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Shelf life of chill stored pangasius (*Pangasianodon hypophthalmus*) fish fillets: effect of vacuum and polyphosphate

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ABSTRACT

Pangasianodon hypophthalmus, commonly known as pangasius is internationally marketed in the form of frozen fillets. Value addition for domestic markets in the form of fillets, fingers, cutlets and fish balls may be an alternative but the lack of proper cold chain facilities (-18 °C) in the domestic sector is an impediment to market these products. The present study was taken up to determine the shelf life of pangasius fillets in chilled condition (<4 °C) in ice. The proximate composition showed that pangasius fillets are a good source of easily digestible protein (17.24%). Four batches of pangasius fish fillets were prepared for the experiments. The first batch (CC, chilled control) of pangasius fillets were packed individually in polythene pouches; the second batch (VC, vacuum control) vacuum packed in polythene pouches; the third batch (CT, chilled treated) soaked in chill water solution [(1% salt, 2% sodium tri polyphosphate (STPP)] for 30 min and packed in polythene pouches and the fourth batch (VT, vacuum treated) was soaked in chilled water solution containing 1% NaCl, 2% STPP for 30 min and vacuum packed in polythene pouches. All the pouches were stored under chilled condition (4 ºC) in ice. The fillets were analysed for chemical and microbiological parameters at regular intervals *viz*., 0, 3, 6, 9 and 12 days of storage. The phosphate content of CT (4410 ppm) and VT (4120 ppm) fillets at the end of 12 days of chilled storage was lower than the permissible limit of 5000 ppm. PV values were lower, both in STPP treated and in vacuum packed fillets. Total volatile base nitrogen (TVBN) values were lower than 30 mg100 g^{-1} in CC, CT, VC and VT fillets till 9 days of chilled storage and thereafter showed relatively rapid increase. The texture of treated fillets (CT and VT) was firm. The colour of vacuum packed fillets (VC and VT) was relatively darker. The aerobic plate count (APC) of STPP treated fillets (CT and VT) was lower than the corresponding control fillets (CC and VC). The results indicate that pangasius fillets can be stored for a period of 9 days in chilled condition (<4ºC) and soaking the fillets in 1%NaCl and 2% STPP chilled water would improve texture and moisture retention.

Keywords: Fillets, *Pangasianodon hypophthalmus*, Pangasius, Polyphosphate, Vacuum

Introduction

Pangasianodon hypophthalmus, an exotic catfish that is endemic to the waters of Mekong basin in south-east Asia, belongs to the family *Pangasiidae* and commonly known as river or silver stripped cat fish, sutchi catfish and iridescent shark. Total pangasius production in India during 2009-10 was 3,01,066 t . The annual production of pangasius cultured in Andhra Pradesh increased phenomenally and reached 3,00,000 t from a culture area of 15,000 ha in 2009-10 (MPEDA, 2010). Pangasius is being cultured, mainly in the Krishna, West Godavari, East Godavari, Guntur and Nellore districts of Andhra Pradesh. Pangasius farming in Andhra Pradesh represents the fastest growth of a single species farming recorded so far in the aquaculture sector of India.

Pangasius meat has high nutritive qualities and excellent sensory properties. The fish can be filleted easily due to the absence of intra-muscular pin bones. Tender flesh, sweet taste; absence of fishy odour and spines, delicate flavour and firm texture when cooked are the attributes that favour consumer preference for pangasius. Frozen catfish fillets popularly known as ‘basa’ forms the mainstay of export of fishery products from Vietnam to US and Europe. There is a great potential for development of convenience products such as fish fillets, fish fingers, fish cutlets, fish balls, fish wafers, fish pickles, smoked fish, canned fish and fish curry in retort pouches from pangasius (Silva *et al*., 2002; Ninan *et al*., 2011; Rathod and Pagarkar, 2013). The major crisis in pangasius farming is the decrease of market price. One of the avenues for addressing this problem is to increase the consumption of the fish by way of promoting value added products in the domestic markets. However, the marketing of value added products requires infrastructure in the form of cold chain from the point of manufacture to the point where the product is consumed. Generally, these value added products are frozen and stored at -18°C till they are consumed. The lack of cold chain facilities in the domestic sector makes it extremely difficult to market these products.

The present study was taken up with an objective to study the shelf life of pangasius fillets stored under iced condition (<4°C), employing vacuum and sodium tri polyphosphate (STPP) treatments.

Materials and methods

P. hypophthalmus weighing between 1 to 1.2 kg, were procured in fresh condition from fish farm and immediately brought to the laboratory within four hours in chilled condition (<4°C).

The fish were manually filleted on a stainless steel flat surface using sharp knives. The fish were beheaded, gutted, gilled and finally washed thoroughly with 2 ppm chlorinated water. The skin of the dressed fish was first removed by making a length wise cut and pulling the skin manually. The exposed flesh of the fish was then cut parallel to the central bone frame and hanging meat was trimmed off. Fish fillet *i.e*. skinless, boneless, fish loin pieces were used for analysis and storage studies.

Four batches of pangasius fish fillets were subjected to the following treatments. First batch (CC, chilled control) of fillets were packed individually in polythene pouches and stored in chilled condition (<4°C) in ice. Second batch (CT, chilled treated) of fillets were soaked in chilled water containing 1% salt (w/v) and 2% STPP (w/v) for 30 min and were packed in polythene pouches and stored under chilled condition (<4°C) in ice. Third batch (VC, vacuum chilled) of fillets were vacuum packed individually in pouches and stored in chilled condition (<4°C) in ice. Fourth batch (VT, vacuum treated) of pangasius fillets were soaked in chilled water containing 1% salt (w/v) and 2% STPP (w/v) for 30 min and were vacuum packed in pouches and stored under chilled condition (<4°C) in ice. Vacuum packing was done using Sevana's quick seal machine (Sevana Electrical Appliances Pvt Ltd, Kerala, India). Pouches made of 12µ polyester laminated with 300 gauge low density polyethylene were used for packing the fish. Flake ice prepared using ice flaking machine (Icematic, Italy) was used for chilling the fish. The fillets were kept in insulated boxes containing adequate quantity of ice and re-icing was done at regular intervals to maintain the temperature of chilled fillets below 4°C. The fillets were taken out at regular intervals *i.e.,* after 0, 3, 6, 9 and 12 days of storage and analysed for chemical and microbiological parameters. Freshly prepared fish fillet (0 day) was also analysed for proximate composition, biochemical and microbiological parameters.

Moisture, protein, fat, and ash were determined as per standard methods (AOAC, 1990). The loss in moisture was calculated by subtracting the moisture content of control (CC) fillets from that of the treated fillets. Peroxide value (PV) was determined iodometrically (Method # 965.33 AOAC, 1990) and total volatile base nitrogen (TVBN) was determined by the Conway micro diffusion method (Conway, 1947). Salt soluble nitrogen (SSN) (Ironside and Love, 1958) and water soluble nitrogen (WSN) (Winton and Winton, 1958) were estimated. Aerobic plate count (APC) was determined as per Speck (1978) using tryptone glucose agar (TGA) and H_2S producing bacterial count analysed using peptone iron agar (PIA) (Gram *et al.* 1987). All the analyses were done in triplicates and the data were subjected to statistical evaluation using SPSS 16 for Windows. Post-hoc (Tukey HSD) test was employed to find out significant difference among means at $p< 0.05$

Results and discussion

Manual filleting of *P. hypophthalmus* was found to be easier due to the absence of intramuscular pin bones. The flesh of *P. hypophthalmus* was found to be tender, with minimal fish odour. The fillets were pinkish to pinkish white in colour. The proximate composition shows that *P. hypophthalmus* fish fillets are rich source of prote in (17.24%) and indicate their suitability for the preparation of value added products. Moisture content was 78.2%, crude fat 2.84% and ash content was 1.3%. Orban *et al*. (2008) studied the nutritional quality of *P. hypophthalmus* fillets produced in the freshwater basins of Vietnam and reported moisture levels of 80–85%, protein content of 12.6–15.6%, lipid content of 1.1–3.0%, and the total lipids were characterised by low cholesterol levels (21–39 mg 100 g^{-1}).

Changes in moisture content of chilled P. hypophthalmus fillets

Moisture content influences the quality of the products (Sen, 2005). Moreover, moisture content of the final product has economic implications as the retention of moisture by the product increases the gross weight of the product resulting in economic gains. It was observed that the moisture content of STPP treated fillets (CT and VT) was higher (Fig. 1). The difference in moisture percentage ranged between 0.6 and 2.76% in CT fillets (CT-CC) and between 0.74 and 3.73% in VT fillets (VT-VC). Sodium tri polyphosphate ($Na_5P_3O_{10}$, STPP) is an approved food additive with an E-number of E451 (triphosphates) and a permissible level of 5 g kg^{-1} (EC directive, 1995). Sodium tri polyphosphate in combination with salt is generally used in fish and shrimp processing for increasing the water holding capacity of the processed product. Truc and

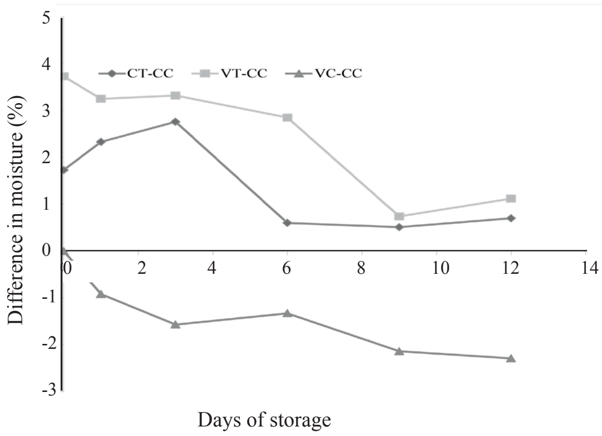


Fig. 1. Changes in moisture content of chilled *P. hypophthalmus* fish fillets

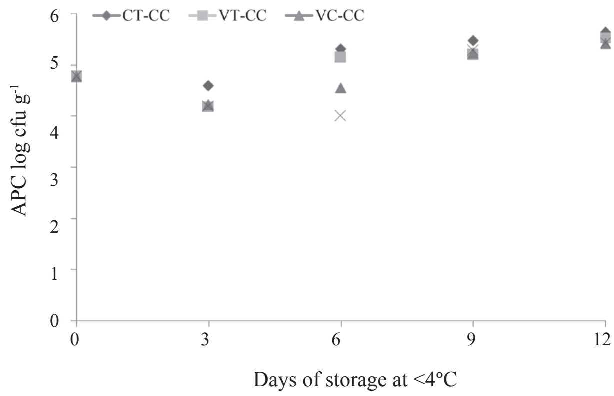


Fig. 2. Changes in aerobic plate count of chill stored *P. hypophthalmus* fish fillets

Muoi (2010) observed that using STPP for pretreatment of *P. hypophthalmus* fillets prior to freezing, had significantly less drip loss than using a mixture of STPP + sodium polyphosphate or STPP + sodium polyphosphate + sodium diphosphate. Soaking of pangasius fillets in chill water with 1% salt and 2% STPP for 30 min increased the phosphate level in the fillets but the phosphate content of CT (4410 ppm) and VT (4120 ppm) fillets at the end of 12 days of chilled storage was lower than the permissible limit of 5000 ppm. The texture of STPP treated fillets (CT and VT) was relatively firm. Vacuum packed (VC) fillets showed a loss in moisture during storage. The loss in moisture ranged between -2.29 and -0.92% in VC fillets (VC-CC). Goulas and Kontominas (2007) reported higher drip loss in vacuum packed chub mackerel (*Scomber japonicus*) fillets when compared to air packed fillets under refrigerated storage. Poor texture and high drip loss of cod fillets packed in vacuum or modified atmospheres indicated that their shelf life was limited by chemical reactions and not only by microbial activity (Dalgaard *et al*., 1993).

Changes in bacteriological quality parameters

Aerobic plate count (APC) indicates the total bacterial load of the sample. The APC showed an initial reduction up to 3rd day, gradual increase till 9th day followed by rapid increase between 9th and 12th day of chilled storage of CC, CT, VC and VT fillets (Fig. 2). APC of STPP treated fillets (CT and VT) were always lower than the corresponding untreated fillets (CC and VC). This could be attributed to the antibacterial effect of STPP. Lee *et al*. (1994) reported 0.5% STTP to be inhibitory to *Staphylococcus aureus*. The ability of polyphosphates to chelate metal ions appears to play an important role in their antimicrobial activity. Polyphosphates also inhibit cell division by blocking cell septation. Gram positive bacteria are generally more susceptible to phosphates than Gram negative bacteria. Although polyphosphates are highly inhibitory to a variety of food borne pathogens, Oliver and Kaper (2001) observed that 1% tripolyphosphate has no lethal effect on *Vibrio vulnificus*. Survival of *V. cholerae* at low temperatures was increased by the addition of 0.5% of heated pyrophosphate and metaphosphate, probably by decreasing the lethality of the cold injury to the cells (Wong *et al*. 1995). Ozogul *et al*. (2004) reported that bacteria grew more quickly in fish stored in air than when vacuum packed at 0 °C. Highest APC value obtained was in CC (4.4 x 10^5 cfu g^{-1}) at the end of 12 days of chilled storage. However, it was observed that the APC values of CC, CT, VC and VT *P. hypophthalmus* fillets were less than the acceptable level of 5 x 10^5 cfu g^{-1} (EIC, 1995; FSSAI, 2011) even after the end of 12 days of chilled storage at less than 4°C. The spoilage microbiota in the processing lines of frozen *P. hypophthalmus* fillets in Vietnam was studied by Tong Thi *et al*. (2013) and reported that the genera *Aeromonas*, *Acinetobacter*, *Lactococcus* and *Enterococcus* were prevalent at various processing steps. H_2S producing bacteria counts were <10 cfu g^{-1} in CC, CT, VC and VT fillets during 12 days of chilled storage of *P. hypophthalmus* fillets indicating minimal spoilage. Rao and Khasim (2009) suggested that counts of H_2S producing bacteria >10^3cfu g^{-1} indicated spoilage in freshwater fish, rohu.

Changes in biochemical quality parameters

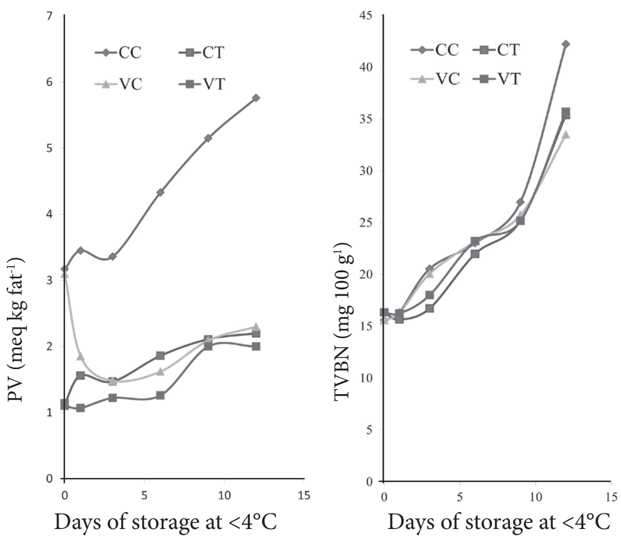
Peroxide value (PV) gives a measure of oxidative rancidity. The PV of vacuum packed fish (VC, VT) was lower than the corresponding air packed fillets. The peroxide values of VC and VT at the end of first day of chilled storage was 1.85 meq kg fat^{-1} and 1.07 meq kg fat^{-1} respectively whereas the PV of CC and CT were 3.45 meq kg fat^{-1} and 1.56 meq kg fat^{-1}, respectively.

The PV reached 5.76 meq kg fat^{-1} in CC at the end of 12 days of chilled storage but the PV was 2.2 meq kg fat^{-1}, 2.3 meq kg fat^{-1} and 2 meq kg fat^{-1} in CT, VC and VT, respectively. PV of STPP treated fillets (CT, VT) were lower than corresponding untreated fillets (Fig. 3a) during the entire period of chilled storage. The low PV values in CT fillets can be attributed to the protective effect of STPP on fats. Lower PV in VC and VT fillets indicates the effectiveness of vacuum packaging in arresting fat oxidation process. Vacuum packing eliminates oxidation and preserves delicate flavours and is widely used in food industry (Gopal *et al*., 1999). Ahvenainen and Malkki (1985) reported that the shelf life in vacuum packages was twice that in cardboard packages. However, the PV of CC, CT, VC and VT fillets during the entire storage period of 12 days were lower than the preferred value of 10 meqkg fat^{-1} (Connell, 1975) thus indicating better quality of fat. The PV of CT fillets was almost similar to that of VT fillets suggesting that using STPP would be an inexpensive way to protect fat quality of *P. hypophthalmus* fish fillets than going for expensive vacuum packaging. Manju *et al.* (2007) suggested that vacuum packaging alone, without preservative treatment would not be of much use. According to Mohan *et al.* (2008) by using O_2 scavenger inside the package, the use of a vacuum packing machine can be avoided and they also found that O_2 scavenger extended the product's shelf-life up to 20 days.

The TVBN values of all the fillets showed a gradual increase during chilled storage (Fig. 3b). However, the TVBN values of CC, CT, VC and VT fillets were less than acceptable value of 30 mg 100 g^{-1} (Connell, 1975) till the end of 9 days of storage indicating acceptable quality. The TVBN values of CC, CT, VC and VT fillets were above 30 mg 100g^{-1} by the end of 12 days of chilled storage thereby making them unacceptable.

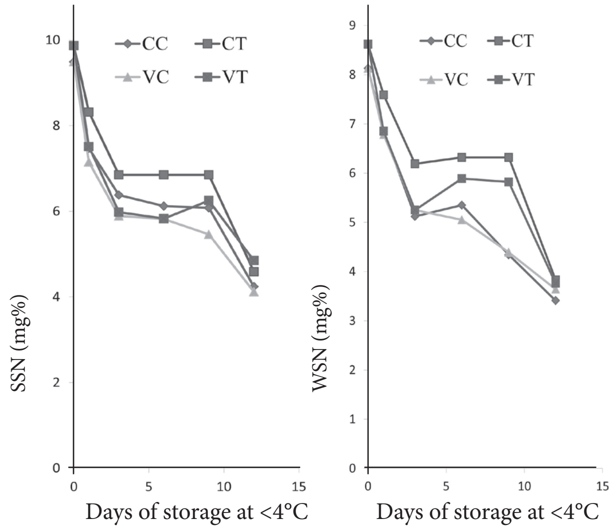
Changes in water soluble nitrogen (WSN) and salt soluble nitrogen (SSN)

The WSN and SSN values of the CC, CT, VC and VT fillets decreased with increase in storage time (Fig 4 a, b) but the rate of decrease was lower in STPP treated fillets. This might be due to the cryoprotective property of STPP. Addition of STPP in frozen tra (*P. hypophthalmus*) fillets helped in reducing drip loss by improving the retention of water by the protein in fish without absorbing too much water into final products (Truc and Muoi, 2010). The SSN values in CC decreased from 9.49 (0 d) to 4.24% (12 d) whereas the change in SSN in CT fillets was from 9.87 (0 d) to 4.59% (12 d). Similarly SSN values in VC decreased from 9.49 (0) to 4.12% (12 d) whereas that in VT fillets was from 9.87 (0 d) to 4.85% (12 d). WSN values showed similar trend; decrease being relatively lower in STPP treated fillets. Similar observations were reported by Channarong *et al.* (2007) while studying the changes in quality of hybrid catfish fillet stored at 4°C and by Thippeswamy *et al.* (2002) while studying the iced storage of Indian milk fish (*Chanos chanos*). Chilling is an effective way of reducing spoilage in fish but it has to be done quickly and hygienically. Immediate chilling of fish ensures high quality value added products (Huss, 1995). Up to 35% yield of high value products can be expected from fish processed within 5 days of storage in ice, after which a progressive decrease in the utility



(a) (b)

Fig. 3. Changes in biochemical quality parameters during chilled storage of *P. hypophthalmus* fish fillets
a) PV b) TVBN



(a) (b)

Fig. 4. Changes in salt soluble nitrogen (SSN) and water soluble nitrogen (WSN) of chilled *P. hypophthalmus* fish fillets
a) SSN b) WSN

was observed with increase in storage days and beyond 9 days of ice storage no high value added products could be processed (Venugopal and Shahidi, 1998). Noseda *et al*. (2012) reported a shelf life of 10 days for vacuum packaged and chill stored *P. hypophthalmus* fillets. Manju *et al*. (2007) reported a shelf life of about 8 days and 15 days respectively for air packed and vacuum packaged pearlspot (*Etroplus suratensis*) during chill storage.

The results of this study show that chilled storage (<4 °C) of pangasius fillets using ice provide a minimum shelf life of 9 days. Treating the fillets with 2% STPP and 1% NaCl proved to be beneficial as it increased moisture retention, lowered bacterial and biochemical spoilage. The information generated would help in preparing and marketing value added products from *P. hypophthalmus* more economically using only ice.

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