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Biochemical and shelf life characteristics of air packed pangasius (*Pangasianodon hypophthalmus*) during chill storage

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Abstract
The effect of sodium benzoate on the shelflife of air packed pangasius (*Pangasianodon hypophthalmus*) during chill storage at 4±2 °C was studied. Based on the cumulative observations of biochemical, microbiological and sensory parameters the shelflife of pangasius fish was predicted. In the present study, organoleptic, biochemical and microbiological quality changes were observed in chilled stored air packed pangasius fish steaks treated with 0.0, 0.5, 1.0, 1.5 and 2.0% sodium benzoate. Organoleptic, biochemical and microbiological analysis showed that chilled stored air packed pangasius fish steaks with treatment of 2 % sodium benzoate had longer shelf life of 16 days. Whereas untreated air packed pangasius fish steaks had a shelf life of 12 days. The findings of the present study, clearly suggest that a combination of sodium benzoate and storage at chilled temperature could be used to prolong the shelf life of pangasius.

Keywords: Air packaging, pangasius catfish, chill storage, sodium benzoate

1. Introduction
Fish is considered a valuable source of protein in the human diet. This is mainly because theynaturally contain a large amount of essential n-3 polyunsaturated fatty acids (PSUFA). Fish in particular have specific and unique nutritional values and occupy a special place in the human diet (Burger and Gochfeld 2009) [4]. As a result, demand for fish products is increasing in both domestic and international markets (Pagarkar *et al*. 2011; Rathod *et al*. 2018) [22, 26]. International trade has also played an important role in providing consumers with more choices. *Pangasianodon hypophthalmus* belongs to the family Pangasiidae and has important economic significance in India (Padiyar *et al.*, 2014) [21]. *P. hypothalamus* is native to the Mekong Basin in Thailand, Cambodia, and Vietnam (Michael and McGee, 2014) [15]. The Asian striped fish, *P. hypophthalmus*, is considered a premium aquaculture species in tropical regions and the main aquaculture product on the world market (Michael and McGee, 2014) [15]. Other tropical countries in Asia have successfully used pangasius fish farming as a means to increase aquaculture production and promote food security. These Asian countries include Thailand, Cambodia, Myanmar, Indonesia, Malaysia, Bangladesh, India and the Philippines. There is great scope to increase the consumption by developing different value added products from Pangasius due to a mild flavor, white flesh colour, firm cooked texture, low fat content (Orban *et al.*, 2008) [19] chilled and frozen sutchi catfish fillets are usually suitable for export markets. Flesh of *P. hypophthalmus* has white or pale pink flesh and the advantages of no fishy smell, small bones and thin skin. These characteristics and their standard sizes on the market have led to the preparation of value-added products, fish fillets, especially to meet the consumer needs. Due to the high nutritional value and excellent sensory characteristics of Pangasius fish meat, people's acceptance of Pangasius fish is increasing every day. Due to social and cultural changes in recent years, there is a lot of room for fish consumption. Sodium benzoate ($NaC_6H_5CO_2$) is used as a preservative in the food industry. It is found naturally in cranberries, plums, plums, cinnamon, cloves and apples. The FDA generally identifies sodium benzoate and benzoic acid as food additives as GRAS (Sara *et al*., 1994) [29]. Due to the antibacterial properties of sodium benzoate, it has been used to prevent the growth of bacteria and mold. It helps to extend the shelf life of fish for a long period of time without affecting

the texture, taste and appearance of the fish (Omojowo *et al*. 2010) [18]. The purpose of this research was to study the effect of sodium benzoate with air packaging on the shelf life of pangasius (*P. hypophthalmus*) steaks at chilled temperatures.

2. Materials and methods

2.1 Raw material

Fresh Pangasius samples were obtained from the local fish market in Ratnagiri. The fish were washed, immediately packed in a 1: 1 insulated box with crushed ice, and then shipped to the fish processing hall of college of fisheries Ratnagiri. Measured the length and weight of the fish. The whole fish was washed with chlorinated water. The fish was descaled, the fins were removed, and the head was decapitated. After being washed with 2 ppm chlorine water by Shalima (1997) [30], the steak was 1.5 - 2.0 cm thick. The steaks were divided into 5 groups one group was left untreated, and the remaining 4 groups were immersed in sodium benzoate solutions at concentrations of 0.5, 1.0, 1.5 and 2% for 5 minutes. After proper draining packaging was carried out in packaging material made up of polyethylene with a capacity of 200 g each and of size 30.0 × 12.0 cm was used for packaging of Pangasius fish steaks.

The air packed samples with or without treatment were coded as untreated air packed steaks (A), 0.5% sodium benzoate treated air packed steaks (B), 1.0% sodium benzoate treated air packed steaks (C), 1.5% sodium benzoate treated air packed steaks (D) and 2.0% sodium benzoate treated air packed steaks (E) respectively. Immediately after packaging, samples were kept in chilling machine which having temperature set at 4 ± 1^0C. Samples were taken every alternate day. Analyze the sensory, biochemical and microbiological characteristics of all samples during refrigeration.

2.2 Chemical analysis

All the chemicals used for the study were of analytical grade. The proximate composition *viz.* moisture, crude protein, crude fat and ash content, also the residue of sodium benzoate in pangasius fish steaks were determined by (AOAC, 2005) [1]. The pH, total volatile basic nitrogen (TVB-N), peroxide value (PV), and free fatty acid content in refrigerated samples were measured to study the biochemical quality characteristics of cat fish steaks. Record the pH of the filtrate with a pH meter (AOAC, 2005) [1]. TVB-N was determined by micro diffusion method of Conway (1950) [5]. Oxidative stability of chilled stored pangasius fish steaks was also measured using titrimetric determination of the amount of peroxide or hydro-peroxide groups, the initial product of lipid oxidation (peroxide value). The peroxide value was expressed as milli equivalent of O2/kg fat (AOAC, 2005) [1].

2.3 Microbiological analysis

Microbiological analysis was carried out for the enumeration of total viable count (TVC). Total viable counts (TVC) were determined in Plate Count Agar by the spread plate method (AOAC 2002) [1].

2.4 Sensory evaluation

Sensory evaluation was based on the various sensory characters. A panel of 10 judges did sensory analysis of stored samples during the chilled storage of fish steaks. The samples were evaluated by a 9 point hedonic scale (ISI, 1975) [7].

Table 1: Quality of fresh pangasius (*P. hypophthalmus*) fish muscle

quality parameter	Pangasius fish muscle
Sensory score for overall acceptability (9point hedonic scale)	9.00
pH	5.87 ± 0.01
TVB-N (mg/100g)	4.78 ± 0.01
PV (meq of O_2/kg)	2.96 ± 0.02
FFA	0.655± 0.002
Total plate count (cfu/g)	4.83 X 102

3. Results and Discussion

3.1 Proximate composition of fresh pangasius fish meat

The moisture, crude protein, fat, and ash in the fresh seasoning meat were 74.32%, 17.02%, 7.50%, and 1.04%, respectively (Table. 2). Viji *et al.* (2014) [32] reported the similar results of the proximate composition of fresh sutchi catfish meat was found to be 77 % moisture, 16.5% protein, 4% fat and 0.97% ash respectively. In another study which was carried out by Rathod *et al.* (2013) [25] reported the moisture, protein, fat, and ash content in fresh Pangasius fish meat were 76.62, 14.37, 6.76, and 2.25 %. Patil *et al.* (2014) [23] reported the moisture, crude protein, fat, and ash content estimated in fresh Pangasius meat were 73.29, 12.03, 13.47, and 1.21% respectively. Large variations occur in proximate composition and are influenced by several factors like the species of fish, diet, fishing ground, season, sex, and sexual maturity and spawning. Considerable variations in the proximate composition have been found to occur between fish of the same species also.

Table 2: Proximate composition of fresh pangasius (*P. hypophthalmus*) muscle

Composition (%)	Pangsius fish muscle
Moisture	74.32 ± 0.03
Crude protein	17.02 ± 0.08
Fat	7.50 ± 0.03
Ash	1.04 ± 0.02

3.2 Sensory Changes

The sensory quality scores of chilled stored air-packed pangasius steaks, At the end of 12 day, the overall acceptability score for sample A of chilled pangasius fish steaks showed a trend from 9.0 to 4.5. At the end of 14 day, the overall acceptability score for sample B, C, D were 9.0 to 4.6, 9.0 to 4.5 and 9.0 to 4.4 respectively. The E values were 9.0 to 4.0 respectively at the end of 16 day (Fig. 1). Kedar *et*

al. (2016) [10] reported the overall acceptability score for all catla steak samples without vacuum-packed, vacuum- packed, with and without sodium benzoate showed declining trend. Binsi *et al*. (2015) [3] also reported the declining trend however the sensory deterioration was delayed in vacuum packed sample compare to air packed sample. Meenakshi *et al*. (2010) [14] also reported declined trend of overall acceptability scores for common carp (*Cyprinus carpio*) stored at chilled temperature.

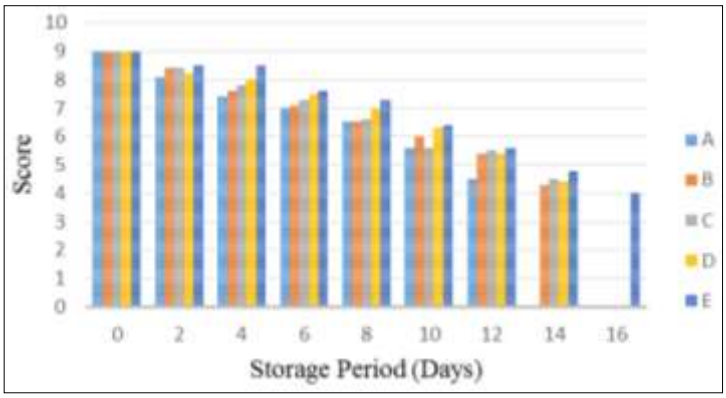

Fig 1: Organoleptic score for overall acceptability of chilled stored air packed pangasius fish steaks

3.3 Changes during chill storage

3.3.1 pH

The pH values for all samples of chilled stored catla fish steaks showed increasing trend from 5.89 to 6.33 in A, 5.85 to 6.30 in B, 5.86 to 6.20 in C, 5.84 to 6.24 in D and 5.88 to 6.32 in E at the end of 16 day (Fig.2). Similar results of pH content found by Guimaaraes *et al*. (2015) [6] the change in pH value of Pangasius fillets which ranging from 5.88 to 6.63. Noseda *et al*. (2012) [17] observed initial pH values of 6.63 in Pangasius fillet. Relekar *et al*. (2019) [27] also reported the initial pH of Pangasius meat cutlet was 6.2 which increase up to 7.12 at the end of 18 days. Juvekar (2007) [9] also reported an increasing trend in pH of air-packed black king fish mackerel (*Rachycentron canadus*) flesh stored at 5 ± 1^0C. The reduction in pH can be ascribed to the dissolution of CO_2 in fish muscle, where it is converted into lactic acid which leads to a drop in pH. The accumulation of lactic acid in the muscle followed by a breakdown of glycogen, the major energy source, through the anaerobic pathway leads to the accumulation of lactic acid (Viji *et al*., 2014) [32].

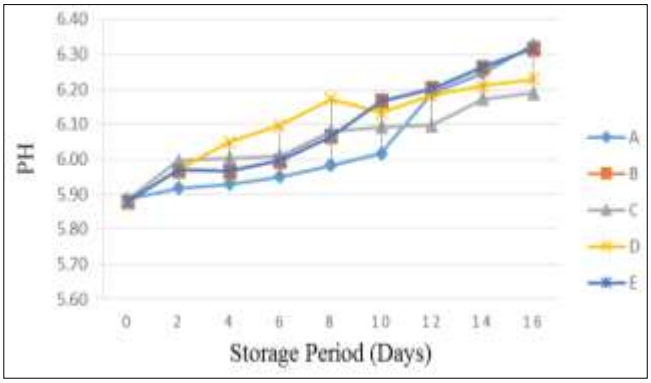

Fig 2: Changes in pH of chilled stored air packed pangasius fish steaks

3.3.2 Total volatile base nitrogen (TVB-N)

The TVB-N (mg / 100g) content of all samples of refrigerated pangasius steak showed an increase from 4.77 to 27.21 in A, 4.77 to 24.97 in B, 4.78 to 24.47 in C, 4.76 to 23.74 in D and 4.79 to 20.21 in E at the end of 16 day (Fig.3). Similar results are obtained by Kumar *et al*. (2015) [12] which shows a gradual increase in TVBN in all samples. TVBN content of fillets increased from an initial value of 4.57 to 41.12 mg/100g in case on chilled air-packed samples. Similar results also reported by Shariat *et al*. (2013) [31] reported that TVB-N content of pangasius fillets increase in range from 18 to 33.5 mg/100 g in air packed samples.

Kedar *et al*. (2016) [10] also reported the increase TVB-N content in all the air packed samples of chilled stored catla fish steaks. Ozogul *et al*. (2004) [20] reported the initial TVBN content of sardines was 5mg/100g in air packed samples it showed increasing trend and reached up to 15 mg/100g. TVBN serves as an indicator for the assessment of the freshness of fish. TVB-N formation was reported to increase with time of storage. TVB-N is the most common index of quality universally used for deciding the state of the freshness of fish (Balachandran, 2001) [2].

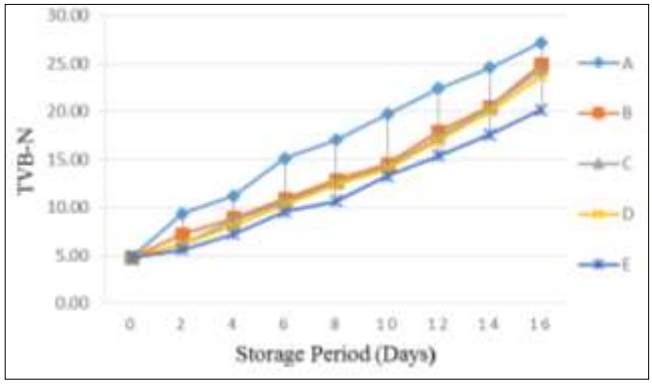

Fig 3: Changes in TVB-N content of chilled stored air packed pangasius fish steaks

3.3.3 Peroxide Value (PV)

The peroxide value (meq of O2/ kg) for all samples of chilled stored pangasius fish steaks showed increasing trend from 2.94 to 11.59 in A, 2.96 to 8.53 in B, 2.99 to 7.98 in C, 2.94 to 7.96 in D and 2.95 to 6.46 in E at the end of 16 day (Fig.4). PV was used for determining the primary product of lipid oxidation. Peroxide value (PV) is used to express the oxidative state of lipid-containing foods. It measures the first stage of oxidative rancidity. Similar results are obtained for the initial PV value of Pangasius by Islami *et al*. (2014) [8] which was 2.2 meqO_2/kg. Kedar *et al*. (2016) [10] also reported the increased PV content in all the air packed samples of chilled stored catla fish steaks. PV value for Pangasius meat

cutlet ranging from 2.34 to 4.04 meqO_2/kg reported by Relekar *et al*. (2019) [27]. Kolekar *et al*. (2013) [11] observed peroxide value (PV) in chilled catla fishball in curry showed an increasing trend from 2.27 to 9.47 meq of O2/Kg. Lahreche *et al*. (2019) [13] reported the initial PV of filleted frigate tuna kept under vacuum condition at refrigerated storage was 1.45 and 2.61 meq O_2/kg of fat for dark and white muscles respectively. Peroxide value (PV) was used for determining the primary product of lipid oxidation and its express the oxidative state of lipid-containing foods. It measures the first stage of oxidative rancidity.

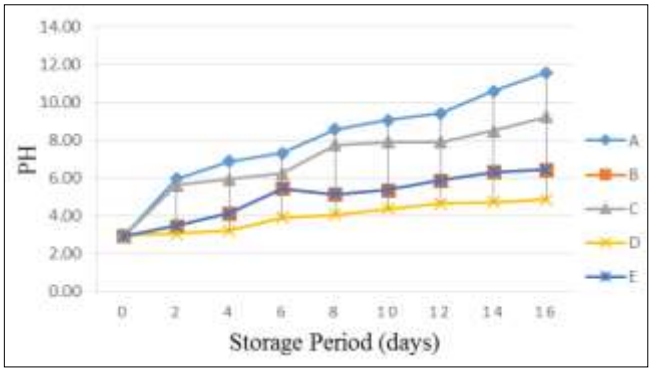

Fig 4: Changes in PV of chilled stored air packed pangasius fish steak

3.3.4 Free Fatty Acid (FFA)

Fatty Acid (% of oleic acid) content for all samples of chilled stored pangasius fish steaks showed increasing trend from 0.65 % to 2.89 % in A, 0.65 % to 2.28 % in B, 0.65 % to 1.62 in C, 0.65 % to 1.49 % in D and 0.65 % to 1.36 % in E at the end of 16 day (Fig.5).

Similar results observed by Viji *et al*. (2014) [32] reported the FFA values ranged from 0.91% to 5.07% for chilled steaks whereas for iced steaks FFA values ranged from 0.53% to 3.65%. Relekar *et al*. (2019) [27] also reported the increase in FFA of Pangasius meat cutlets ranging from 1.28% to 5.02 % at the end of 18 days. Mohan *et al*. (2019) [16] observed initial FFA values of Indian oil sardine were 2.38%. The formation of FFA proceeds during storage probably due to the action of lipases and phospholipases. The progressive increase in the % FFA values over the storage time showed the progressive breakdown of lipids in fish muscle with time. Lipid hydrolysuis is caused by the lipolytic enzymes present in the tissues. Accumulation of free fatty acids results in further deterioration in quality. Free fatty acids accelerate protein denaturation (Bhalchandran, 2001) [2].

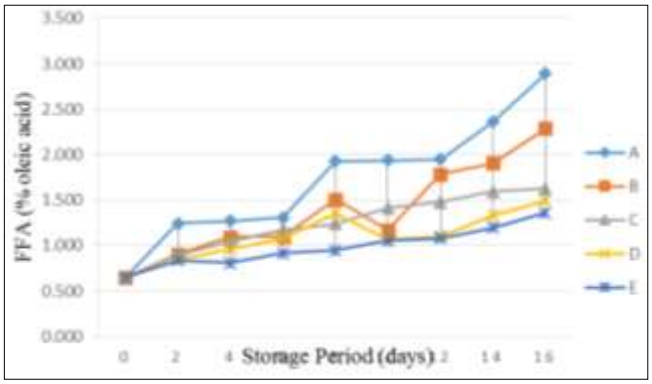

Fig 5: Changes in FFA of chilled stored air packed pangasius fish steaks

3.4 Microbiological analysis

3.4.1 Total Plate Count (TPC)

The total number of plates (cfu / g) for all refrigerated pangasius fish fillet samples showed a trend increase from 4.82 x 10^2 to 1.36 x 10^5 in A, 4.80 x 10^2 to 9.99 x 10^4 in B, 4.80 x 10^2 to 2.85 x 10^4 in C, 4.85 x 10^2 to 1.71 x 10^4 in D and 4.87 x 10^2 to 1.53 x 10^4 in E at the end of 16 day (Fig 6).

Zhang *et al*. (2015) [33] reported a similar increase in TPC (log cfu/g) ranging from3 d 7 log CFU/g on day 6 during chilled storage. Similar results obtained by Binsi (2013) [3] Fresh pabda catfish meat had a total viable count of 4.6 log10 cfu/g. During chill storage, bacteria grew quickly in CPE samples than in VP samples. Kedar *et al*. (2016) [10] reported the increasing trend for total plate count (cfu/g) for all samples of chilled stored catla fish steaks. Kumar *et al*. (2015) [12] reported the initial content of TPC (log cfu/g) of raw silver pomfret fillets was 4.63 log cfu/g.

The TPC is widely used to assess the bacterial quality of fish and a pathogenic bacterium indicates the inadequacy of hygiene and sanitation during processing, transport, and storage (Sanjeev, 2000) [28].

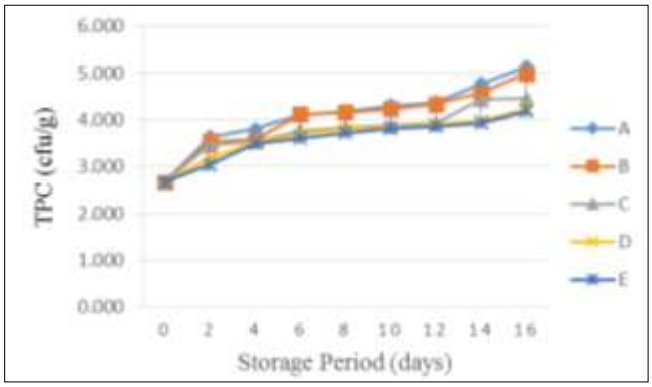

Fig 6: Changes in TPC values of chilled stored air packed pangasius fish steak

4. Conclusion

The results of present study revealed that air packaging alone, without treatment, would not be of much effective under the reported experimental conditions. The findings of the present study, clearly suggest that a combination sodium benzoate and storage at chilled temperature could be used to prolong the shelf life of pangasius. Therefore, it was concluded that 5-minute dip treatment of 2.0 % sodium benzoate can be used to maintain and enhance the shelf life up to 16 days of Pangasius fish (*P. hypophthalmus*) steaks during chilled storage.

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