INFLUENCE OF MORINGA OLEIFERA LEAF EXTRACT ON MICROBIAL AND QUALITY PARAMETERS OF PANGASiUS HYPOPHTHALMUS MINCE UNDER FROZEN STORAGE (-18±2°C)

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(Accepted 18 July 2017)

ABSTRACT: Pangasius hypophthalmus is one of the most important commercial fishes with high nutritive qualities and excellent sensory properties such as tender flesh, sweet taste, absence of fishy odor and spines, delicate flavor and firm texture during cooking that help to gain its consumer preference. Moringa oleifera (commonly called drumstick) is one of such most important terrestrial plants which exhibit antibacterial, antifungal as well as antioxidant properties which influence its application in food preservation. In the present study, three different concentrations (5%, 10% and 15%) of Moringa oleifera leaves extract were used as antimicrobial and antioxidant agent to study the shelf life of minced meat prepared from Pangasius hypophthalmus during frozen storage at ×18±2°C for a period of 5 months. The treatments showed significantly (p<0.05) improved values of the quality parameters such as TVBN, TMA and TBARS as compared to control throughout the frozen storage period. TPC was also found to be reduced significantly (p<0.05) in treated samples. Presence of different phytochemicals that have effective antimicrobial properties of M.oleifera extract against wide range of bacteria which were responsible for accumulation of ammonia and volatile bases in fish flesh, decreased protein breakdown, thus lowering TVBN and TMA values. The antioxidant properties of polyphenols present in the M. oleifera leaf extract retarded the formation of malonaldehydes. From the present study, it can be concluded that 15% concentration of M. oleifera leaves extract is considered as most effective to maintain the quality of the minced Pangasius hypophthalmus during five months frozen storage at -18±2°C.

Key words: Moringa oleifera, Pangasius hypophthalmus, frozen storage, antimicrobial, antioxidant.

INTRODUCTION

Fish is highly perishable and is readily susceptible to chemical and microbial deterioration (Gram and Huss, 2001) due to presence of polyunsaturated fatty acids and essential amino acids in a large quantity. Pangasius hypophthalmus is one of the most important commercial fishes with high nutritive qualities and excellent sensory properties such as tender flesh, sweet taste, absence of fishy odor and spines, delicate flavor and firm texture during cooking that help to gain its consumer preference. Increasing market demand for this fish proves its acceptance by the consumers almost all over the world (Phan et al, 2009). With the availability of this fish in large quantity, there is a good potential for development of convenience products such as fish cutlets, fish fingers, canned fish and fish curry in retort pouches (Rathod and Pagarkar, 2013).

Lipid oxidation is a major cause of muscle food deterioration leading to subsequent off-flavours, unpleasant odours, texture, discoloration and decrease in nutritious value (Frankel, 1998). To retain the good quality characteristics for longer and extend the shelf life during frozen storage of fish, chemical preservatives such as butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT), have been widely used. Parallely, numerous studies are focused on using natural ingredients to enhance fish quality and shelf life in order to avoid the use of harmful synthetic preservatives (Khanedan et al, 2011). Many plant tissues are good sources of phytochemicals, notably phenolic and flavonoids (Gorinstein et al, 2005), that can act as the best alternative to the mutagenic food additives.

Moringa oleifera (commonly called drumstick) is one of such most important terrestrial plants which exhibit antibacterial, antifungal as well as antioxidant properties which influence its application in food preservation. Due to the presence of many important substances like ascorbic acid, estrogenic substances, beta sitosterol, iron, calcium, phosphorus, copper, vitamin A, B and C, alpha tocopherol, beta carotene, protein and essential amino acids like methionine, cystine, tryptophan and lysine, drumstick leaves can be considered as a dietary supplement. Ethanolic extract of M. oleifera showed a
broad spectrum antimicrobial property against many pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* sp., *Pseudomonas aeruginosa*, *Cornebacterium* sp., *Klebsiella pneumonia* and *Acinetobacter* sp. (Rajamanickam and Sudha, 2013) due to presence of phytochemicals such as flavonoids, saponins, tannins and other phenolic compounds (Sato et al, 2004).

Along with its anti-inflammatory, anti-helminthic, antifungal, pro-cogulant and flocculating properties, *Moringa* leaves have been reported to be a good source of natural antioxidants and thus, enhance the shelf life of fat containing foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Siddhuraju and Sudha, 2013) due to presence of phytochemicals such as flavonoids, saponins, tannins and other phenolic compounds (Sato et al, 2004).

The fishes (*Pangasius hypophthalmus*) procured from Howrah fish market were beheaded, descaled, filleted and skinned manually. The fillets were fed into silent cutter and the mince thus collected was washed thoroughly by following three washing cycles using chilled water. The meat was pressed using screw press to drain off the excess water. Mince was prepared under good hygienic and sanitary conditions to prevent any cross contamination and was used for further studies.

**Determination of the shelf life of the washed mince treated with *Moringa oleifera* leaf extract under frozen (-18±2°C) storage condition**

The study was conducted in a completely randomized design for four treatments, prepared by manually mixing the raw mince with *Moringa oleifera* leaf extract at different concentrations (5%, 10% and 15%) and were blended for 1 minute (Hazra et al, 2012) and keeping another sample without any treatment. Samples were then placed in Styrofoam trays and stored at ×18±2°C for 5 months. The treatments were (i) Pangasius mince without any treatment i.e. control (C), (ii) mince treated with *Moringa oleifera* extract at 5% concentration (5 ml extract/100 g mince) (S1), (iii) mince treated with *Moringa oleifera* extract at 10% concentration (10 ml extract/100 g mince) (S2), (iv) mince treated with *Moringa oleifera* extract at 15% concentration (15 ml extract/100 g mince) (S3).

Proximate composition of the raw *Pangasius* minced meat was determined only once before frozen storage that includes protein, fat, ash and moisture content. Moisture of the experimental samples was measured by Moisture Balance (Precisa, Dietikon, Switzerland). Total nitrogen was estimated by Kjeldahl method (AOAC, 1995). Crude protein value was calculated by multiplying the total nitrogen value by a factor of 6.25. Estimation of total lipid was done by the method described by Bligh and Dyer (1959). The ash content was measured by the method of AOAC (1995). All the results were expressed on wet weight basis.

The treatments were subjected in triplicate for Total Plate Count (TPC) and physicochemical analyses at fortnight interval starting from day 0. TPC was determined by spread plating appropriate dilutions on Total Plate Count Agar (Hi-media) (Nath et al, 2014) and results expressed as log cfu/g. The physicochemical indices used to analyse the shelf life of fish mince wasTotal volatile basic nitrogen or TVBN (by the method described by Nath et al, 2014), Tri Methyl Amine or TMA (by the method described by AOAC, 1995) and Thio-Barbituric Acid or TBA value (by the method described by Tarladgis et al, 1960).

**RESULTS AND DISCUSSION**

The moisture, protein, lipid and ash contents of fresh *Pangasius hypophthalmus* minced meat were estimated to be 76.25%, 17.42%, 4.37% and 1.24% respectively (Figure 1) which reveals that the fish had low moisture, high protein and moderate fat content. Viji et al (2014) reported that the proximate composition of the fresh...
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Sutcli catfish meat was found to be 77% moisture, 16.5% protein, 4% crude fat and 0.97% ash, which supports the findings of the present study. Rao et al. (2013) reported that the proximate composition of *P. hypophthalmus* fish fillet was 17.24%, 78.2%, 2.84% and 1.3% for protein, moisture, crude fat and ash content respectively. Similar findings were also established by Rathod and Pagarkar (2013), where moisture and protein contents of *Pangasius* were reported to be 76.6% and 14.4% respectively. High protein content (15.97%) in *Pangasianodon hypophthalmus* was also reported by Islami et al. (2014). Proximate composition of fishes is highly variable mainly due to species, catching season, environment, diet, sex and age (Boran and Karaçam, 2011). Mushahida-Al-Noor et al. (2012) affirmed that different diets result in a wide variation in proximate composition of *Pangasius hypophthalmus* i.e. 74.10% to 79.15% moisture content, 15.50% to 16.60% protein, 4.08% to 8.08% lipid content and 1.20% to 1.24% ash content.

The maximum acceptable count for fresh water fish is 10³ CFU/g as recommended by International Commission on Microbiological Specification for Foods. The changes in TPC in all the samples showed a gradual increase from an initial value of 3.56 log CFU/g and finally reaching to 6.92 log CFU/g, 6.67 log CFU/g, 6.46 log CFU/g and 5.89 log CFU/g for control, S1, S2 and S3 respectively after 5 months of frozen storage (Figure 2). The TPC in control sample was significantly (P<0.05) higher than the treatments over a storage period of 5 month suggesting that the *M. oleifera* leaf extract possesses antimicrobial properties that resulted in growth inhibition of bacteria. This can be supported by the findings of Sato et al. (2004) who reported that the leaves of *M. oleifera* contain a number of phytochemicals such as flavonoids, saponins, tannins and other phenolic compounds that have antimicrobial activities against microorganisms. During the study, a sudden decrease in TPC value was encountered in all the samples on the 15th day of storage which may be due to the effect of cold shock of freezing temperature on the microorganisms present in the minced meat. This corroborates with the findings of Ranken (2000), who showed that the TPC value of the chicken meat ball samples reduced during the first 20 days of frozen storage due to the effect of freezer temperature on the microbes in extending their lag phase. After 20 days of storage, a significant increment of the microbes occurred due to the adaptability of the microbes to the freezer temperature (Sinhamahapatra et al., 2013). The results of the present study are in agreement with the findings of Hazra et al. (2012) also reported that there is a significant (p<0.05) reduction in TPC in cooked ground buffalo meat treated with *Moringa* leaf extract at 1%, 1.5% and 2% levels (log 2.95±0.29 CFU/g, 2.72±0.17 CFU/g and 2.65±0.19 CFU/g respectively) as compared to control (log 2.96±0.22 CFU/g) during refrigerated storage. Likewise, Adeyemi et al. (2013) also reported that after eight weeks of storage of smoked dried African catfish (*Clarias gariepinus*), the microbial load in the control was found to be ≥3.0×10⁶ CFU/g, where as in the samples treated with 1%, 2% and 3% *M. oleifera* marinade (MOM), the microbial load was 5.0×10⁷ CFU/g, 6.0×10⁷ CFU/g and 7.0×10⁶ CFU/g respectively stored under room temperature. Falowo et al. (2016) reported that the raw ground beef meat treated with *Moringa* leaf stalk extract showed comparatively lower Total Viable Count (5.28±0.53 log CFU/g) than the control (5.80±1.05 log CFU/g) after 3 days of storage at 4°C. In the present study, S3 exhibited the lowest TPC at the end of the 5 months storage period under frozen storage (-18±2°C) suggesting that 15% concentration of *Moringa oleifera* leaves extract possesses the best antimicrobial properties against fish mince (Figure 2).

TVBN serves as an indicator for the assessment of the freshness of fish. Enzymes from spoilage microorganisms, particularly proteolytic enzyme, can metabolize the amino acids of the fish muscle producing a wide variety of volatile compounds resulting in off flavours and odors. The total chemical compounds of trimethylamine or TMA (produced by spoilage bacteria), dimethylamine or DMA (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites), and other volatile basic nitrogenous compounds associated with seafood spoilage are measured in TVB-N test (Huss, 1995). The TVB-N value between the ranges of 30–35 mg/100 g for flesh is generally regarded as the limit of acceptability for ice stored cold-water fish for human consumption (Castro et al., 2006). In the present study, the initial TVBN content was 10.20 mg % that finally raised to 29.12 mg %, 28.53 mg %, 25.77 mg % and 24.85 mg % for control, S1, S2 and S3 respectively at the end of 150 days of frozen storage (-18±2°C) (Figure 3), thus, remained within the acceptable limits of 30–35 mg/100 g. The TVBN in control sample was significantly (p<0.05) higher than the treatments over a storage period of 5 month suggesting a significant influence of *Moringa* extract in reducing TVBN value which may be due to the presence of different phytochemicals that have effective antimicrobial properties against wide range of bacteria, which are responsible for accumulation of ammonia and volatile bases in fish flesh. The increase in...
TVBN values during the first month could be attributed to bacterial activity and endogenous enzymes of fish (Ibrahim and El-Sherif, 2008). Hossain et al (2005) reported that in Thai Pangasius (Pangasius sutchi) TVBN value was found to be 24.25 mg/100g at 20°C day of ice storage which was within the range of acceptable value. But, at the end of 25th day, the TVBN value increased to 40.1 mg/100 g which exceeded the recommended level. Manthey (1988) established that lower storage temperature may result in lower increase in TVBN value during the storage period. This can be supported by the finding of Anderson (2008), who reported that in frozen hilsa fish stored at –20°C, TVBN value increased from 5.60 to 27.20 mg/100 g at the end of 75 days. Suvanich et al (2000) stated that the increase in TVBN values in of minced fish at the end of frozen storage was may be due to the breakdown of endogenous compounds into non-protein N-compounds (Vareltzis et al, 1997). But, studies on storage of different frozen fish species, it was suggested that TVB-N values might change depending on the spoilage flora and analysis method (Dalin et al, 2013). TVBN value may also depend on the species, catching methods, season and region, age and sex of the fish (Nasopoulou et al, 2012). In the present study, S3 exhibited significantly (p<0.05) lowest values of TVBN at the end of the 5 months storage period under frozen storage (-18±2°C) suggesting that 15% concentration of Moringa oleifera leaves extract is most effective in maintaining the acceptable level of TVBN values in fish mince (Figure 3).

TMA is the most commonly used volatile amine in the fish industry for evaluating freshness and spoilage in fish, since it is produced from bacterial utilization of trimethylamine oxide (TMAO), a naturally occurring osmoregulatory substance found in the fish species. Formation of Trimethylamine (TMA) is caused by reduction of Trimethylamine oxide (TMAO) by bacterial activity and partly by intrinsic enzymes and often used as index of freshness of marine fish (Villareal and Pazo, 1990). The maximum permissible limit of TMA is 5 mg N/100g flesh beyond which seafood will develop an objectionable odour and taste (EOS, 2005). In the present study, the initial TMA content was 0.57 mg N/100g which finally increased to 4.69 mg N/100 g, 4.11 mg N/100 g, 3.82 mg N/100 g and 3.58 mg N/100 g for control, S1, S2 and S3 respectively after 5 months of frozen storage (-18 ±2°C) (Figure 4) retaining all the values within limit. TMA accumulation is a result of bacterial breakdown of TMAO and this occurs to a significant level only during logarithmic phase of microbial growth (Huss, 1988). During frozen storage, the increase in TMA content of all the samples is due to action of spoilage bacteria and endogenous enzyme, which imparts an unpleasant “fishy” odor (Kilinc and Cakli, 2004). Ntseba et al (2005) reported that freezing inhibits bacterial activity and thereby inhibits TMA accumulation. Hozbor et al (2006) found a strong correlation between the microbiological changes in sea salmon stored in ice and other quality indices like TMA-N, TVN-N and Histamine, indicating that, with the decrease in microbial activity, there is simultaneous decrease in TMA value. In the present study, it is clearly noticed that all the treatments showed significantly (p<0.05) lower values of TMA than the control throughout the storage period among which S3 (15% concentration of Moringa oleifera extract) was lowest which may be due to the ability of Moringa extract to reduce microbial load, thereby decreasing protein breakdown and hence lower values of TMA were resulted. This finding can be supported by the report of Siddhuraju and Becker (2003) stating that the plant has broad spectrum antimicrobial activity against both gram-positive and gram-negative bacteria due to the presence of antibiotic compounds in it.

TBA is the secondary breakdown product of lipid oxidation and is widely used as an indicator of the degree of lipid oxidation (Ucak et al, 2011). The level of tissue malonaldehyde, a secondary degradation product of lipid present, is often measured in order to assess the extent of lipid peroxidation that has occurred in biological systems (Khayat and Schwall, 1983). A TBA value in the range of 1–2 mg malonaldehyde/kg of fish sample is usually taken as the limit of acceptability (Lakshmanan, 2000) beyond which (2 mg malonaldehyde/kg) the product will smell and taste. During frozen storage (-18±2°C), the initial TBA value was 0.23 mg MDA/kg which finally increased to 1.30 mg MDA/kg for control, 1.24 mg MDA/kg for S1, 1.13 mg MDA/kg for S2 and 1.09 mg MDA/kg for S3 sample after 5 months (Figure 5), all remained within optimum limit. This may be due to the antioxidant properties of polyphenols present in the Moringa oleifera extract that retarded the formation of malonaldehydes as reported by Sarah et al (2010). O’Byrne et al (2002) showed that polyphenols in Moringa extract act as chain breaking peroxyl radical scavengers which lead to the inhibition of lipid peroxidation and also prevent low density lipoprotein oxidation. Hazra et al (2012) reported that the cooked ground buffalo meat treated with 1%, 1.5% and 2% Moringa oleifera leaves extract showed significantly (p<0.05) lower TBA values as compared to control during refrigerated storage (4±1°C) among which the lowest value observed in 1.5% extract treated sample. The increase in TBA value during the storage may be processed.
attributed to the partial dehydration of fish and to the increased oxidation of unsaturated fatty acids. The increase in TBA values of *Moringa oleifera* treated samples was significantly (p<0.05) lower than the control sample throughout the storage period of 5 months suggesting that *Moringa* leaves extract can extend the shelf life of fish samples by inhibiting lipid oxidation in fish. The present result is in agreement with the findings of Pari *et al* (2007) stating that *Moringa oleifera* leaves extract can potentially be used as preservatives as well as antioxidants in food industry due to its antibacterial and antioxidative activities. TBARS values first increased during frozen storage due to the accumulation of products from lipid oxidation especially Malonaldehyde (MA) and malondialdehyde (MDA) and then a lower increase rate due to interaction of MA and MDA with protein, amino acid, glycogen etc. resulting in lower amount of free MDA (Goulas and Kontominas, 2007). Under frozen temperatures (–5.15°C to –18.15°C), there is a decrease in activity of spoilage microorganisms and enzymes present in fish products, delaying the formation of metabolites from protein degradation (Bazarra *et al*, 2015). However, low temperatures do not prevent lipid oxidation totally due to the action of endogenous

*Results are mean of three determinations (n=3) with s.d. # Values vary significantly (p<0.05) among the treatments.

**C= Control, S1= 5ml M. oleifera extract/100g mince, S2= 10 ml M. oleifera extract/100g mince, S3= 15 ml M. oleifera extract/100g mince.
lipoxygenases even under frozen conditions (Karlsdottir et al., 2014), which explains the deterioration of lipid during frozen storage. The present study reveals that among the treated samples, 15% concentration always showed significantly (p<0.05) better result than the other treated samples during measuring product stability and rancidity by TBA assay.

From the present study, it is observed that, although, all the samples (both control and treatments) showed a trend of deterioration during 5 months frozen storage period at -18±2°C, whereas the rate was much lower in case of M. oleifera leaves extract treated samples than control. Among the treatments, the quality of the minced meat sample treated with 15% concentration of Moringa leaves extract (S3) was found to be the best in terms of TPC, TVBN, TMA-N, TBARS and protein solubility at the end of frozen storage. Hazra et al. (2012) reported that among 1%, 1.5% and 2% levels of aqueous solution of crude extract of drumstick, 1.5% concentration was the most effective for treating cooked ground bufalo meat during refrigerated storage at 4±1°C. According to Adeyemi et al. (2013), 3% (W/V) Moringa oleifera marinade (MOM) was found to be better than 1% and 2% MOM when treated with smoked dried African catfish (Clarias gariepinus) stored at ambient temperature for two months. Hence, from this present study, it can be concluded that 15% concentration of Moringa oleifera leaves extract is considered as most effective to maintain the quality of the minced Pangasius hypophthalmus during five months frozen storage at -18±2°C.

REFERENCES


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