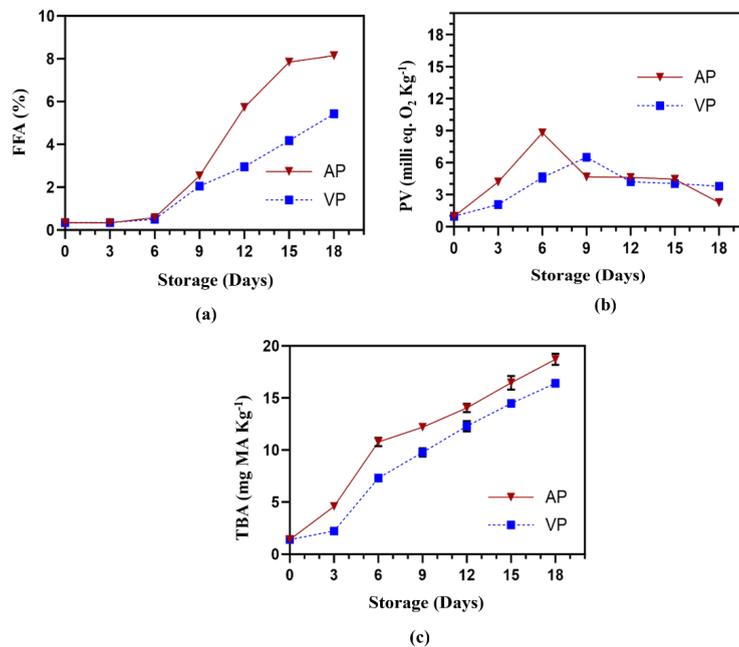


Fig. 3. Changes in mean ( $\pm$  SD) values of (a) free fatty acid (FFA) (b) peroxide value (PV) and (c) thiobarbituric acid value (TBA) of air packed (AP) and vacuum packed (VP) spotfin flathead *Grammoplites suppositus* during chilled storage over 18 days ( $n = 3$ ).

### Changes in peroxide values (PV)

The peroxide values exhibited a distinctive pattern during storage, with an initial value of  $0.96 \pm 0.02$  milli equivalent  $O_2 \text{ kg}^{-1}$  gradually increasing until the 6<sup>th</sup> day for AP samples and 9<sup>th</sup> day for VP samples, respectively (Fig. 3b). A significant ( $P < 0.05$ ) difference between the peroxide values of AP and VP samples was evident as early as the 3<sup>rd</sup> day. A sharp decline in PV occurred between the 9<sup>th</sup> and 18<sup>th</sup> day of chilled storage for the AP samples and between the 12<sup>th</sup> to 18<sup>th</sup> day of storage for the VP samples (Fig. 3b). The PV did not exceed  $20 \text{ meq } O_2 \text{ kg}^{-1}$  for AP and VP samples during the entire storage duration.

### Changes in thiobarbituric acid (TBA) values

The gradual increase in thiobarbituric acid (TBA) values during the iced storage of AP and VP samples is depicted in Figure 3c. Initially, the TBA value was  $0.42 \pm 0.005 \text{ mg malonaldehyde kg}^{-1}$ . However, from the 9<sup>th</sup> day, a noteworthy distinction ( $P < 0.05$ ) between the TBA values of AP and VP samples was observed, with AP samples dominating the TBA levels. Usually, TBA values within the range of 1–2 mg malonaldehyde  $\text{kg}^{-1}$  of fish muscle are considered the threshold at which an unpleasant odour develops (Gopakumar, 2002). On the 18<sup>th</sup> day, the TBA values of AP and VP samples were recorded as  $2.07 \pm 0.08$  and  $1.6 \pm 0.05$ , respectively ( $P < 0.05$ ).

### Changes in colour and texture profile

The changes in colour values of the sample are depicted in Table 1. The colour of the fish flesh turned from white to yellowish during the storage period. Across the storage period,  $L^*$  values showed a decreasing pattern, with a significant distinction ( $P <$

$0.05$ ) between the samples from the 3<sup>rd</sup> day. The  $a^*$  values showed a decreasing trend whereas  $b^*$  values exhibited a fluctuating pattern. However, the  $L^*$ ,  $a^*$ , and  $b^*$  values remained higher for VP samples than AP samples (Table 1). The shifts in texture profile values of both samples are depicted in Table 2. The VP samples consistently showed higher values for hardness than AP samples during the chilled storage. Cohesiveness also decreased during the entire storage period for both AP and VP samples. Springiness declined in both samples during the chilled storage. Gumminess and chewiness also decreased (Table 2), with VP samples showing higher values than AP samples.

### Changes in microbial count

By the 15<sup>th</sup> and 18<sup>th</sup> days, the TVC counts of AP samples reached  $8.21 \pm 0.01 \text{ log cfu g}^{-1}$ , and VP samples reached  $7.14 \pm 0.03 \text{ log cfu g}^{-1}$  ( $P < 0.05$ ) (Fig. 4a), exceeding the acceptable limit of  $10^7 \text{ cfu g}^{-1}$  (ICMSF, 1986). This coincided with the sensory rejection point and the increase in volatile base production ( $r = 0.92$ ). The psychrotrophic counts for AP samples and VP samples reached  $9.2 \pm 0.02 \text{ log cfu g}^{-1}$  and  $8.11 \pm 0.06 \text{ log cfu g}^{-1}$ , respectively, on the 15<sup>th</sup> day of storage. However, on the 18<sup>th</sup> day, it increased to  $9.34 \pm 0.04 \text{ log cfu g}^{-1}$  for AP samples and  $8.54 \pm 0.04 \text{ log cfu g}^{-1}$  for VP samples ( $P < 0.05$ ) (Fig. 4b). Over the entire storage duration, LAB counts remained  $< 10 \text{ cfu g}^{-1}$  in both AP and VP samples (Fig. 5a). *Pseudomonas* count of  $5.17 \text{ log cfu g}^{-1}$  and  $4.63 \text{ log cfu g}^{-1}$  was reported for AP and VP samples, respectively on the day of sensory rejection (Fig. 5b). The  $H_2S$ -producing bacterial counts were  $5.63 \text{ log cfu g}^{-1}$  and  $4.63 \text{ log cfu g}^{-1}$  for AP and VP samples, respectively (Fig. 5c). The initial count of *Brochothrix* for AP and VP samples were  $4.58 \text{ log cfu g}^{-1}$  and  $4.57 \text{ log cfu g}^{-1}$ , respectively. At the time of sensory rejection,

Table 1. Changes in mean ( $\pm$  SD) values for lightness (L\*), red-greenness (a\*) and yellow-blueness (b\*) of air packed (AP) and vacuum packed (VP) spotfin flathead *Grammoplites suppositus* during chilled storage. Values with different superscripts (a, b) for AP and VP samples indicate significant differences based on the least significance difference (LSD) test (n = 3).

Storage (Days)	Sample	L*	a*	b*
0	AP	58.51 $\pm$ 0.10 <sup>a</sup>	8.71 $\pm$ 0.05 <sup>a</sup>	10.51 $\pm$ 0.06 <sup>a</sup>
	VP	58.51 $\pm$ 0.10 <sup>a</sup>	8.96 $\pm$ 0.02 <sup>b</sup>	9.54 $\pm$ 0.03 <sup>b</sup>
3	AP	57.82 $\pm$ 0.06 <sup>a</sup>	8.33 $\pm$ 0.07 <sup>a</sup>	10.27 $\pm$ 0.03 <sup>a</sup>
	VP	58.58 $\pm$ 0.03 <sup>b</sup>	8.53 $\pm$ 0.04 <sup>b</sup>	9.23 $\pm$ 0.02 <sup>b</sup>
6	AP	56.38 $\pm$ 0.07 <sup>a</sup>	7.75 $\pm$ 0.03 <sup>a</sup>	11.82 $\pm$ 0.07 <sup>a</sup>
	VP	57.84 $\pm$ 0.02 <sup>b</sup>	6.08 $\pm$ 0.04 <sup>b</sup>	10.62 $\pm$ 0.03 <sup>b</sup>
9	AP	52.52 $\pm$ 0.04 <sup>a</sup>	-2.41 $\pm$ 0.06 <sup>a</sup>	9.65 $\pm$ 0.02 <sup>a</sup>
	VP	53.32 $\pm$ 0.42 <sup>b</sup>	5.90 $\pm$ 0.04 <sup>b</sup>	9.61 $\pm$ 0.07 <sup>a</sup>
12	AP	48.54 $\pm$ 0.08 <sup>a</sup>	-2.50 $\pm$ 0.11 <sup>a</sup>	10.59 $\pm$ 0.02 <sup>a</sup>
	VP	50.66 $\pm$ 0.52 <sup>b</sup>	-2.38 $\pm$ 0.02 <sup>a</sup>	10.41 $\pm$ 0.05 <sup>b</sup>
15	AP	48.65 $\pm$ 0.10 <sup>a</sup>	-1.74 $\pm$ 0.03 <sup>a</sup>	12.13 $\pm$ 0.11 <sup>a</sup>
	VP	47.58 $\pm$ 0.03 <sup>b</sup>	-2.20 $\pm$ 0.03 <sup>b</sup>	12.27 $\pm$ 0.06 <sup>a</sup>
18	AP	47.16 $\pm$ 0.57 <sup>a</sup>	-1.56 $\pm$ 0.04 <sup>a</sup>	10.36 $\pm$ 0.09 <sup>a</sup>
	VP	48.89 $\pm$ 0.29 <sup>b</sup>	-1.35 $\pm$ 0.03 <sup>b</sup>	9.87 $\pm$ 0.06 <sup>b</sup>

Table 2. Changes mean ( $\pm$  SD) values for the texture profile values (hardness, cohesiveness, springiness, chewiness and gumminess) of air packed (AP) and vacuum packed (VP) spotfin flathead *Grammoplites suppositus* during chilled storage. Values with different superscripts (a, b) for AP and VP samples indicate significant differences based on least significance difference (LSD) test (n = 3).

Storage (Days)	Sample	Hardness (kgf)	Cohesiveness	Springiness (mm)	Chewiness (kgf.mm)	Gumminess (N)
0	AP	18.36 $\pm$ 1.05 <sup>a</sup>	0.28 $\pm$ 0.04 <sup>a</sup>	0.96 $\pm$ 0.01 <sup>a</sup>	4.59 $\pm$ 0.03 <sup>a</sup>	4.19 $\pm$ 0.12 <sup>a</sup>
	VP	20.86 $\pm$ 1.56 <sup>b</sup>	0.30 $\pm$ 0.05 <sup>a</sup>	0.98 $\pm$ 0.01 <sup>a</sup>	5.05 $\pm$ 0.13 <sup>b</sup>	4.40 $\pm$ 0.21 <sup>a</sup>
3	AP	15.79 $\pm$ 1.17 <sup>a</sup>	0.27 $\pm$ 0.02 <sup>a</sup>	0.88 $\pm$ 0.05 <sup>a</sup>	4.24 $\pm$ 0.26 <sup>a</sup>	3.96 $\pm$ 0.42 <sup>a</sup>
	VP	20.28 $\pm$ 1.63 <sup>b</sup>	0.29 $\pm$ 0.03 <sup>a</sup>	0.90 $\pm$ 0.02 <sup>a</sup>	5.17 $\pm$ 0.39 <sup>b</sup>	4.23 $\pm$ 0.10 <sup>a</sup>
6	AP	15.99 $\pm$ 1.54 <sup>a</sup>	0.21 $\pm$ 0.03 <sup>a</sup>	0.80 $\pm$ 0.16 <sup>a</sup>	4.10 $\pm$ 0.30 <sup>a</sup>	3.61 $\pm$ 0.19 <sup>a</sup>
	VP	18.52 $\pm$ 0.95 <sup>b</sup>	0.25 $\pm$ 0.05 <sup>a</sup>	0.82 $\pm$ 0.04 <sup>a</sup>	4.76 $\pm$ 0.42 <sup>b</sup>	4.04 $\pm$ 0.37 <sup>a</sup>
9	AP	13.57 $\pm$ 1.41 <sup>a</sup>	0.20 $\pm$ 1.02 <sup>a</sup>	0.66 $\pm$ 0.05 <sup>a</sup>	3.89 $\pm$ 0.62 <sup>a</sup>	3.32 $\pm$ 0.26 <sup>a</sup>
	VP	16.61 $\pm$ 0.80 <sup>b</sup>	0.22 $\pm$ 0.02 <sup>a</sup>	0.80 $\pm$ 0.06 <sup>b</sup>	4.39 $\pm$ 0.29 <sup>a</sup>	3.97 $\pm$ 0.49 <sup>a</sup>
12	AP	12.66 $\pm$ 2.35 <sup>a</sup>	0.17 $\pm$ 0.03 <sup>a</sup>	0.58 $\pm$ 0.14 <sup>a</sup>	3.75 $\pm$ 0.47 <sup>a</sup>	3.29 $\pm$ 0.05 <sup>a</sup>
	VP	15.51 $\pm$ 0.87 <sup>b</sup>	0.21 $\pm$ 0.02 <sup>a</sup>	0.73 $\pm$ 0.09 <sup>a</sup>	4.09 $\pm$ 0.56 <sup>a</sup>	3.94 $\pm$ 0.23 <sup>b</sup>
15	AP	11.97 $\pm$ 1.68 <sup>a</sup>	0.15 $\pm$ 0.03 <sup>a</sup>	0.50 $\pm$ 0.13 <sup>a</sup>	3.30 $\pm$ 0.20 <sup>a</sup>	3.10 $\pm$ 0.39 <sup>a</sup>
	VP	14.48 $\pm$ 1.02 <sup>a</sup>	0.17 $\pm$ 0.12 <sup>a</sup>	0.71 $\pm$ 0.04 <sup>a</sup>	3.95 $\pm$ 0.61 <sup>a</sup>	3.52 $\pm$ 0.17 <sup>a</sup>
18	AP	10.18 $\pm$ 2.01 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>a</sup>	0.35 $\pm$ 0.20 <sup>a</sup>	2.87 $\pm$ 0.26 <sup>a</sup>	2.75 $\pm$ 0.47 <sup>a</sup>
	VP	12.44 $\pm$ 1.54 <sup>a</sup>	0.14 $\pm$ 0.02 <sup>a</sup>	0.53 $\pm$ 0.11 <sup>a</sup>	3.59 $\pm$ 0.38 <sup>a</sup>	3.17 $\pm$ 0.14 <sup>a</sup>

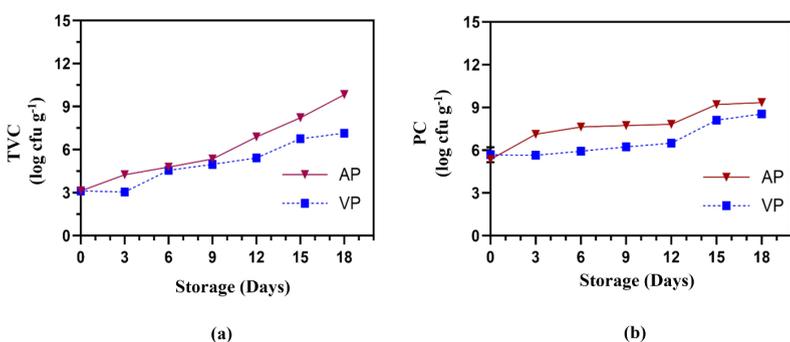


Fig. 4. Changes in mean ( $\pm$  SD) values for (a) total viable count (TVC), (b) psychrotrophic count (PC) during chilled storage of spotfin flathead *Grammoplites suppositus* in air packed (AP) and vacuum packed (VP) conditions (n = 3).

AP and VP samples reached a value of 6.68 log cfu g<sup>-1</sup> and 6.17 log cfu g<sup>-1</sup>, respectively ( $P < 0.05$ ) (Fig. 5d), which revealed that *Brochothrix* constitutes a relevant spoilage bacterium even in vacuum-packed seafood.

### Changes in sensory scores

The changes in sensory scores of the fish during chilled storage are given in Figure 6. Over the initial days of storage, the fish exhibited an elastic texture

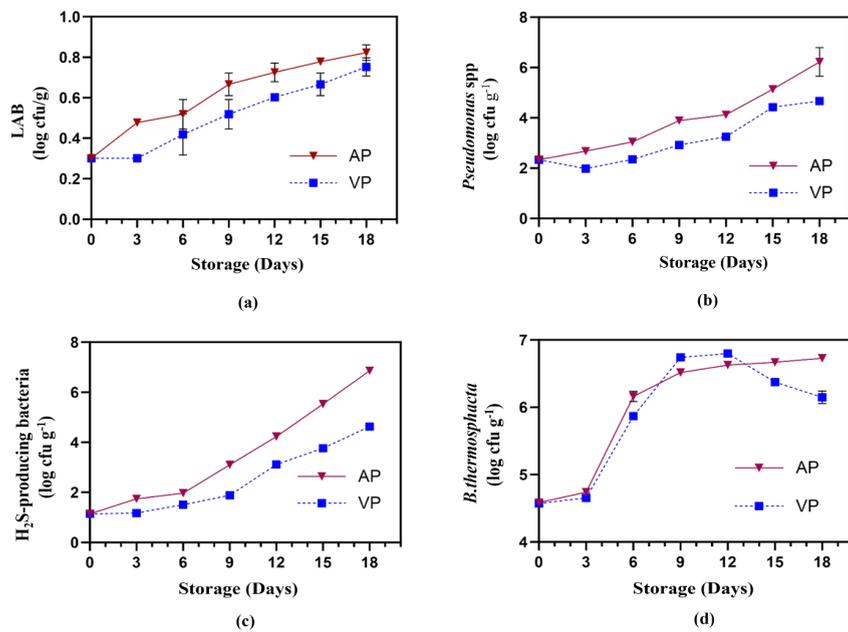


Fig. 5. Changes in mean (± SD) values (a) lactic acid bacteria (LAB) (b) *Pseudomonas* spp. (c) H<sub>2</sub>S producing bacteria, and (d) *Brochothrix thermosphacta* counts during chilled storage of spotfin flathead *Grammoplites suppositus* in air packed (AP) and vacuum packed (VP) conditions (n = 3).

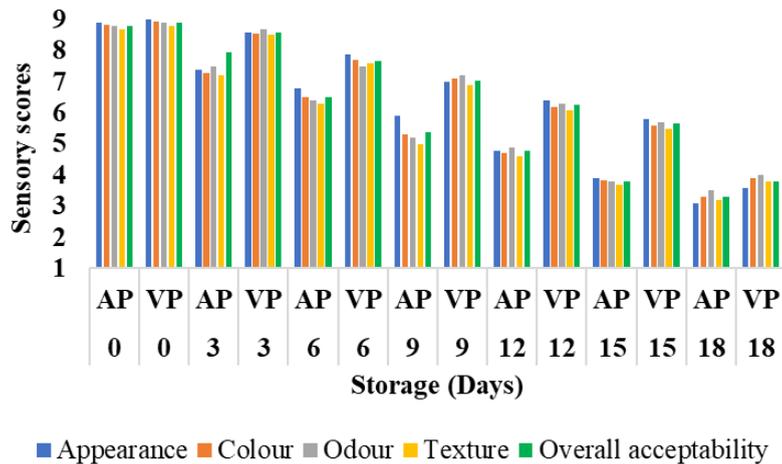


Fig. 6. Changes in mean (± SD) values for sensory scores of spotfin flathead *Grammoplites suppositus* in air packed (AP) and vacuum packed (VP) conditions during chilled storage (n = 3).

and possessed a fresh white appearance with an overall acceptability score of 8.8. However, sensory changes, especially off odours pronounced quickly in the AP samples. The colour of fish flesh changed to yellow, and the texture scores dropped for the AP samples by the 12<sup>th</sup> day of chilled storage. However, the VP samples remained fresh up to the 15<sup>th</sup> day of storage. On the nine-point hedonic scale, a score on or below "4" indicates a 'dislike', which was taken as the limit of sensory rejection during the study, according to Monteiro et al. (2013). The sensory scores of AP and VP samples dropped to 3.4 and 3.8 by the 15<sup>th</sup> and 18<sup>th</sup> day, respectively. The sensory rejection of the samples correlated well with the TVC values. Considering the microbiological and sensory limits, the shelf life of spotfin flathead was 12–15 days for VP and 15–18 days for AP samples.

## Discussion

The changes in quality attributes of air and vacuum-packed *G. suppositus* during chilled storage were evaluated. From the findings, it was evident that vacuum packaging combined with chilled storage improved the storage stability of gutted spotfin flathead, as confirmed through physicochemical, microbiological and sensory evaluation. According to Patil et al. (2020), vacuum packaging is widely used in the food sector, as it maintains the quality attributes of food products by reducing shrinkage, preserving colour and delaying spoilage. Chilled storage is a popular method to preserve the quality of seafood by reducing bacterial proliferation, thereby increasing the storage life (Manju et al., 2007). Reports are available regarding the importance of vacuum packing in extending the shelf life of fish in chilled, refrigerated

and frozen conditions (Aberoumand and Baesi, 2020; Nguyen et al., 2023; Babić Milijašević et al., 2023).

## Physiochemical parameters

The deterioration in fish quality is associated with the formation of various undesirable compounds through a series of physiochemical and microbiological changes during storage (Ocano-Higuera et al., 2011). In the study, vacuum packaging showed a significant effect on all the physiochemical quality parameters of the fish. The reduced TVBN content observed in VP samples (Fig. 2b) can be attributed to the limited oxygen presence in the vacuum pack, which inhibited microbial growth and the generation of volatile compounds. Similar observations were reported by Rodrigues et al. (2016) during vacuum-packed storage of rainbow trout. Similarly, a gradual reduction of TMA content in VP samples (Fig. 2a) was noticed with increasing storage time. This might be due to the inhibition of spoilage bacteria such as *Shewanella*, *Photobacterium*, *Alteromonas* and Enterobacteriaceae, responsible for the conversion of trimethylamine oxide (TMAO) into TMA (Huss, 1995). According to Summers et al. (2017), the enzymatic and non-enzymatic degradation of TMAO generates TMA, dimethylamine (DMA) and formaldehyde (FA), which contribute to the quality losses in fish products stored under chilled and frozen conditions. The changes in TMA and TVBN content during the study were consistent with Binsi et al. (2015) who highlighted the effect of chilled storage in retarding spoilage. The accumulation of TMA-N and TVBN compounds led to an increase in the pH of the fish muscle, which accelerated bacterial proliferation, leading to a decreased shelf life of the AP samples, aligning with the findings of Sallam (2008). Conversely, the anoxic condition in VP samples caused the pH values to be persistently lower, due to the effective inhibition of aerobic bacterial growth (Fig. 1).

Lipid oxidation leads to quality losses in fish, characterised by the production of off-flavour and odour, reduction of shelf life and reduced nutritional value (Secci and Parisi, 2016). According to Hernandez et al. (2009), storage temperature, packaging condition (Jezek and Buchtova, 2007) and amount of fat in tissue are the main factors responsible for lipid oxidation in fish. In the study, the VP samples exhibited lower peroxide values (Fig. 3b), highlighting the role of packaging in reducing lipid oxidation. The decrease in PV in both samples towards the end of storage can be attributed to the breakdown of hydroperoxides into aldehydes and ketones, contributing to the development of undesirable odorous compounds (Hamilton et al., 1997). The increased TBA content, a secondary product of lipid oxidation, was observed more in AP samples because of the oxidation of unsaturated fatty acids over the storage duration (Fig. 3c). According to Secci and Parisi (2016), the susceptibility of polyunsaturated fatty acids to oxidative degradation increases the TBA value, which

could be mitigated by natural antioxidants, metal-chelating compounds, and ideal storage conditions. Even though the TBA values in VP samples were within the limit of acceptability over the storage duration, the minor TBA content detected in VP samples could be attributed to the accidental transmission of oxygen into the pack from the atmosphere (Mohan et al., 2019).

However, TBA values cannot accurately provide the rate of lipid oxidation as malonaldehyde has the potential to react with other constituents leading to lesser TBA values (Ozogul et al., 2011). However, TBA value is a useful index due to its correlation with the sensory attributes during the storage of fish products as reported by Li et al. (2023). Furthermore, lipid oxidation and hydrolysis produce free fatty acids, which are undesirable as they can be transformed into odorous volatile compounds. The lesser FFA content in VP samples is due to the inhibition of lipolytic aerobic bacteria, thus minimising lipid hydrolysis, as documented by Mohan et al. (2019). A marked difference in FFA values between AP and VP samples can be linked to increased bacterial activity and the production of numerous volatile bases in the AP samples which was resisted in the vacuum packaged samples (Fig. 3a).

The change in colour of fish muscle during storage is an important factor attributed to the autolytic, microbial and lipid oxidation occurring in fish muscle leading to chromatophore disruption (Lakshmanan et al., 2002). The colour of the fish muscle turned from white to yellowish, more pronounced in the AP samples as the storage progressed. However, the  $L^*$ ,  $a^*$  and  $b^*$  values indicated that the VP samples during chilled storage exhibited better colour retention compared to AP samples (Table 1). The results were in line with Chowdhury et al. (2017) for vacuum-packed seabass fillets. The texture of the fish muscle is a significant factor that determines the quality and acceptability of fish. The factors influencing the texture of fish muscle include the rate and extent of post-mortem muscle shortening, post-mortem reduction in pH, and proteolysis causing the breakdown of myofibrillar protein (Huss, 1995). The hardness values of the samples declined with storage time (Table 2). This decrease is due to the weakening of connective tissue, and an increase in microbial load, affecting pH and water-holding capacity (Manju et al., 2007). The VP samples consistently showed higher values for hardness than AP samples, aligning with Genc et al. (2013) for vacuum-packaged meagre fillets.

Cohesiveness did not show significant differences during the entire storage period between AP and VP samples, consistent with the findings of Hernandez et al. (2009) for meagre fillets. Springiness was lower in the AP samples due to reduced fish muscle elasticity during chilled storage (Manju et al., 2007). Gumminess and chewiness also showed higher values for VP samples than AP samples, aligning with the findings on vacuum-packed seabream fillets (Ayala et al., 2011).

Overall, vacuum packaging of fish samples combined with chilled storage had a beneficial effect on the texture profile, maintaining their original characteristics similar to the observations made by Chowdhury et al. (2017). Hansen et al. (2009) reported that the vacuum-packed samples of Atlantic salmon fillets appeared to be even firmer than the samples packed under modified atmospheric pressure.

## Microbiological parameters

Microbial spoilage of fish during chilled storage leads to the production of various odd-flavoured compounds, including biogenic amines, sulphides, organic acids, alcohols, and ketones (Dalgaard et al., 2006). In the study, TVC was retarded in the VP samples compared to AP samples (Fig. 4a). The results confirm the findings of Esteves et al. (2021) and Genc et al. (2013) during low-temperature storage of fish. During storage, the carbon dioxide content in the vacuum pack rises as a result of the surface bacteria utilising the residual oxygen in the pack and the metabolic processes occurring in the fish muscle. This reduces the growth of gram-negative bacteria in vacuum-packed samples as reported by Eustace (1981). The psychrotrophic bacteria consistently dominated the bacterial population for both AP and VP samples throughout the storage period (Fig. 4b). Similar findings were observed by Sivertsvik et al. (2002) during chilled storage of cod, where psychrotrophic bacteria became predominant within the microbial community after 1–2 weeks.

However, vacuum packaging slowed down the proliferation of psychrotrophic bacteria compared to the AP samples. Over the entire storage duration, lower LAB counts were noticed in both samples (Fig. 5a). This might be due to the dominance of psychrotrophic bacteria over the mesophilic LAB during storage, as reported by Jorgensen and Huss (1989). During the storage period, there was a gradual increase in *Pseudomonas* count, observed in both AP and VP samples (Fig. 5b), similar to the findings of Amegovu et al. (2012) during chilled storage of Nile perch. A slow rise in pH levels and low carbohydrate availability during storage may have promoted the growth of *Pseudomonas* and other pH-sensitive psychrotrophic bacteria in the fish samples (Francoise, 2010).

The results of the current study agree with the findings of Zhang et al. (2015) who reported significantly lower counts of *Pseudomonas* in vacuum-packed common carp compared to air pack samples during storage at 4 °C. The H<sub>2</sub>S-producing bacteria was also lower in the VP samples throughout the storage period (Fig. 5c). A similar retarding count of sulphide-producing bacteria was observed in vacuum packed triggerfish fillets stored at refrigeration temperature as reported by Esteves et al. (2021). This reduction in the growth of H<sub>2</sub>S-producing bacteria could be due to the siderophores produced by *Pseudomonas*, which

retarded the growth of H<sub>2</sub>S-producing bacteria, as reported by Chytiri et al. (2004) in rainbow trout. *Brochothrix thermosphacta* is a major spoilage bacterium occurring in raw meat, packed either in aerobic or vacuum-packed condition (Feiner, 2006). In this study, a rapid increase in *B. thermosphacta* counts was noticed in VP samples until the 12<sup>th</sup> day of storage after which it dominated in the AP samples. The aerobic metabolism of glucose by *B. thermosphacta* leads to sour odour generation whereas under anaerobic conditions it produces lactic acid, ethanol and certain fatty acids, causing off flavour (Dainty and Hofman, 1983). Similar observation on the dominance of *B. thermosphacta* during chilled storage under various packaging conditions was reported by Mohan et al. (2010).

## Sensory parameters

The sensory attributes declined in both AP and VP samples over the storage period (Fig. 6). However, the VP samples consistently exhibited higher organoleptic scores than AP samples throughout the storage period. The vacuum packaging showed efficacy in reducing texture deterioration compared to the AP samples, aligning with the observations of Chowdhury et al. (2017). The VP samples maintained sensory acceptability for up to 18 days, surpassing the AP samples, which were acceptable only up to 15 days of storage. In this study, the sensory rejection correlated well with TVBN and TVC. Comparable findings were reported by Remya et al. (2018) where the sensory score was in good accordance with the microbial counts and volatile base production. Vacuum packaging combined with chilled storage extended the shelf life of *G. suppositus* to 15–18 days, compared to 12–15 days for air packed samples. This exceeds the 11–13 day shelf life reported by Genc et al. (2013) for meagre *Argyrosomus regius* fillets and is comparable to the 16 days shelf life of mackerel (Scombridae) during chilled vacuum-packed storage (Viji et al., 2016).

## Conclusion

In the study, significant changes in the physiochemical, microbiological, and sensory attributes of air and vacuum-packed gutted *G. suppositus*, during chilled storage were analysed. Gutting and vacuum packaging, along with chilled storage (2–4 °C), extended the shelf life of *G. suppositus*. The VP samples maintained a shelf life of 15–18 days, compared to the 12–15 days in the AP samples. Gutting and low temperature storage delayed spoilage in AP and VP samples. However, vacuum packaging further retarded volatile base production, lipid oxidation, and microbial growth, contributing to an extended shelf life in VP samples, as confirmed through sensory analysis. These findings demonstrate that combining vacuum packaging with chilled storage can be a useful strategy for maintaining *G. suppositus* quality and increasing its storage stability. As an underutilised fish species, this research marks a

significant step towards the understanding of its post-harvest stability, facilitating its preservation for a longer period.

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