



Influence of Treatment with Sodium Benzoate and Packaging on quality of Catla (*Catla catla*) steaks during Chilled Storage

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ABSTRACT

Catla (*Catla catla*) steaks treated with sodium benzoate (SB) 0.5-2 % packed under aerobic (AP) and vacuum packaging (VP) were evaluated for biochemical, microbiological and sensory quality changes during chilled storage conditions for 27 days. The results were plotted against time, determining best fit order with corresponding regression equation to predict and compare with experimental findings. The formation of volatile amines (TVB-N) was low in samples treated with SB under VP, while no significant difference was observed in pH values. Peroxide value and salt soluble nitrogen values were significantly influenced under VP and SB treatment. Total plate count values significantly increased in all samples, but were within the maximum permissible limits. *E. coli*, *S. aureus* and *Salmonella* were not detected in the samples during storage. Zero order kinetics exhibited best fit for changes in biochemical and microbiological quality. Sensory evaluation scores had high correlation with storage period and low relative error (P_0) for control samples under AP and VP. Therefore, combination of SB at 2.0% and VP can be used effectively as an intervention for the preservation of chilled stored Catla steaks.

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Introduction

Fish is regarded as one of the major source of quality animal proteins, rich in PUFA, with several health benefits within purchase reach of common man, said importance has led to increase in fish consumption globally (FAO 2006). Fish is highly perishable, due to continuous action of several endogenous enzymes and microorganisms that degrade proteins and develop fat rancidity causing rapid deterioration (Rodrigues et al., 2016). Catla, a low fat fish, is rich in several omega-3 fatty acids & amino acids mostly consumed fresh or processed into various value added products (Pawar et al., 2019; Mohanty et al., 2014).

Global freshwater aquaculture production has registered a ten-fold growth during past decades. Catla (*Catla catla*) is a prominent member of Indian major carps (IMC). It contributes majority among the cultured freshwater fish production with more preference to fresh fish with moderate size catch (1-2 Kg). The compatibility of Catla in polyculture is well established & its low tropic feeding habit reduces the cost of production and makes Catla the best choice for aquaculture.

Catla is generally sold in fresh form or transported to fish deficit areas about 2000-3000 km distant places from the production site. India is the largest Catla producer globally, producing 2.96 million tonnes, with an incremental production of 40% (FAO 2012; FAO-FishStat 2019). However, no post harvest processing & value addition facilities are available in Catla (FAO 2006). Moreover, there is lack of information about the quality changes of Catla steaks (FAO 2006).

Demand for fresh or minimally processed seafood is on the rise globally & domestically. The increasing demand for fresh fish has intensified the search for methods & technologies for maintaining freshness & quality preservation. Recently, food packaging has developed due to increased demands on product safety, shelf life extension, cost-efficiency, environmental issues & consumer convenience (Pagarkar et al., 2015). Vacuum packaging, is a well-established technology, helps extend shelf life by eliminating air (especially oxygen) from the package (Rodrigues et al. 2016).

Sodium benzoate is categorised under sodium salt, widely used as a food preservative. It is classified among the generally recognised as safe (GRAS) list of preservatives & first chemical preservative permitted by United States Food & Drug Administration for food application (Wibbertmann et al. 2000). The preservative & antimicrobial property of sodium benzoate has been demonstrated by several studies earlier (Lee et al., 1965; Mirshekari et al., 2016; Shahmohammadi et al., 2016).

The current study examines the influence of sodium benzoate treatment on Catla fish steaks packed aerobically & vacuum on sensory, biochemical & microbiological quality during chilled storage (0 to -2°C). Experimental

data were used to derive regression equations based on best fit order of reaction for changes in quality helpful in predicting shelf life.

Materials and Methods

Sample Preparation

Freshly caught Catla (*Catla catla*) fishes were, iced immediately (1:1) packed in insulated boxes & transferred immediately to the laboratory (within 4h). The average weights of fishes were 1.2 to 1.5 kg & 35 to 45 cm length. Upon arrival, fishes were prepared as shown in Figure 1, similar to our earlier publication Kedar et al. (2016).

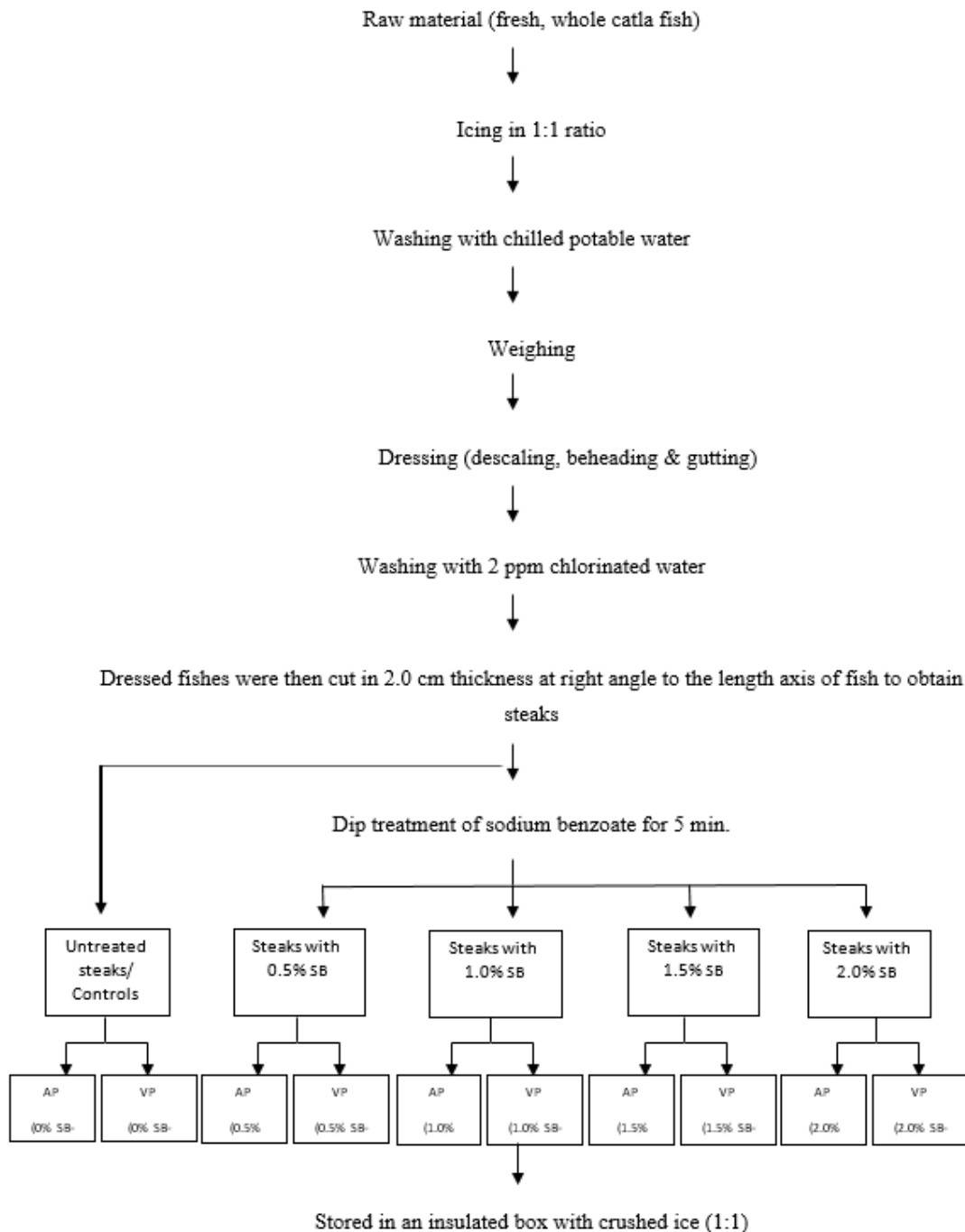


Figure 1. Flow chart for sample preparation

Briefly, fishes were washed thoroughly, dressed & washed with 2 ppm chlorinated water. Fishes were cut into steaks of 1.5 to 2.0cm thickness using entire dressed fish. Further steaks were divided into 10 batches for different treatments using food grade sodium benzoate (SB) (Ms. Thomas Baker (chemicals) Ltd. Mumbai) of appropriate concentration by dipping for 5 min at ambient conditions & 3 steaks were packed in one bag aerobically (AP) using sealing machine & vacuumed (VP) by vacuum sealing machine (Model FV-400) procured from Ms. M. M. M. Buxabhoj & Co., Mumbai, in nylon laminated polyethene bags (30 x 12 cm) having 200 g capacity.

Samples were labelled as 0% SB-AP (acted as control for air packaging), 0.5% SB-AP, 1.0% SB-AP, 1.5% SB-AP, 2.0% SB-AP, 0% SB-VP (acted as control for vacuum packaging), 0.5% SB-VP, 1.0% SB-VP, 1.5% SB-VP, 2.0% SB-VP. Immediately after packaging, samples were iced in the ratio of 1:1 (fish steak: ice) in insulated boxes. Firstly a 5 cm thick layer of crushed ice was put at the bottom, then packs containing the sample & ice were put alternatively & the uppermost portion covered with a layer of ice. Insulated fish boxes were placed at room temperature, with outlet at the bottom facilitating removal of water from the melting ice. Re-icing was done every day after proper draining of ice melt water to supplement the loss due to melting. In India, majority of fishes are preserved using ice due to its practicability as well as economic aspect & lacking proper supply chain for local market. The storage was conducted in insulated boxes using crushed ice (Prabhakar et al. 2019). Total of 30 pouches for each treatment were prepared & stored at chilled conditions, samples were drawn at random at every 3 days interval analysed for sensory, biochemical & microbiological quality.

Sensory Evaluation

Sensory evaluation was carried out by ten panellists based on appearance, colour, texture, odour, taste & overall acceptability on 9 point hedonic scale with 9 (highest quality score representing like extremely) to 1 (lowest suggesting dislike extremely) & score of < 5 was regarded as score for unacceptability (ISI, 1975). Samples were drawn at random for evaluation from each treatment, while for taste evaluation samples were removed from packaging material and cooked in boiling 2% salt solution for 5 minutes followed by cooling to room temperature & presented in coded plates.

Biochemical Composition

The samples drawn at random from different treatment was analysed for changes in proximate composition. The moisture content was determined by drying the sample ($100 \pm 5^\circ\text{C}$) using hot air oven until the difference in successive reading is not more than one mg. Protein content of sample was estimated using standard Kjeldahl method. While, crude fat content was estimated by extraction with petroleum ether as solvent using soxhlet apparatus & ash content by incinerating sample at 550°C for 5hr. using muffle furnace AOAC (2005). Changes in pH were measured using a digital pH meter, from filtrated of homogenized 5g sample in 20ml distilled water AOAC (2005). Total volatile basic nitrogen (TVB-N) content of the Catla samples was determined using Conway micro

diffusion units as described by Beatty & Gibbons (1937) and results were expressed as nitrogen mg/100g of sample. Peroxide value (PV) used for measuring lipid rancidity as per AOAC (2005) briefly, samples treated with saturated potassium iodide solution, causing liberation of iodine from peroxides determined by titrating with sodium thiosulphate using starch indicator and results were expressed as milli-equivalent of O_2/kg of sample. Salt soluble nitrogen (SSN) was extracted as described by Dyer, French & Snow (1950). Briefly, 10g of fish sample homogenized in 90ml Dyer's buffer consisting of 0.02M sodium bicarbonate (pH 7.5). The homogenate was decanted, the filtrate thus obtained by analysed for nitrogen content & expressed as mg of nitrogen/100g.

Microbiological Analysis

Microbiological analysis was conducted by homogenizing 10 g of sample with physiological saline (0.85% NaCl) 90 ml. The homogenized samples were further diluted progressively for required concentration. Total plate count (TPC) was determined by spreading 1ml of homogenate on petri plate containing plate count agar (PCA) performing incubation at 35°C for 48 h. *Escherichia. coli* was evaluated using Tergitol-7 agar medium by pour plate method & incubation at 35°C for 24 h. For *S. aureus* & *Salmonella* enumerations, Baird Parker agar (BPA) & Bismuth Sulphate agar (BSA) were used with incubation at 35°C for 48 h (AOAC 1995). After incubation, plates exhibiting typical colony characters were counted & multiplied with dilution factor reciprocal, for arriving at log CFU/g values.

Data Analyses

All experiments were performed in triplicate unless and otherwise stated. The readings were obtained as a mean of minimum three readings. The obtained data was analysed with Analysis of Variance (ANOVA) tool available in MS-Excel 2013, significant difference (95% level of confidence; $P < 0.05$) and post hoc tested by applying Student-Newman-Keuls (SNK) test. Furthermore, values of different measured indices were plotted versus time during the study to determine the best fit order of reaction to obtain equation & predict the shelf life using the equation given by Mizrahi (2004) as given below. Where, t_s being shelf life, D the index of deterioration, D_0 initial level of index of deterioration & K forming the kinetic constant.

$$t_s = \frac{D - D_0}{K}$$

Results and Discussion

Changes in Proximate Composition

Fresh Catla fish muscle had 79.26% moisture, 18.55% crude protein, 1.2% fat & 0.99% ash content. The changes in the proximate composition of the Catla steaks during 27 days of chilled storage as presented in Table 1. The results indicated Catla to be low fat fish with high protein content in agreement with previous findings of Mohanty et al. (2014), making it a popular choice amongst health conscious consumers. Hence, assessing quality characters & prolonging the shelf life with acceptable quality is important. The results of proximate composition indicated

slight but non-significant increase in moisture content. Rate of increase in moisture content was inversely related with concentration of SB. The possible reason for this slight increase in moisture might be the improved water imbibing capacity of proteins due to lowered denaturation, matching the changes in pH. Protein content reduced significantly in all samples, but at varying rates which were positively influenced by increase in SB concentration and VP condition. While, fat and ash composition increased slightly, but the rates were rapid for AP in comparison to VP sample. The slight variation among the reported findings of Memon et al. (2011) & Bhattacharyya et al. (2019) might be due to changes in feeding habits, season of catch & slaughtering practices followed.

Sensory Evaluation

Sensory evaluation is an important & relevant evaluation parameter which provides the actual quality preferences of the evaluator. The freshly prepared Catla steak samples were characterized by typical fish odour with pinkish white colour and firm flesh with juicy interior upon cooking. The results for changes in sensory parameters of treated Catla steaks during storage are summarized in Figure 2. The sensory parameters declined as storage period progresses, colour faded, and coarseness increased with decrease in juiciness of cooked sample. The changes in appearance, colour & texture were not adequate to establish in depth quality analysis. Odour changed from fresh slight fish to pungent/putrid. No significant differences ($P > 0.05$) were found in overall acceptability of samples treated differently during the entire storage period. Based on overall acceptability AP control samples were acceptable until 12 days, while VP control for 15 days. Results also found a direct relation of SB concentration on overall acceptability, but the rate was higher for VP (24 days) as compared to AP (15 days) samples. Our findings were comparable to results of Li et al. (2011) for yellow grouper (*Epinephelus awoara*) fillets stored under vacuum. The study reports the highest overall acceptability (shelf life) for treated vacuum packed samples in comparison to

Houicher et al. (2015); Li et al. (2018); Lahreche et al. (2019) which may be attributed to low fatty nature of Catla fish. Results of sensory evaluation suggests Catla an important freshwater fish, can be stored for additional 12 days by treatment with SB when packed under vacuum conditions at chill storage conditions with maintained desirable quality characters.

The Data for changes in overall acceptability, odour & taste were adequately modelled by zero order reaction, exhibiting a strong linear correlation ($R^2 > 0.8412, 0.8326$ & 0.8585) during storage period Figure 2 (B to D) inset. Which was further used to compare the experimental shelf life with predicted by shelf life equation Table 2. Predicted shelf life satisfactorily agreed with experimental data with low relative error ($P_0, \%$) $< 0\%$ in samples (0.5% SB-VP to 2.0% SB-VP) treated with SB & VP for estimation based on taste followed by overall acceptability & odour respectively. The predicted shelf life, based on sensory evaluation supports the actual findings for their applicability in retail chain.

Biochemical Composition

Changes in pH Values

The initial pH Catla fish steak was found to be 5.6 which increased gradually as illustrated Table 3A,B. The pH increased in all samples but no significant differences ($P > 0.05$) was observed among treatments in the beginning of storage. The rate of increase was found to be higher in AP samples in comparison to VP samples. The possible reason for the rise of pH in AP samples might be biochemical & microbiological decomposition, generating alkaline compounds such as ammonia & other nitrogenous bases. The slower rate for increase in pH for fish packed under vacuum is explained by Mohan, Abin, Kishore, Panda & Ravishankar (2019a) as predominance of lactic acid producing bacteria under vacuum conditions. It was evident that pH values in all samples were within the maximum prescribed limits (7.1) indicative of muscle decomposition as prescribed by Khalafalla, Ali, & Hassan (2015).

Table 1. Results of percentage change in proximate composition of Catla (*Catla catla*) steaks during 27 days storage

Initial Proximate composition						
P	0% SB-AP	0.5% SB-AP	1.0% SB-AP	1.5% SB-AP	2.0% SB-AP	
M	79.26±0.04	79.26±0.06	79.25±0.05	79.24±0.01	79.25±0.05	
CP	18.55±0.02	18.54±0.04	18.55±0.07	18.56±0.05	18.57±0.08	
F	1.2±0.01	1.21±0.02	1.22±0.02	1.22±0.05	1.23±0.01	
A	0.99±0.03	0.99±0.01	0.98±0.01	0.98±0.02	0.95±0.05	
P	0% SB-VP	0.5% SB-VP	1.0% SB-VP	1.5% SB-VP	2.0% SB-VP	
M	79.26±0.03	79.25±0.07	79.25±0.05	79.25±0.09	79.24±0.01	
CP	18.57±0.03	18.57±0.05	18.58±0.07	18.59±0.01	18.59±0.02	
F	1.2±0.04	1.21±0.03	1.22±0.04	1.22±0.07	1.23±0.08	
A	0.97±0.02	0.97±0.05	0.95±0.04	0.94±0.06	0.94±0.02	
Final Proximate composition*						
M	80.14±0.02	80.14±0.04	80.12±0.01	80.11±0.04	80.07±0.03	
CP	17.25±0.06	17.25±0.07	17.3±0.05	17.31±0.04	17.42±0.07	
F	1.56±0.01	1.56±0.02	1.55±0.04	1.55±0.05	1.52±0.01	
A	1.05±0.03	1.05±0.02	1.03±0.04	1.03±0.01	0.99±0.05	
M	80.08±0.01	80.08±0.02	80.06±0.04	80.06±0.05	80.05±0.07	
CP	17.36±0.06	17.38±0.05	17.42±0.07	17.44±0.04	17.45±0.05	
F	1.54±0.02	1.52±0.05	1.52±0.07	1.51±0.07	1.51±0.02	
A	1.02±0.04	1.02±0.07	1.00±0.04	0.99±0.04	0.99±0.05	

P: Parameter, m: Moisture (%), CP: Crude protein (%), F: Fat (%), A: Ash (%), Results expressed value arrived from using mean values (n = 7). *Final proximate composition is the day of sample spoilage based on sensorial rejection.

Table 2. Shelf life (Days) of Catla steaks based on sensory evaluation

Parameter	Shelf life in (Days)	Samples									
		A	B	C	D	E	F	G	H	I	J
Overall acceptability	Experimental Shelf life	12	15	15	15	15	15	24	24	24	24
	Predicted shelf life	12	13	12	12	13	12	22	22	24	25
	Relative error P ₀ (%)	0	15	25	25	15	25	9	9	0	4
Odour	Experimental Shelf life	12	15	15	15	18	15	24	24	24	24
	Predicted shelf life	12	12	13	12	14	13	23	22	23	28
	Relative error P ₀ (%)	0	25	15	25	28	15	4	9	4	14
Taste	Experimental Shelf life	12	15	15	15	18	15	24	24	24	24
	Predicted shelf life	12	12	12	12	13	12	22	22	24	23
	Relative error P ₀ (%)	0	25	25	25	38	25	9	9	0	4

A: 0% SB-AP, B: 0.5% SB-AP, C: 1.0% SB-AP, D: 1.5% SB-AP, E: 2.0% SB-AP, F: 0% SB-VP, G: 0.5% SB-VP, H: 1.0% SB-VP, I: 1.5% SB-VP, J: 2.0% SB-VP

The results of changes in pH fitted a zero order reaction with a weak linear relation ($R^2 > 0.533$) during storage period Table 5 suggesting low importance of pH for quality estimation in Catla steaks similar to findings of Li et al. (2011). Lowest relative errors (P₀,%) were observed in experimental & calculated pH values (>2) in all samples with maximum predicted life for sample J.

Total volatile base-nitrogen (TVB-N) assessment

The rate of TVB-N formation increased significantly ($P < 0.05$) during the storage of Catla steak & confirmed by SNK test (Table 3A,B). The maximum prescribed limit for TVB-N is 30 mg/100 g in fish muscle as suggested by the Commission of the European Community (1995) & Macéet al. (2012). The maximum prescribed limits were reached on 9th day for control samples, while treated samples required 21 days to reach the maximum permissible levels, matching the sensorial rejection data, except for sample 2.0% SB-VP that exhibited excellent control of TVB-N values. The reduced rate of TVB-N evolution in VP samples treated with SB as compared to AP treated sample treated with SB could be possibly due to antimicrobial effect of SB & reduced oxygen atmosphere created by vacuum packaging. Results in similar line were also reported by Macéet al. (2012) for vacuum packed Salmon steaks & Rodrigues et al. (2016) for Rainbow trout. TVB-N is built up a total of ammonia, monoethyl amine, dimethylamine (DMA) & trimethylamine (TMA) & other compounds evolved as a result of spoilage due to endogenous autolytic enzymes & progressive proteolysis caused by microorganisms imparting off-flavours, considered as index quality parameter used for determining shelf life in seafood's.

A linear zero order model was fitted to the data for TVB-N changes as shown in Table 5. ($R^2 > 0.80$), based on maximum acceptable limits of TVB-N shelf life was predicted to be 68 days. Least relative error (P₀, %) 0% were observed in sample 2.0% SB-VP exhibiting highest R^2 values & best fit of given data.

Peroxide Value (PV) determination

PV measures peroxides & hydroperoxides liberated as a product of primary lipid oxidation that is considered as an indicative of oxidative rancidity. Increase in PV leads to generation of off-flavours, presenting reduced quality of fats. PV is one of the methods used for determining the shelf life of food products as suggested by Okpala, 2014. The changes in PV during storage are shown in Table 3A,B. The initial values of PV were 2.36 ± 0.03 meq of O₂/kg, which increased with storage period but within maximum permissible limits 10 meq of O₂/kg as proposed by Sudalayandi and Manja. (2011). The rate of PV

evolution was lower for VP in comparison to AP owing to positive effect of vacuum packaging, eliminating oxygen in the packs, that enhances the level of lipid oxidation (Hasegawa et al.1992). The results were in conformation with findings of Mohan, Abin, Kishore, Panda & Ravishankar (2019a) for Indian oil sardine (*Sardinella longiceps*) during iced storage. Also, the rapid initial increase in PV could be due to rapid initial decomposition rate followed by a smaller but significant growth.

There was significant difference ($P < 0.05$) amongst different treatments, further SNK test indicated samples 0.5% SB-VP, 1.0% SB-VP, 1.5% SB-VP & 2.0% SB-VP were significantly different, but no significant difference was noted amongst the samples. The changes in PV were best modelled by zero order reaction exhibiting strong linearity ($R^2 > 0.809$) for treated samples (Table 5) with acceptable P₀ values (< 4) & predicted shelf life of 255 days. The low fatty nature of Catla treated with SB & supplemented with VP may be the probable reason. Fish lipids are highly unsaturated and highly prone to oxidation, results also highlighted lower applicability of PV for their use in shelf life prediction in fish based systems. Also such large values of predicted shelf life suggests prediction should not be predicted on single factor alone.

Salt soluble nitrogen (SSN) estimation

As shown in Table 3A,B, SSN content of all treated Catla steak samples decreased significantly ($P < 0.05$) during the storage period, with SNK test confirming sample 2.0% SB-VP to be superior than other samples. In present work, SSN of samples showed a declining trend, indicative of protein denaturation during chill storage & autolytic degradation (Qian et al., 2018; Cropotova et al., 2019). The decline rate for SSN was lowest in sample 2.0% SB-VP due to high concentration of SB, exhibiting antimicrobial property & lowering the rate of microbial decomposition. VP creates environment which, inhibits growth of microorganisms by eliminating oxygen. Also, prevents oxidation by reducing the capability of microbes to oxidatively deaminate nitrogen, leading to reduced solubility of myofibrillar proteins (Sunet et al., 2018). Data showing a similar trend in vacuum packed fishes were reported (Duun and Rustad 2008; Qian et al., 2018).

The reduction of SSN is linearly correlated with storage period ($R^2 > 0.83$, data not shown) for all treated samples with highest for sample 2.0% SB-VP ($R^2 = > 0.95$, data not shown) as best fit. Which is due to the antimicrobial property of SB & unavailability of oxygen in VP, changes in SSN were in accordance with microbiological spoilage & TVB-N evolution.

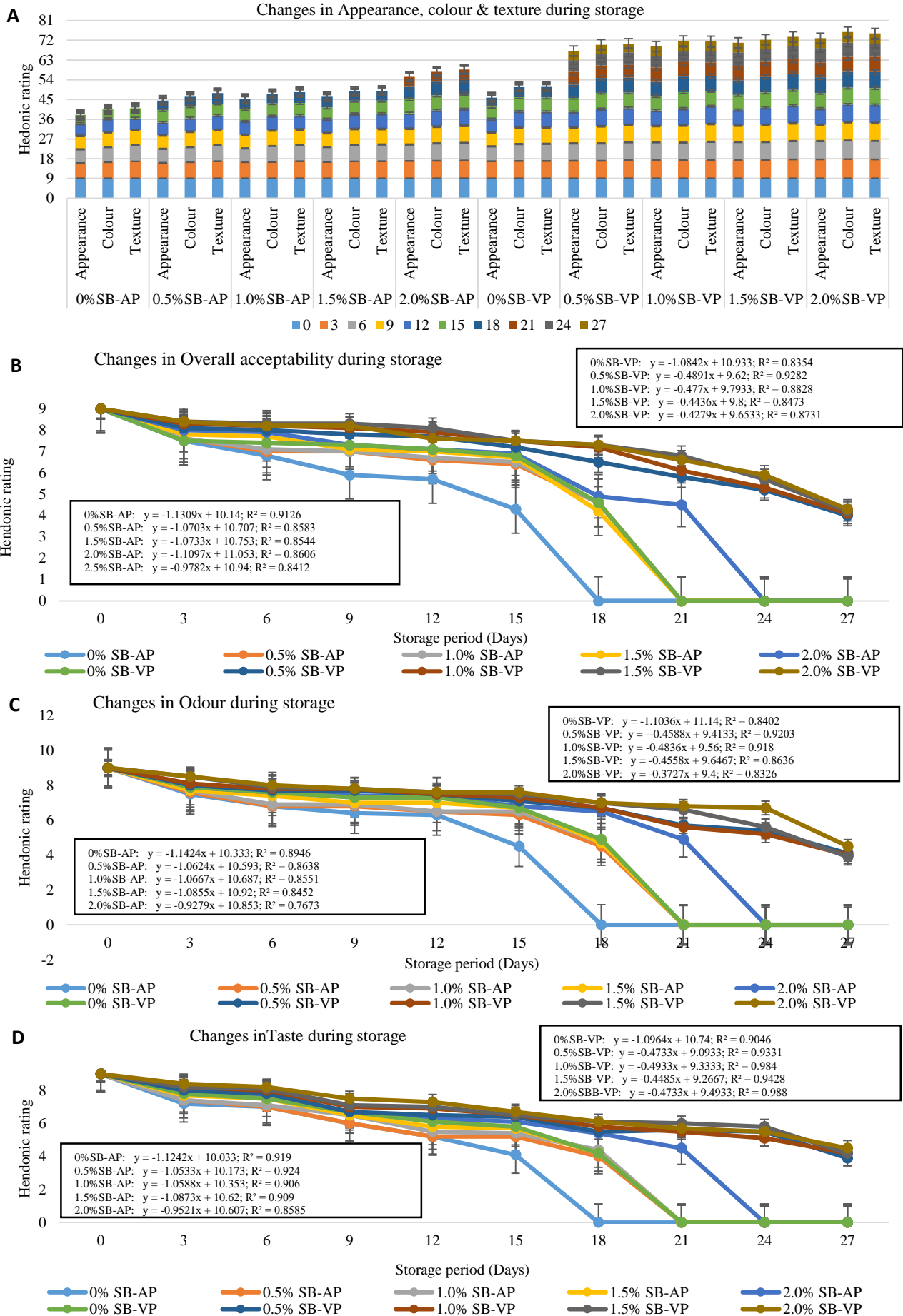


Figure 2. Changes in sensory evaluation parameters A- Appearance, colour & texture, B- Overall acceptability, C- our and D- Taste with its apparent zero order equations inset

Table 3A. Changes in biochemical composition in Catla (*Catla catla*) steaks during storage¶

Days	Air Packed Samples, 0% SB-AP			
	pH	TVB-N	PV	SSN
0	5.56 ± 0.01aA	7.15 ± 0.01aA	2.37± 0.03aA	65.42± 0.01aA
3	5.61 ± 0.01aB	21.45 ± 0.03aB	7.42± 0.01aB	43.81± 0.01aB
6	5.73 ± 0.02aC	27.42 ± 0.01aC	8.65± 0.01aC	39.32± 0.01aC
9	5.88 ± 0.03aD	30.35 ± 0.01aD	8.95± 0.02aCD	33.78± 0.01aD
12	5.91 ± 0.01aE	32.65 ± 0.01aE	9.05± 0.03aD	33.35± 0.01aD
15	5.93 ± 0.02aE	33.51 ± 0.01aF	9.12 ± 0.01aDE	31.24± 0.02aE
18	5.96 ± 0.02aF	35.86 ± 0.01aG	9.35 ± 0.02aEF	31.16± 0.02aE
21	6.18 ± 0.02aG	38.21 ± 0.01aH	9.56 ± 0.01aF	29.14± 0.02aF
24	6.20 ± 0.01aG	39.48 ± 0.02aI	10.80 ± 0.21aG	28.35 ± 0.03aF
27	6.51 ± 0.01aH	39.71 ± 0.01aI	11.16 ± 0.02aH	28.13 ± 0.02aF
Days	Air Packed Samples, 0.5% SB-AP			
0	5.56 ± 0.01aA	7.14 ± 0.02aA	2.37 ± 0.02aA	65.43 ± 0.02aA
3	5.79 ± 0.02bB	13.63 ± 0.02bB	5.41 ± 0.01bB	53.15 ± 0.03bB
6	5.83 ± 0.02bC	18.50 ± 0.09bC	5.84 ± 0.02bC	47.01 ± 0.01bC
9	5.86 ± 0.02bD	22.16 ± 0.01bD	6.15 ± 0.02bD	40.01 ± 0.01bD
12	5.98 ± 0.01bE	26.55 ± 0.02bE	6.25 ± 0.03bDE	38.17 ± 0.01bE
15	6.01 ± 0.01bF	28.42 ± 0.01bF	6.42 ± 0.01bDE	36.06 ± 0.03bF
18	6.10 ± 0.12bG	30.81 ± 0.01bG	6.88 ± 0.02bF	34.34 ± 0.02bG
21	6.13 ± 0.01bH	32.78 ± 0.02bH	7.75 ± 0.02 bG	32.42 ± 0.01bH
24	6.13 ± 0.02bH	33.24 ± 0.02bI	7.95 ± 0.02bG	31.23 ± 0.02bI
27	6.47 ± 0.01bI	36.74 ± 0.02bJ	8.85 ± 0.02bH	30.15 ± 0.03bJ
Days	Air Packed Samples, 1.0% SB-AP			
0	5.55 ± 0.03aA	7.14 ± 0.01aA	2.37 ± 0.03aA	65.43 ± 0.01aA
3	5.82 ± 0.01cB	13.57 ± 0.01bB	5.40 ± 0.06bB	53.75 ± 0.02bcB
6	5.85 ± 0.01bC	18.35 ± 0.03bC	5.75 ± 0.02bC	48.36 ± 0.01cC
9	6.01 ± 0.01cD	22.14 ± 0.02cD	5.85 ± 0.02cC	41.95 ± 0.02cD
12	6.02 ± 0.01cD	26.38 ± 0.02cE	5.94 ± 0.02cC	39.07 ± 0.01bE
15	6.06 ± 0.02cE	28.29 ± 0.01bF	6.32 ± 0.02bE	36.95 ± 0.02bcF
18	6.11 ± 0.01bF	30.76 ± 0.01bG	6.38 ± 0.01cdE	34.62 ± 0.01bG
21	6.16 ± 0.02aG	31.52 ± 0.01cH	7.74 ± 0.01bF	33.47 ± 0.01bcH
24	6.19 ± 0.02cH	32.62 ± 0.01bI	8.18 ± 0.03bG	31.96 ± 0.03bI
27	6.43 ± 0.02cI	35.56 ± 0.02cJ	8.68 ± 0.01bH	30.64 ± 0.01bJ
Days	Air Packed Samples, 1.5% SB-AP			
0	5.55 ± 0.03aA	7.13 ± 0.02aA	2.36 ± 0.01aA	65.45 ± 0.36aA
3	5.88 ± 0.02dB	13.53 ± 0.02bB	5.40 ± 0.02bB	54.85 ± 0.02cdB
6	5.97 ± 0.02cC	18.28 ± 0.02bC	5.72 ± 0.02bC	49.25 ± 0.03cdC
9	6.03 ± 0.01cD	21.70 ± 0.44dD	5.78 ± 0.01ceC	42.76 ± 0.02cD
12	6.05 ± 0.03dDE	26.34 ± 0.02cE	6.25 ± 0.02bD	42.16 ± 0.02cD
15	6.06 ± 0.02cE	28.26 ± 0.01bF	6.35 ± 0.02bDE	36.16 ± 0.02cE
18	6.06 ± 0.03cE	30.68 ± 0.02bG	6.58 ± 0.02dE	34.64 ± 0.02bF
21	6.07 ± 0.02cEF	31.47 ± 0.02cH	7.15 ± 0.02cF	33.24 ± 0.02cG
24	6.09 ± 0.02dF	32.60 ± 0.12bcI	7.90 ± 0.02bG	31.93 ± 0.02bH
27	6.42 ± 0.01cG	34.42 ± 0.01dJ	8.56 ± 0.03cH	30.65 ± 0.02bI
Days	Air Packed Samples, 2.0% SB-AP			
0	5.55 ± 0.01aA	7.13 ± 0.01aA	2.36 ± 0.02aA	65.45 ± 0.02aA
3	5.91 ± 0.01eB	12.95 ± 0.01bB	3.39 ± 0.02cB	54.95 ± 0.03dB
6	6.06 ± 0.02dC	17.21 ± 0.01cC	4.70 ± 0.03cC	49.95 ± 0.02dC
9	6.13 ± 0.02dD	20.98 ± 0.02eD	4.75 ± 0.02dC	42.80 ± 0.01cD
12	6.13 ± 0.02eD	25.71 ± 0.01dE	5.18 ± 0.01dD	42.65 ± 0.01cD
15	6.19 ± 0.02dE	28.02 ± 0.01bF	5.30 ± 0.12eD	41.65 ± 0.03dE
18	6.20 ± 0.06dE	30.64 ± 0.01bG	5.32 ± 0.02eD	34.66 ± 0.02bF
21	6.23 ± 0.02dF	31.26 ± 0.02cH	5.88 ± 0.03dE	33.48 ± 0.03cG
24	6.35 ± 0.01eG	32.46 ± 0.03cI	6.95 ± 0.02cF	31.95 ± 0.01bH
27	6.41 ± 0.01cH	33.55 ± 0.03eJ	7.42 ± 0.01dG	30.69 ± 0.02bI

Results expressed are mean values ± SD, n = 3, P<0.05. Different letters (the first small) indicates significant difference along the row; Different letter (the second capitalized) indicates significant difference along the column

Table 3B. Changes in biochemical composition in Catla (*Catla catla*) steaks during storage

Days	Vacuum Packed Samples, 0% SB-VP			
	pH	TVB-N	PV	SSN
0	5.56 ± 0.01Aa	7.15 ± 0.02aA	2.37 ± 0.01aA	65.42 ± 0.01aA
3	5.81 ± 0.01bB	13.54 ± 0.01bB	5.41 ± 0.01bB	52.95 ± 0.03bB
6	5.83 ± 0.02bB	18.51 ± 0.01bC	5.85 ± 0.03bC	45.75 ± 0.02eC
9	5.89 ± 0.02aC	21.43 ± 0.02dD	5.96 ± 0.01eCD	42.79 ± 0.03cD
12	5.93 ± 0.02aD	26.91 ± 0.01eE	6.15 ± 0.01bD	41.71 ± 0.01cD
15	6.01 ± 0.01bE	28.56 ± 0.01bF	6.60 ± 0.23dE	40.61 ± 0.01eE
18	6.02 ± 0.01eEF	30.95 ± 0.02bG	7.52 ± 0.02fF	32.88 ± 0.01eF
21	6.03 ± 0.02eF	32.65 ± 0.03bH	7.91 ± 0.03bG	28.64 ± 0.01aG
24	6.19 ± 0.02cG	33.47 ± 0.01bI	8.12 ± 0.01bG	29.91 ± 0.01cG
27	6.37 ± 0.01dH	36.18 ± 0.02bJ	9.75 ± 0.02eH	29.24 ± 0.02bG
Days	Vacuum Packed Samples, 0.5% SB-VP			
0	5.55 ± 0.03aA	7.13 ± 0.02aA	2.36 ± 0.03aA	65.44 ± 0.02aA
3	5.84 ± 0.01cB	12.24 ± 0.02cB	2.39 ± 0.02dA	56.14 ± 0.01eB
6	5.92 ± 0.02eC	16.16 ± 0.02dC	2.58 ± 0.32dAB	50.34 ± 0.01dC
9	5.96 ± 0.02eD	20.75 ± 0.03eD	2.65 ± 0.01fB	48.98 ± 0.02dD
12	5.98 ± 0.02bD	23.92 ± 0.01fE	3.85 ± 0.02eC	45.17 ± 0.02dE
15	5.98 ± 0.01eD	27.71 ± 0.01bF	3.90 ± 0.05eC	41.33 ± 0.02dF
18	5.98 ± 0.01aD	29.45 ± 0.01cG	4.98 ± 0.01gD	39.88 ± 0.01dG
21	6.07 ± 0.02cE	31.33 ± 0.12cH	5.24 ± 0.02eE	37.92 ± 0.01dH
24	6.17 ± 0.03cF	32.42 ± 0.01bI	5.86 ± 0.02dF	36.16 ± 0.03dI
27	6.35 ± 0.03dG	33.51 ± 0.01eJ	6.25 ± 0.32fG	35.94 ± 0.02cI
Days	Vacuum Packed Samples, 1.0% SB-VP			
0	5.55 ± 0.01aA	7.13 ± 0.01aA	2.36 ± 0.02aA	65.44 ± 0.02aA
3	5.94 ± 0.01fB	10.90 ± 0.01dB	2.39 ± 0.02dA	57.35 ± 0.01fB
6	6.00 ± 0.06fC	16.40 ± 0.01dC	3.56 ± 0.01eBC	51.84 ± 0.05fC
9	6.04 ± 0.01cD	20.65 ± 0.01eD	3.62 ± 0.02gB	49.03 ± 0.02dD
12	6.06 ± 0.03dD	23.85 ± 0.03fE	3.74 ± 0.01eBC	45.67 ± 0.01dE
15	6.06 ± 0.02cD	27.65 ± 0.03cF	3.85 ± 0.02eBC	41.86 ± 0.01dF
18	6.14 ± 0.04fE	29.38 ± 0.02cG	4.15 ± 0.02hD	40.15 ± 0.03dG
21	6.16 ± 0.05aEF	31.24 ± 0.02cH	4.22 ± 0.01fD	39.54 ± 0.01eG
24	6.17 ± 0.01cF	32.26 ± 0.01cF	4.92 ± 0.02eE	37.54 ± 0.02eH
27	6.32 ± 0.01eG	33.35 ± 0.01eJ	5.25 ± 0.01gF	36.36 ± 0.02eI
Days	Vacuum Packed Samples, 1.5% SB-VP			
0	5.55 ± 0.01aA	7.12 ± 0.01aA	2.35 ± 0.03aA	65.42 ± 0.01aA
3	5.96 ± 0.03fB	10.43 ± 0.01dB	2.39 ± 0.01dA	58.68 ± 0.02gB
6	5.99 ± 0.02fC	15.34 ± 0.01eC	3.49 ± 0.35eB	52.30 ± 0.03fC
9	6.06 ± 0.02fD	20.30 ± 0.06eD	3.58 ± 0.02gB	52.13 ± 0.02eC
12	6.08 ± 0.02fD	23.72 ± 0.01fE	3.66 ± 0.01eB	46.14 ± 0.02dD
15	6.08 ± 0.02cD	27.58 ± 0.02cF	3.84 ± 0.02eCD	41.95 ± 0.03dE
18	6.13 ± 0.02bE	29.09 ± 0.02cG	4.05 ± 0.02hDE	40.38 ± 0.02dF
21	6.13 ± 0.02bE	31.17 ± 0.01cH	4.12 ± 0.01fE	39.75 ± 0.02eF
24	6.17 ± 0.02cF	32.16 ± 0.02cI	4.88 ± 0.01eF	37.65 ± 0.02eG
27	6.30 ± 0.17eG	33.32 ± 0.01cJ	5.18 ± 0.02gG	36.45 ± 0.03cH
Days	2.0% SB-VP			
0	5.55 ± 0.03aA	7.12 ± 0.01aA	2.35 ± 0.01aA	65.42 ± 0.01aA
3	6.00 ± 0.06gB	8.38 ± 0.02eB	2.39 ± 0.01dAB	64.85 ± 0.02hAB
6	6.17 ± 0.01gC	9.25 ± 0.02fC	2.45 ± 0.01dAB	64.35 ± 0.03gBC
9	6.19 ± 0.02gD	9.45 ± 0.01fCD	2.56 ± 0.01fABC	63.64 ± 0.02fCD
12	6.20 ± 0.06gD	10.36 ± 0.02gD	2.62 ± 0.02fBD	62.99 ± 0.02eDE
15	6.21 ± 0.01dD	11.66 ± 0.01dE	2.74 ± 0.01fCE	62.26 ± 0.01eE
18	6.21 ± 0.01dDE	13.86 ± 0.01dF	2.82 ± 0.03iDF	60.97 ± 0.01eF
21	6.23 ± 0.02dEF	13.32 ± 0.01dF	2.95 ± 0.02gEGH	59.68 ± 0.02fG
24	6.25 ± 0.03fFG	15.71 ± 0.01dG	3.05 ± 0.03fFGH	58.95 ± 0.03fG
27	6.27 ± 0.01fG	15.93 ± 0.02fG	3.12 ± 0.01hH	56.48 ± 0.04dH

Results expressed are mean values ± SD, n = 3, P<0.05. Different letters (the first small) indicates significant difference along the row; Different letter (the second capitalized) indicates significant difference along the column

Table 4. Changes in microbiological quality of Catla (*Catla catla*) steaks during storage*

SP	TPC (log10), Air Packed Samples				
	0% SB-AP	0.5% SB-AP	1.0% SB-AP	1.5% SB-AP	2.0% SB-AP
0	2.73 ± 0.02aA	2.73 ± 0.04aA	2.72 ± 0.03aA	2.72 ± 0.01aA	2.72 ± 0.02aA
3	3.85 ± 0.01aB	3.72 ± 0.01bB	3.51 ± 0.01cB	3.48 ± 0.03cB	3.46 ± 0.04cB
6	4.18 ± 0.03aC	4.16 ± 0.23aC	3.65 ± 0.02bC	3.65 ± 0.05bC	3.66 ± 0.21bC
9	4.38 ± 0.03aC	4.32 ± 0.61aD	4.20 ± 0.05aD	3.82 ± 0.01bD	3.81 ± 0.18bD
12	4.55 ± 0.01aD	4.51 ± 0.05aE	4.83 ± 0.10bE	4.15 ± 0.04cE	4.10 ± 0.14cE
15	5.76 ± 0.02aE	4.83 ± 0.10bF	4.59 ± 0.03cF	4.45 ± 0.05dF	4.42 ± 0.01dF
18	5.89 ± 0.05aF	5.14 ± 0.05bG	5.12 ± 0.61bG	5.07 ± 0.07bG	4.93 ± 0.04cG
21	5.92 ± 0.017aF	5.24 ± 0.07bG	5.22 ± 0.16bG	5.19 ± 0.03bdH	5.19 ± 0.05bdH
24	6.55 ± 0.01aG	6.25 ± 0.01bH	6.22 ± 0.07bH	6.21 ± 0.02bcI	6.12 ± 0.01cI
27	6.95 ± 0.02aH	6.66 ± 0.04bI	6.65 ± 0.03bI	6.64 ± 0.01bJ	6.62 ± 0.00bJ
SP	TPC (log10), Vacuum Packed Samples				
0	2.731 ± 0.04aA	2.73 ± 0.04aA	2.72 ± 0.01aA	2.72 ± 0.03aA	2.72 ± 0.01aA
3	3.52 ± 0.18cB	3.41 ± 0.11cdB	3.37 ± 0.02dB	2.79 ± 0.02eA	2.74 ± 0.07eA
6	4.20 ± 0.04cC	3.66 ± 0.0bC	3.64 ± 0.11bC	3.62 ± 0.15bB	2.79 ± 0.02dAB
9	4.51 ± 0.12cD	3.80 ± 0.27bD	3.79 ± 0.05bD	3.77 ± 0.04bC	2.86 ± 0.03dBC
12	4.74 ± 0.04bE	3.85 ± 0.02dD	3.80 ± 0.17dD	3.78 ± 0.18dC	2.88 ± 0.0eBC
15	4.84 ± 0.02bE	4.40 ± 0.01dE	3.82 ± 0.08eD	3.79 ± 0.09eC	2.93 ± 0.08fC
18	5.02 ± 0.01cF	4.75 ± 0.05dF	4.51 ± 0.01eE	4.06 ± 0.01fD	2.97 ± 0.01gC
21	6.15 ± 0.01cG	5.14 ± 0.60dG	4.95 ± 0.02eF	4.64 ± 0.04fE	3.15 ± 0.15gD
24	6.34 ± 0.04dH	5.62 ± 0.15eH	5.11 ± 0.01fG	4.85 ± 0.07gF	3.45 ± 0.02hE
27	6.80 ± 0.14eI	6.78 ± 0.05dI	6.24 ± 0.04eH	5.85 ± 0.15fG	3.75 ± 0.01gF

SP: Storage Period (days), *Results expressed are mean values ± SD, n = 3, P<0.05. Different letters (the first small) indicates significant difference along the row; Different letter (the second capitalized) indicates significant difference along the column.

Changes in microbiological quality

All tested samples showed a statistically significant ($P<0.05$) increase in total plate count (TPC) log values (Table 4), from initial values of 2.73 log. CFU/g indicating appropriate initial hygienic handling practices & probable effect of treatment with chlorinated water. Further confirmed by SNK test, which showed significant difference among samples 1.0% SB-VP, 1.5% SB-VP & 2.0% SB-VP from others. Data for growth in TPC log. values were modelled with zero order equation showing significant linear increase ($R^2>0.804$ with storage period (Table 5) with a predicted shelf life of 129 days for sample 2.0%SB-VP with low relative error ($P_0, \%$) suggesting data fit. The values for TPC increased by 100% in all samples except sample 2.0% SB-VP, where the rise was meagre 27% indicating the positive anti-microbial effects of SB with increasing concentration as found by Seman et al. (2008). These effects are due to undissociated benzoic acid molecule interfering permeability of cell membrane & VP eliminating available oxygen with pronounced effect of lower temperature maintained (Lee et al.1965; Rodrigues et al. 2016). Samples exhibited high microbiological quality in reference to maximum permissible limit (7 log CFU/g) set by the International Commission in Microbial Specification for Foods (ICMSF 1998). Trend similar to our findings were also reported in vacuum packed fishes (Dalgaard et al. 1993; Mohan et al. 2019b). *E. coli*, *S. Aureus* & *Salmonella* were not detected matching findings of González-Rodríguez et al. (2002) for vacuum packed freshwater fish.

Conclusion

In the present study sodium benzoate & vacuum packaging effectively retarded the chemical, microbiological & sensorial quality deterioration of Catla fish steaks stored during chilled storage conditions. Results of sensorial evaluation suggested that treatment with sodium benzoate alone improved the overall acceptability by 20%, while the combination of sodium benzoate & vacuum packaging improved overall acceptability by 50% in comparison of air packed control & 62.5% for vacuum pack control samples. Results of TVB-N PV & microbiological analysis suggested synergistic effects of SB and VP maintaining the quality and extending shelf life. Results suggest Catla steak samples treated with 2.0% of SB packed under vacuum were superior to other treatments based on sensory, biochemical and microbiological quality evaluated. Experimental data best fitted zero order regression with an R^2 value of 0.968 for TVB, 0.99 for PV & 0.804 for TPC. Results for comparison of experimental data with predicted data was acceptable based on low relative error ($P_0, \%$) <0. Considering the practical applicability, using combination of preservation methods is suggested for shelf life extension for Catla steaks, thus scaling up feasibility. Furthermore, the specific spoilage microorganisms & headspace analysis in vacuum packed sample, biogenic amine formation with changes in colour & texture profile of samples needs to be assessed.

Table 5. Correlation coefficient (R^2) values, regression equation with experimental and predicted values for predicting shelf life values for Catla (*Catla catla*) steaks during storage.

P	Sample	R^2 value	Experimental value	Predicted value at	Relative error P_0 (%)	Predicted Shelf life
pH (7.1)	0% SB-AP	0.93	6.51	6.36	2	50
	0.5% SB-AP	0.906	6.47	6.33	2	59
	1.0% SB-AP	0.92	6.43	6.39	0	57
	1.5% SB-AP	0.716	6.42	6.22	3	77
	2.0% SB-AP	0.811	6.41	6.44	0	64
	0% SB-VP	0.904	6.37	6.27	1	66
	0.5% SB-VP	0.828	6.35	6.26	1	74
	1.0% SB-VP	0.757	6.32	6.30	0	79
	1.5% SB-VP	0.716	6.3	6.29	0	84
	2.0% SB-VP	0.533	6.27	6.36	1	89
TVB-N (30)	0% SB-AP	0.807	39.71	43.96	10	23
	0.5% SB-AP	0.943	36.74	38.71	5	22
	1.0% SB-AP	0.934	35.56	38.67	8	22
	1.5% SB-AP	0.924	34.42	37.37	8	23
	2.0% SB-AP	0.927	33.55	36.97	10	23
	0% SB-VP	0.94	36.18	38.62	6	22
	0.5% SB-VP	0.947	33.51	36.67	9	23
	1.0% SB-VP	0.943	33.35	36.64	9	23
	1.5% SB-VP	0.946	33.32	36.58	9	22
	2.0% SB-VP	0.968	15.93	16.02	0	68
PV (10)	0% SB-AP	0.666	11.16	11.61	3	34
	0.5% SB-AP	0.836	8.85	8.79	0	42
	1.0% SB-AP	0.809	8.68	8.65	0	43
	1.5% SB-AP	0.823	8.56	8.47	1	45
	2.0% SB-AP	0.916	7.42	7.26	2	48
	0% SB-VP	0.873	9.75	9.31	4	37
	0.5% SB-VP	0.953	6.25	6.18	1	47
	1.0% SB-VP	0.921	5.25	5.13	2	77
	1.5% SB-VP	0.921	5.18	5.05	2	79
	2.0% SB-VP	0.99	3.12	3.11	0	253
TPC (7)	0% SB-AP	0.958	6.95	7.02	1	29
	0.5% SB-AP	0.946	6.66	6.43	3	34
	1.0% SB-AP	0.949	6.65	6.37	4	33
	1.5% SB-AP	0.96	6.64	6.34	4	32
	2.0% SB-AP	0.958	6.62	6.28	5	47
	0% SB-VP	0.957	6.8	6.73	0	31
	0.5% SB-VP	0.931	6.78	6.12	9	33
	1.0% SB-VP	0.9	6.24	5.63	9	40
	1.5% SB-VP	0.899	5.85	5.31	9	43
	2.0% SB-VP	0.804	3.75	3.47	7	129

P: Parameter (max. permissible limits)

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