



Shelf life Characteristics of *Pangasianodon hypophthalmus* Fillets Treated with *Moringa oleifera* (Lam) Leaf extract

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Abstract

Effect of dip treatment in *Moringa oleifera* (Lam) leaf extract (MOL) on the quality and shelf life of vacuum packed *Pangasianodon hypophthalmus* fillets was evaluated during chilled storage at $2 \pm 1^\circ\text{C}$. The control (untreated) and treated groups with 5% (v/v) and 10% (v/v) *Moringa oleifera* leaf extract (MOL) were examined periodically at 0, 3, 6, 9, 12, 15, 18 days during chilled storage until rejection by sensory, physicochemical and microbiological methods. The study revealed that the *Moringa oleifera* extract is a good source of phenolic compounds with significant antioxidant potential. The total phenolic content was found to be 183.75 mg GAE 100 g⁻¹. MOL was found to have high antioxidant potential. The pH and TBA values of control group were significantly higher ($p < 0.05$) than treated groups. Among the treated samples 10% treatment showed the lowest value. *Moringa oleifera* leaf extract was also found to have strong antimicrobial potential which could retain the quality attributes of fillets during the storage time. The dip treatment with 5 and 10% MOL improved the shelf life of fillets by 6 days compared to control under vacuum packed condition. In microbiological view point this treatment can be effectively used as a safe bio-preservative to extend the shelf life of vacuum packed pangasius fillets under chilled condition without any adverse effect on the sensory acceptance of the treated fillets.

Keywords: Biopreservation, chilled storage, vacuum packaging; *Moringa oleifera*

Received 17 May 2019; Revised 15 October; Accepted 23 October 2019

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Introduction

Consumption of fishery products is on the rise all over the world as it is a rich source of high-quality protein, essential vitamins, polyunsaturated fatty acids (Verbeke et al., 2005). Despite its nutritional contents, the high levels of moisture and pH, free amino acids, polyunsaturated fatty acids and the presence of various naturally present autolytic enzymes makes fish a highly perishable product even under refrigerated conditions (Cakli et al., 2007). To address this problem and to meet the increasing demand for high quality ready to cook fish products with extended shelf-life, various innovative techniques were developed which ensures quality as well as safety (Maftoonazad & Badii, 2009). In this regard, several synthetic chemical additives like benzoates, sulphites, nitrites, sorbates, formaldehyde, parabens, butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) are being used indiscriminately in the food industry as preservatives (Anand & Sati, 2013; Sharma, 2015). But continuous usage of such chemicals including antibiotics as preservatives in food industry results in increased evidence of cancer, other foodborne illness, and development of multidrug resistance (MDR) in bacterial strains (Van et al., 2015). Recently, the consumer awareness and concerns regarding the usage of chemical preservatives results in numerous efforts to find natural alternatives to prevent bacterial and fungal growth in foods. Biopreservation is a novel technology which extends the shelf-life and safety of food products by the use of natural products like essential oils, phytoextracts, animal enzymes, microbial bacteriocins, organic acids and naturally occurring polymers (Garcia et al., 2010; Viji et al., 2015a; Binsi et al., 2017).

Moringa Oleifera (Lam) belongs to family *Moringaceae* is a native to North-western India. It is often called

as 'Miracle tree' or 'Tree of life' because of its wide range of medicinal uses with high nutritional value (Morimitsu et al., 2000; Gopalakrishnan et al., 2016). Its high antioxidant and antimicrobial potential were reported by many authors (Chandrasekhar et al., 2006; Sreelatha & Padma, 2009; Anwar et al., 2007; Kawo et al., 2007; Abalaka et al., 2012; Sayeed et al., 2012). *M. oleifera* leaves are being used as a natural preservative for shelf life extension of various food products like goat meat patties (Das et al., 2012), pork patties (Muthukumar et al., 2014), smoke-dried catfish (Adeyemi et al., 2013) and raw beef meat packed in MAP (Shah et al., 2015) etc. *Pangasius* (*Pangasianodon hypophthalmus*) is emerging as a significant component of global whitefish supply (Viji et al., 2015). Their delicate flavour, absence of fishy odour and intramuscular spines offers a great potential for the various 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; Rathod & Pagarkar, 2013; Ninan et al., 2015). There are limited reports on the use of *Moringa oleifera lam* leaf extract as a natural source of antimicrobial substance in fish processing. Therefore, this study evaluates the antibacterial and antioxidant potential of *Moringa oleifera* leaf extract in preservation of fish fillet. Rao et al. (2013) reported that *Pangasius* fillets can be stored for a period of 9 days in chilled condition (<4°C). In the present study evaluates the antibacterial and antioxidant potential of *Moringa oleifera* leaf extract for extension of shelf life in vacuum packed *Pangasius* fillets during chilled storage was evaluated.

Materials and Methods

Fresh *M. oleifera* leaves were collected and extract was prepared according to Rahman et al. (2009). The yield of MOL is 29% and this was used further for the preparation of 5% (v/v) and 10% (v/v) solution in distilled water with 2°C. *Pangasius* fillets dipped in water with 2°C was used as a control. Quantitative estimation of polyphenols was done for *Moringa* leaf juice and fish fillets dipped in 5% (v/v) and 10% (v/v) MOL to study the extent of juice penetration to fish tissues by the spectrophotometric technique based on Folin Ciocalteu assay (Singleton & Rossi, 1965).

Fresh *P. hypophthalmus* were procured from the local market, Mysore, Karnataka was immediately brought

to the laboratory in chilled condition. The fish were eviscerated, manually filleted and washed thoroughly. The yield of the fish fillet was 44%. Fish fillets were again cut into pieces of equal size approximately weighing 30 gm each, *i.e.* skinless, boneless; fish pieces were used for analysis and storage studies. Fish fillets were randomly divided into two groups; the first group was treated with 5% *Moringa oleifera* leaf juice (MOL) while the second group was treated with 10% MOL. Fish fillets dipped in distilled water acted as control. The fish was dipped for 15 min in twice the volume of the MOL solution maintained at 2°C for the effective incorporation.

Treated fish fillets and control was vacuum packaged in LDPE (Low density poly ethylene) bags in triplicates under 90% vacuum using a vacuum cum modified atmospheric packaging machine (Sevana, India), and stored at 2±1°C in the refrigerator. Samples were drawn at regular intervals and were subjected to sensory, physicochemical and microbiological evaluation. pH of the homogenized sample in distilled water (1: 5 W/V) was determined using a glass electrode digital pH meter (Cyberscan pH tutor, Eutech instruments, India). Color of the homogenized fish samples were estimated using Hunter colourimeter (Hunter lab, Reston, VA, USA) in terms of L, a* and b* values (Shand, 2000).

The texture of *Pangasius* fillets was analysed using Universal Testing Machine (LLYOD instruments, Inc. USA). TBA values of *Pangasius* fillets were determined as per Tarladgis method (1960). The OD was measured at 538 nm using UV/vis spectrophotometer, Parkin Elmer, USA). Aerobic plate count (APC) was performed on Trypticase soy agar (TSA) (Bd and Difco, USA) for control and *Pangasius* fillets dipped in 5% (v/v) and 10% (v/v) *Moringa oleifera* leaf juice solution periodically every three days until rejection. All TSA plates were incubated at 37°C for 48 h. Sensory analysis of *Pangasius* fillets was carried out by five semi-trained panelists based on attributes like color, texture, and aroma and were asked to assign a score of 1-9 according to Meilgaard et al., 1999. Data were subjected to analysis of variances (one way- ANOVA) according to Knapp & Miller (1992) using (SPSS Statistics 16.0) software program. Data were expressed as mean values with standard deviation. Differences in the mean values of the various treatments were determined by Duncan test, and the significance was defined at (p< 0.05).

Results and Discussion

The total phenolic content (TPC) of *M. oleifera* leaf extract (MOL) was 183.75 mg Gallic acid equivalent 100 g⁻¹. Nambiar et al. (2013) reported a TPC of 141.59 to 185.32 mg GAE 100 g⁻¹. A higher TPC value of *M. oleifera* leaf aqueous extract was reported as 45.81- 48.36 mg GAE g⁻¹ previously (Das et al., 2012; Sreelatha & Padma, 2009). These differences might be due to differences in the procedures followed for the extraction of phenolic compounds, season, stage of leaf development and maturity, genetic variability and post-harvest handling of the leaf samples (Sayeed et al., 2012). In this study, the total phenolic content of Pangasius fillets dipped in 5% (v/v) and 10% (v/v) *M. oleifera* juice solution was 28.75 mg GAE 100 g⁻¹ and 72.5mg GAE 100 g⁻¹ respectively. This clearly indicates that the extract was incorporated effectively in to fish fillet within the dipping time of about 15 min.

Generally, pH changes can be used as a spoilage indicator in fishery products. The initial value of muscle pH was 6.26 (Table 1). The pH values of untreated group increased significantly (p<0.05) during storage and showed 0.66 units difference at the end of storage. The pH values of 5 and 10% MOL treated samples also increased significantly (p<0.05) during storage and expressed a difference of 0.47 units and 0.2 units respectively at the end of storage. Normally, the post-mortem pH fish meat is 7 or slightly lower, immediately after the catch (Huss et al., 1995). But the pH might be increased due to stress which the fish encountered during harvesting or due to accumulation of microbial metabolites like biogenic amines (Gill et al., 1983; Abbas et al., 2008).

Table 1. Changes in mean pH values of *P. hypophthalmus* fillets under chilled conditions Control-Untreated, MOL : Treated with *Moringa oleifera* leaf extract

Days of storage	Control	5% MOL	10% MOL
0 th day	6.26 ± 0.06 ^c	5.97 ± 0.06 ^b	5.86 ± 0.06 ^a
3 rd day	6.38 ± 0.06 ^c	6.18 ± 0.02 ^b	6.01 ± 0.06 ^a
6 th day	6.42 ± 0.02 ^c	6.31 ± 0.00 ^b	6.18 ± 0.06 ^a
9 th day	6.54 ± 0.06 ^c	6.47 ± 0.00 ^b	6.26 ± 0.06 ^a
12 th day	6.67 ± 0.00 ^c	6.58 ± 0.01 ^b	6.32 ± 0.06 ^a
15 th day	6.83 ± 0.01 ^c	6.64 ± 0.02 ^b	6.38 ± 0.01 ^a
18 th day	6.92 ± 0.06 ^c	6.73 ± 0.01 ^b	6.46 ± 0.07 ^a

The pH values of the untreated group were significantly higher (p<0.05) than 5% and 10% MOL treated samples. 10% MOL treated samples showed the lowest pH value throughout the study. The low pH of the treated samples observed throughout the storage period might also be due to acidic nature of *M. oleifera* extract with pH 5.57.

TBA index gives a measure of malonaldehyde formed in the muscle as a result of oxidation of lipid peroxides. The maximum acceptable limit of TBA is 2 mg malonaldehyde/kg of fish beyond which the fish may develop unpleasant odour and taste (Wenjiao et al., 2014). The TBA value of all the groups showed an increase in malonaldehyde content on storage as shown in Fig. 1. The initial TBA-RS values of control, fillets dipped in 5% (v/v) and 10% (v/v) MOL were 0.037±0.03, 0.023±0.02 and 0.021±0.04 respectively. These values increased progressively (p<0.05) and reached 0.264±0.05, 0.211±0.01 and 0.176±0.01 respectively on 18th day of storage. TBA-RS values of control were significantly higher (p<0.05) than treated groups while 5% (v/v) MOJ treated samples showed lower TBA-RS value than 10% treated sample throughout the study. The present study revealed that MOL was effective in controlling the lipid oxidation.

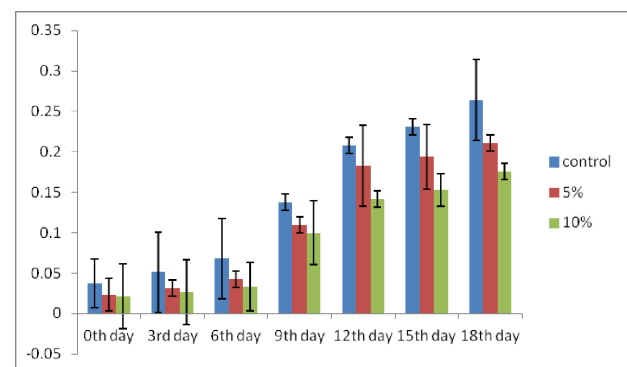


Fig. 1. Changes in mean values of fillets TBA (mg malonaldehyde kg⁻¹ of fish) during storage period

The changes in aerobic plate count (APC) with the storage period for control and treated fish fillets are given in Table 2. The initial APC was found to be 4.2 cfu g⁻¹ and thereafter showed a significant reduction (p<0.05) on the third day of storage. The APC increased continuously and reached 10⁶ cfu g⁻¹ on 9th and 15th day for control and treated sample respectively. This reduction might be mainly due to a sudden shift in the temperature to 2±1°C. Similar results were reported previously (Rao et al.,

2013; Binsi et al., 2016; Rao et al., 2017). The extension of shelf life for the treated samples might be due to antimicrobial properties of *M. oleifera* leaf extract (Suarez et al., 2003; Bukar et al., 2010).

Table 2. Changes in mean values of aerobic plate count (APC) during storage period

Days of storage	Control (log ₁₀ cfu g ⁻¹)	5% MOL (log ₁₀ cfu g ⁻¹)	10% MOL (log ₁₀ cfu g ⁻¹)
0 th day	4.25 ± 0.00 ^c	4.22 ± 0.03 ^{ab}	4.17 ± 0.06 ^a
3 rd day	3.97 ± 0.03 ^c	3.60 ± 0.00 ^b	2.80 ± 0.10 ^a
6 th day	5.72 ± 0.03 ^c	4.25 ± 0.05 ^b	3.90 ± 0.00 ^a
9 th day	6.13 ± 0.06 ^c	5.35 ± 0.05 ^b	4.98 ± 0.03 ^a
12 th day	NA	5.97 ± 0.03 ^c	5.58 ± 0.03 ^b
15 th day	NA	6.53 ± 0.03 ^c	6.25 ± 0.22 ^b
18 th day	NA	NA	NA

The texture of fish is one of the primary quality attributes for consumer acceptability. Hardness 1 and 2 were found to be decreasing in control as well as treated samples during storage (Table 3). Similar results were reported for chitosan treated double filleted Indian oil sardine and sodium acetate dip treated pearl spot during chill storage (Mohan et al., 2012; Manju et al., 2007). The results of the present study were also in agreement with those of Viji et al. (2015) on the quality characteristics and shelf life of sutchi catfish (*Pangasianodon hypophthalmus*) steaks and fresh water catfish during chilled storage (Binsi et al., 2015).

Table 3. Changes in Hardness 1 and 2 of *P. hypophthalmus* fillets during storage period

Hardness	Treatments	Initial (Kgf) (0 th day)	Final (Kgf) (18 th day)
Hardness 1	Control	1.36±0.09 ^a	1.02±0.05 ^a
	5% MOL	1.45±0.24 ^a	1.24±0.06 ^b
	10% MOL	1.35±0.28 ^a	1.30±0.06 ^b
Hardness 2	Control	1.24±0.10 ^a	0.77±0.15 ^a
	5% MOL	1.42±0.03 ^b	1.24±0.07 ^b
	10% MOL	1.22±0.10 ^a	1.17±0.04 ^b

Lightness value (L*) was initially high for 10% MOL treated sample compared to untreated and 5%

treated sample, but decreased significantly during storage (Table 4). The initial high lightness value of treated sample could be attributed to low pH of the MOL used for dip treatment which resulted in the leaching of muscle pigments during treatment. Likewise low pH of chitosan resulted in lightness for the chitosan treated double filleted Indian oil sardine during chilled storage (Mohan et al., 2012). In contrast Lopez-Caballero et al. (2005) reported that there are not many differences in colour of codfish patties coated with chitosan.

Table 4. Changes in fillets colour during storage period

Colour	Treatments	Initial (0 th day)	Final (18 th day)
L*	Control	54.22±0.26 ^a	48.36±0.127 ^a
	5% MOL	54.47±0.16 ^a	51.74±0.63 ^b
	10% MOL	56.59±0.09 ^b	53.10±0.02 ^b
a*	Control	7.15±0.03 ^a	6.98±0.04 ^a
	5% MOL	7.03±0.06 ^a	6.64±0.29 ^a
	10% MOL	7.10±0.10 ^a	6.84±0.40 ^a
b*	Control	26.21±0.46 ^a	24.32±0.50 ^a
	5% MOL	25.87±0.21 ^a	24.02±0.16 ^a
	10% MOL	26.27±0.44 ^a	24.21±0.31 ^a

Mean values of sensory scores of the samples over the storage period by the panel for colour, texture, aroma and for overall acceptability are shown in Table 5. A decrease in quality of fish samples was noticed by the panellists during storage on the 6th day while 5 and 10% MOL treated fillets was found to be acceptable till 9th day of storage. Initially, the colour score for 10% *M. oleifera* treated samples were less due to the high intensity of greenish colour compared to that of control and 5% treated samples. But 10% *M. oleifera* treated samples scored high for its aroma throughout the period of storage studies as it imparted a masking effect on fishy smell. Further studies are needed to study the underlying mechanism of flavour masking. This study concluded that the *Moringa oleifera* extract is a good source of phenolic compounds with significant antioxidant and antimicrobial potential. Hence 5% MOL can be used as a safe bio preservative which can replace the use of synthetic antioxidants and antimicrobials to extend the shelf life of *Pangasius* fillets under chilled conditions.

Table 5. Changes in sensory scores of fillets during storage period

Attributes	Treatments	0 th day	3 rd day	6 th day	9 th day	12 th day	15 th day	18 th day
Colour	Control	8.16±0.11 ^b	7.64±0.38 ^a	3.92±0.25 ^a	1.96±0.59 ^a	1.34±0.38 ^a	1.06±0.05 ^a	1.00±0.00 ^a
	5% MO	8.16±0.15 ^b	8.14±0.23 ^b	6.7±0.23 ^b	2.98±0.64 ^b	2.44±0.27 ^b	1.54±0.03 ^b	1.28±0.19 ^b
	10% MO	7.68±0.31 ^a	8.14±0.11 ^b	7.7±0.26 ^c	3.42±0.43 ^b	3.04±0.64 ^b	1.8±0.35 ^b	1.74±0.19 ^c
Texture	Control	8.74±0.15 ^b	7.46±0.51 ^a	3.94±0.11 ^a	1.46±0.45 ^a	1.36±0.11 ^a	1.14±0.36 ^{ab}	1.00±0.00 ^a
	5% MO	8.32±0.26 ^{ab}	8.26±0.21 ^b	6.9±0.16 ^b	3.06±0.57 ^b	2.3±0.16 ^b	1.28±0.30 ^a	1.26±0.13 ^b
	10% MO	7.98±0.58 ^a	8.18±0.16 ^b	7.46±0.38 ^c	3.3±0.32 ^b	3±0.16 ^c	2.1±0.84 ^c	1.48±0.29 ^b
Aroma	Control	7.32±0.28 ^a	6.98±0.52 ^a	3.92±0.08 ^a	1.10±0.14 ^a	1.34±0.38 ^a	1.10±0.12 ^a	1.00±0.00 ^a
	5% MO	8.06±0.45 ^b	8.26±0.15 ^b	7.24±0.23 ^b	3.46±0.52 ^b	3.12±0.66 ^b	1.26±0.33 ^a	1.28±0.13 ^b
	10% MO	8.96±0.05 ^c	8.76±0.11 ^c	7.50±0.31 ^b	3.38±0.44 ^b	3.16±0.43 ^b	2.10±0.33 ^b	1.50±0.32 ^b
Overall acceptability	Control	8.07±0.15 ^a	7.36±0.43 ^a	3.93±0.13 ^a	1.47±0.29 ^a	1.27±0.20 ^a	1.21±0.11 ^a	1.00±0.00 ^a
	5% MO	8.18±0.20 ^a	8.22±0.13 ^b	6.95±0.05 ^b	3.17±0.24 ^b	2.62±0.33 ^b	1.35±0.12 ^a	1.28±0.07 ^b
	10% MO	8.21±0.18 ^a	8.36±0.10 ^b	7.55±0.19 ^c	3.37±0.34 ^b	3.07±0.04 ^c	2.00±0.32 ^b	1.57±0.21 ^c

Acknowledgments

The authors thank Director, Indian Council of Agriculture Research-Central Institute of Fisheries Technology, Cochin and Director, Deference Food Research Laboratory, Mysore for providing facilities to undertake this work.

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