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Microbiological changes of Pangasius hypophthalmus fillets with Moringa oleifera (Lam) leaves in chilled storage condition

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Worldwide fishery products consumption is on the rise as it is a rich source of high-quality protein, essential vitamins, polyunsaturated fatty acids *etc* (Verbeke et al., 2005). But the unique biological composition along with high water content of fishery products make them susceptible to rapid enzymatic and microbial spoilage. Therefore, their shelf life is limited and several synthetic chemical additives are being used indiscriminately as preservatives. But continuous usage of such chemicals including antibiotics results in cancer, other foodborne illness, and development of multidrug resistance (MDR) in bacterial strains (Thomas et al., 2015).

To address such issues, bio-preservation has emerged as 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, naturally occurring polymers *etc (*Pilar et al., 2010). *Moringa Oleifera (Lam)* belongs to family *Moringaceae* is often called as 'Miracle tree' or 'Tree of life' because of its wide range of medicinal uses with high nutritive value. *Moringa* leaf is well known for its potential antibacterial as well as antioxidant activity and is being used as a natural preservative for shelf life extension of various food products. There are limited reports on the use of *Moringa* leaf juice as a natural source of antimicrobial substance in fish processing and this study evaluates the antibacterial and antioxidant potential of *Moringa* leaf juice for extension of shelf life in vacuum packed pangasius fillets during chilled storage at 4 ± 1 °C.

About 5.5 kg of *Pangasius hypophthalmus* were procured in fresh condition from the local market, Mysore, Karnataka and immediately brought to the laboratory in the chilled condition. The fish were cut into pieces of equal size approximately weighing 30 gm and used for analysis and storage studies. Fresh *Moringa* leaves were collected and juice was extracted based on Rahman *et al*. (2009). The yield of juice is 29% and this

was used further for the preparation of 5% (v/v) and 10% (v/v) solution in distilled water at 4ºC. Water at 4ºC was used as a control.

The control (untreated) and treated groups of fillets with 5% (v/v) and 10% (v/v) *Moringa* leaf juice (MOL) were examined periodically at 0, 3, 6, 9, 12, 15, 18 days during chilled storage until rejection by sensory, physicochemical and microbiological methods

This study revealed that the *Moringa* juice is a good source of phenolic compounds with significant antioxidant potential and total phenolic content was found to be 183.75mg GAE /100g. The pH and TBA-RS values showed an increasing trend during the storage time and were significantly higher ($p < 0.05$) in control group than treated and 10% treatment showed the lowest value. *Moringa* leaf juice was also found to have strong antimicrobial and antioxidant potential and can retain the quality attributes during the storage time. The initial TPC was found to be 4.2 log, which showed a significant reduction ($p<0.05$) on the third day of storage and thereafter increased continuously and reached about 6 log on 9th and 15th day for control and treated sample, respectively. The dip treatment for 15 minutes with MOL improved the shelf life by 6 days in vacuum packed condition. Hardness 1 and 2 were found to decrease in control as well as treated samples during storage. Lightness value (L*) was initially high for 10% MOL treated sample compared to untreated and 5% treated sample, but decreased significantly during storage. A decrease in quality of fish samples was noticed by the panelists on storage on the 6th day while the 5% and 10 % *MOL treated* fillets was found acceptable till 9th day of storage.

Treatment of *Moringa oleifera* juice can thus be effectively used as a safe bio-preservative to extend the shelf life of vacuum packed pangasius fillets under refrigerated condition without any adverse effect on the sensory acceptance.

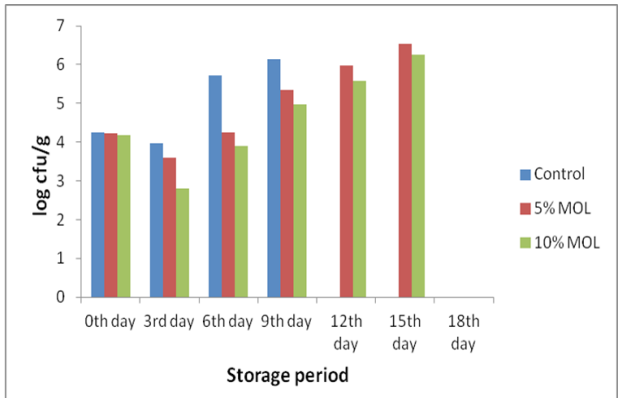

Figure 1. Changes in mean values of total plate count (TPC) during storage period

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Identifying melanosis producing bacteria from shrimp with utilization perspective.

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Melanosis, which is also called as black spot, the enzymatic browning of phenolic compounds in shrimp is considered as a challenge in processing industry. The melanosis happens either due to the innate immune response (Prophenoloxidase system) or due to the tyrosinase producing bacteria in the system (Nirmal *et al.*, 2009). The tyrosinase producing bacteria convert the phenolic compounds into melanin with the help of phenol oxidase enzymes. The final product melanin and the intermediate products such as L-dopa (L-3,4-dihydroxyphenylalanine), dopaquinone and dopamine are commercially important. Tyrosinases are the key enzymes to form the biopolymer melanin which have inherent properties like absorption of UV radiation, metals, sound and also have anti-oxidant and semi-conductor properties are used in the production of complex biopolymers (EMPA., 2010) These bacteria has the potential to be used for tyrosinase enzyme production, biocompost production from fishery products, phenolic waste treatment and also for melanin production (Amonette *et al.*, 2004;., Kafilzadeh et al., 2010;., Cédric *et al.*, 2016.)

In this study, *Penaeus vannamei* was procured from market of Ukkadam, Coimbatore with melanosis. Tyrosin enriched nutrient agar media was used for isolation of colonies. Three isolates i.e TMA7, TMA9, TMA10 showing maximum tyrosinase production were selected for further studies.

Potential tyrosinase producing isolates TMA 7, TMA9 and TMA10 were selected for identification using 16 S rDNA sequencing. Crude DNA was extracted from the young cultures from tyrosinated broth the phenol chloroform method. The forward and reverse primer used are 27F AGAGTTTGATCCTGGCTCAG and 1492R ACGGYTACCTTGTTACGACTT. Amplified product of 1500 bp was sequenced by sanger sequencing. Blast analysis of the Isolates TMA7,TMA9 ,TMA10 shown similarity for *Bacillus sp, Acinetobacter Sp,* and *Bacillus megaterium* respectively. Phylogenetic tree constructed revealed the distances and similarity of these bacteria. The study has provided the evidence *Bacillus sp* could be a potential source of tyrosinase enzyme for application in the shrimp waste industry.

Tyrosinases from microbes are being exploited for a variety of biotechnological and environmen-