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EFFECT OF ALOE VERA TREATMENT ON QUALITY OF INDIAN SHAD (*Tenualosa ilisha*) DURING CHILLED STORAGE

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ABSTRACT

An attempt was made to study the use of *Aloe vera* gel extract treatment on hilsa fish, *Tenualosa ilisha*, storing with ice in insulated box. A dip treatment of 18%, 20% and 22% *Aloe vera* gel extract for 2 hrs before chilled storage in ice was applied to fish. Subsequently, significant changes in value of T3 (22 %), TMA-N, as 1.50 ± 0.16 to 7.62 ± 0.24 (mg/100g) ,TVB-N, 7.35 ± 0. to 19.78 ± 0.34 (mg/100g), PV 0.66 ± 0.09 to 2.17 ± 0.09 (m.equ/kg), FFA, 1.16 ± 0.01 % to 2.98 ± 0.06 % with microbiological and sensory parameters were observed. Moreover, all treated fish showed shelf life of 15 days without noticeable sign of spoilage as compare to 12 days records for untreated fish. Study also revealed that 22% *Aloe vera* gel extract before putting in ice box may extend the shelf life of fish.

Key Words: *Aloe vera*, Antimicrobial, Antioxidant, Chilled storage, Hilsa fish

INTRODUCTION

Fish is an extremely perishable aquatic food item (Agbon *et al.*, 2002). It begins to degrade and irreversible change that result in spoilage. Fish is highly susceptible to deterioration without any preservative or processing measures (Okonta and Ekelemu, 2005). It has been reported that immediately the fish dies, a number of physiological and microbial deterioration set in thereby degrade the fish (Emokpae, 1979). In tropical environments, spoilage will proceed very rapidly. Since it is irreversible, it cannot be stopped completely after death. However, techniques are available to slow down the decomposition or spoilage of fish so that it reaches the consumer in reasonably acceptable condition. Preservation in ice is one of the most efficient ways of retarding spoilage. The rate of deterioration during ice storage of fish varies with species, depends on the concentrations of substrates, metabolites in the tissue, microbial contamination and conditions of storage after catching (Pacheco-Anguilar *et al.*, 2000) .

Hilsa (Tenualosa ilisha) is one of the most important tropical marine fish commonly known as Indian shad. For hilsa, icing is widely preferred method for short term preservation. In resent time, bio-preservation is a novel food preservation method used for extension of shelf life and enhanced safety of foods by the use of natural or controlled microbiota and/or antimicrobial compounds (Ananou *et al.*, 2007). In post harvest technology, bio-preservation aims at extending shelf life of fruits and vegetables by utilizing plant-based products which have been used in food engineering for a long time. *Aloe vera* gel is one of the promising bio-preservative which has a great potential to become a common use for most fresh fruits and vegetables. *Aloe Vera* contains phyto-components compounds such as vitamins, nutrients and anti-nutrient compounds (Maenthalsong *et al.*, 2007). Currently, the most widely used part from *Aloe Vera (L.)* is the part a gel, whereas its peel not yet utilized optimally. Moreover, it has reported that the skin of *Aloe Vera* has antioxidant activity (Miladi and Damak, 2008). Similarly, Aduhsan (2008) reported that in aloin also can act as antioxidant compounds, for preservation of food.

T. ilisha is a commercially important fish, which is usually marketed in the form of chilled whole condition. Extension of shelf life of *T. ilisha* during chilled storage is important parameters not only for its market value but also for further processing it into other value added products in distant market. Extension of chilled storage life of fish with an effective dose of *Aloe vera* treatment, a natural preservative, during chilled storage will be helpful to the processor as well as the consumer for getting quality products. The objectives of current study were to investigate the effect of *A. vera* gel extract dip treatment on hilsa fish (*Tenualosa ilisha*) for extension of shelf life of ice preserved short duration transportation and long term ice preserved storage.

MATERIALS AND METHODS

Fresh hilsa (*Tenualosa ilisha*) measuring were 22.12 ± 0.81 cm ,101.35 ± 2.47 gram procured from

Veraval fishing harbor and transported to laboratory in ice condition (1:1). Fresh *A. vera* harvested from local agricultural farms of Veraval city *A. vera* were taken to laboratory for subsequent preparation.

PREPROCESSING FRESH OF HILSA (*T. ILISHA*) AND PREPARATION OF *A. VERA* GEL

In the laboratory, the fishes were washed with potable chilled water to remove dirt, particles, and individual fish weight and length was recorded accordingly. After washing fishes were used for chilled storage. The gel extraction process was carried out under hygienic condition in the processing hall. The plant was washed with potable water and gel was extracted from core portion of the plant.

DETAILS OF EXPERIMENT

The first experiment was conducted to standardize the dose of *A. vera* gel extract for treatment. Fishes in triplicate were treated respectively with 10, 15 and 20 %, extract for 2 hours, stored in ice box for 15 days. Based on chemical, microbiological and sensory analysis the 20 % treatment was found to be the best.

Second experiment was conducted with 18, 20 and 22 % gel extract treated with fish to evaluate the most effective concentration.

SENSORY ANALYSIS

Sensory evaluation of the samples was conducted every day by five trained panel members. Total plate count (TPC), TVB-N (Total Volatile Base Nitrogen) were carried out at the interval of 3 days for a period of 15 days after chilled storage (Snedecor and Cochran, 1967).

PROXIMATE COMPOSITION

Moisture, ash, crude protein and total lipid of fish samples were determined according to the prescribed methods (AOAC, 2006). TVB-N and TMA free fatty acids (FFA) in the samples were determined following to the standard method (Beatty and Gibbson, 1937; Takagi *et al.*, 1984). The peroxide value of the lipid was determined from the lipid extract using iodometric method (Jacobs, 1958).

MICROBIAL TEST

TOTAL PLATE COUNT

The microbiological characteristic of fresh fish was assessed according to standard method. The fish samples were tested for total plate count of bacteria on NA (nutrient agar). TPC/g sample = Average count x 2 x dilution factor.

SENSORY CHARACTERISTICS

Organoleptic evaluation of the fresh fish was carried out by highly experienced judges on 9-point hedonic scale (Joseph and Iyer, 2006). Analysis was conducted on randomly selected samples immediately after removal from chilled storage.

RESULTS AND DISCUSSION

PROXIMATE COMPOSITION OF FRESH FISH

The proximate composition of the fresh hilsa fish (*T. ilisha*) was analyzed. The protein content was 17.976 ± 0.16 % which more or less coincides with the recent findings (Mazumder *et al.*, 2008).The lipid content of 11.9 ± 0.96% was noted. Saha and Guha in their study of 34 species estimated highest amount 19.4% of fat in hilsa (Saha and Guha, 1989). Moisture content of 67.88 ± 0.36 % was well accordance to the previous findings (Nabi and Hossain 1989). Ash content was found to be 1.94 ± 0.20% which is corollary to the result of Abimbola *et al.* (2010).

QUALITY CHANGES DURING CHILLED STORAGE OF *A. VERA* TREATED FISH

NITROGENOUS COMPOUNDS

CHANGES IN TMA-N DURING CHILLED STORAGE

TMA content of hilsa increased from initial 1.60 ± 0.10 (mg/100g) to 10.78 ± 0.20 (mg/100g) in control on 12thday of chilled storage period whereas in 18%, 20%, 22% treated samples like T1(18%), T2(20%) ,T3(22%) the level of TMA-N increased from 1.60 ± 0.10 (mg/100g) to 9.32 ± 0.08 (mg/100g), from 1.47 ± 0.11 (mg/100g) to 8.34 ± 0.16 (mg/100g), from 1.50 ± 0.16 (mg/100g) to 7.62 ± 0.24 mg% respectively (Table 1). TMA level in all treated sample remained much below as compared to the level of control for the same period. The results showed the effectiveness of *A. vera* treatment in minimizing the development of TMA due to bacterial activity. The results of the present study are in agreement with the findings of Ishida *et al.* (1976) who reported that at low temperature storage, such as refrigeration above 0^{0}C TMA-N formation slows down noticeably. After 12th day TMA-N content in control increased from 10.78 ± 0.20 (mg/100g) to 16.37 ± 0.29 mg/100g on 15th day of chilled storage which is higher than the limit of acceptability as suggested (Connell, 1975), whereas all treated samples were found to be comparatively less, well within the acceptable limit of TMA-N, after 15th day of chilled storage (Fig. 1) than control. This might be attributed to the inhibitory effect of *A. vera* gel extract on the growth of bacteria. The interaction effect of *A. vera* gel treatments and chilled storage period (days) were found to be significant ($p<0.05$).

CHANGES IN TVB-N DURING CHILLED STORAGE

TVB-N is a product of bacterial spoilage and the content is often used as an index to assess the keeping quality and shelf life of seafood products. After 15 days of storage there was significant difference in TVB-N value of control and treated sample. Initial value of TVB-N for T0 was 7.42 ± 0.09 (mg/100g) which reached to 28.98 ± 0.80 (mg/100g) at the end of 15 days of chilled storage period. Whereas in treated T1(18%), T2(20%) ,T3(22%) the level of TVB-N increased from 7.35 ± 0.11 (mg/100g) to 23.24

± 0.91 (mg/100g), from 7.36 ± 0.13 (mg/100g) to 23.45 ± 0.11 (mg/100g), from 7.35 ± 0.11 (mg/100g), to 19.78 ± 0.34 (mg/100g), respectively (Table 2) at the end of 15 days of chilled storage. The result shows that treated samples were still below the limit of acceptability were fresh and suitable for further storage where as untreated sample approaching the critical limit of TVBN cannot be stored further (Fig. 2). This is the positive effect of *A. vera* gel extract treatment on extension of shelf life. Any of sample did not cross the critical limit of 35-40 (mg/100g) of TVB-N including control. However, in treated it was much within the ice stored fish which indicates the effectiveness of treatment in quality of stored samples. The present study showed the positive effect of minimizing TVB-N formation with the increasing *A. vera* gel concentration of treated fish. The 22 % *A. vera* treatment was found most effective dose. The TVB-N change was significant ($p<0.05$) during chilled storage. Ocano-Higuera *et al.* (2009) reported that TVB-N and TMA-N on Cazon fish stored in ice (0 ^{0}C) increased significantly ($p < 0.05$). Similar result have been reported by Shalini *et al.* (2000) that during refrigerated storage of vacuum packed *L. lentjan* fillets treated with different additive like sodium acetate.

INDICES OF RANCIDITY

CHANGES IN FREE FATTY ACID (FFA) DURING CHILLED STORAGE

Formation of free fatty acids (FFA) during frozen storage of seafood is factor that leads to the deterioration of protein quality. It has been reported that FFA value increased in common sole (*Solea solea*) during ice storage (Yesim *et al.*, 2011). In the present study initial value of FFA for T0 was 1.17 ± 0.01 % oleic acid which progressed to 4.95 ± 0.10 % at the end of 15 days of chilled storage period. Significant changes ($p < 0.05$) were observed in in treated T1(18%), T2(20%) ,T3(22%) the level of FFA increased from 1.17 ± 0.01 % to 3.85 ± 0.06 %, from 1.17 ± 0.01 % to 3.18 ± 0.06 %, from 1.16 ± 0.01 % to 2.98 ± 0.06 % respectively during a total of 15 days of chilled storage. Low value of FFA in treated sample in comparison to untreated fish clearly indicates inhibitory effect of *A. vera* gel extract on the lipolysis. Present study shows significant difference of FFA value between untreated sample and treated sample, that might be attributed to antioxidant effect of *A. vera* gel extract contributing factor. *Aloe vera* is rich in bioactive compounds some of which are antioxidants those are broadly used in food engineering as preservative such as mannans, antrachinon, c-glycoside, antron, antrakuinon and lectine (King *et al.*, 1995; Eshun and He, 2004). Low value of FFA in treated fish may be due to such antioxidant compounds present in *A. vera* gel extract.

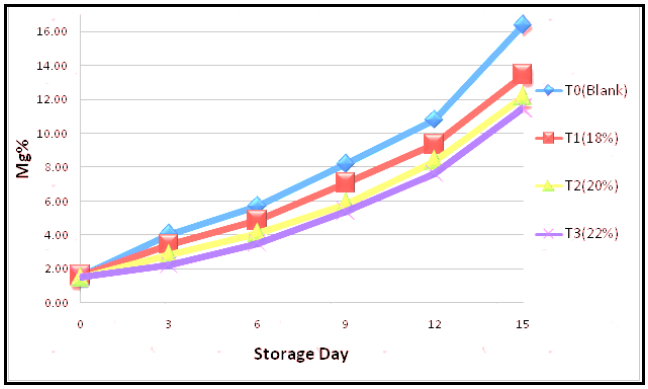

Fig 1: Changes in TMA-N (mg %) during chilled storage study of *T.ilisha*

CHANGES IN PEROXIDE VALUE (PV) DURING CHILLED STORAGE

Peroxide value (PV) is used to express the oxidative state of lipid contents of the foods. PV value is the measure of first stage of oxidative rancidity. In the present study the changes in PV value in all chilled samples showed increased trends with intermittent fluctuation during chilled storage period.

Table 1: Changes in TMA-N (mg %) during chilled storage study of *T.ilisha*

Storage day	T0 (Blank)	T1 (18%)	T2 (20%)	T3 (22%)
0	1.60	1.60	1.47	1.50
3	4.05	3.44	2.80	2.24
6	5.70	4.84	4.07	3.50
9	8.19	7.04	5.84	5.38
12	10.78	9.32	8.34	7.62
15	16.37	13.43	12.14	11.47

Table 2: Changes in TVB-N (mg %) during chilled storage study of *T.ilisha*

Storage day	T0 (Blank)	T1 (18%)	T2 (20%)	T3 (22%)
0	7.42	7.35	7.36	7.35
3	10.23	9.02	8.06	7.46
6	12.85	10.56	9.42	8.81
9	14.77	12.46	11.69	10.96
12	21.73	18.76	16.87	15.97
15	28.98	23.24	20.79	19.78

Initial value of PV for T0 was 0.71 ± 0.12(m.equ/kg), reached to 4.39 ± 0.12 (m.equ/kg) at the end of 15 days of chilled period. In treated sample T1 (18%), T2 (20%), T3 (22%) the level of PV increased from 0.64 ± 0.16 (m.equ/kg) to 3.25 ± 0.12 (m.equ/kg), from 0.66 ± 0.11 (m.equ/kg) to 2.48 ± 0.17 (m.equ/kg), from 0.66 ± 0.09 (m.equ/kg) to 2.17 ± 0.09 (m.equ/kg) respectively during a total of 15 days of chilled storage. Yesim *et al*. (2011) reported that PV value was 15.02 meq kg^{-1} for the common sole stored in ice, significantly ($P<0.05$) increased to maximum value of 35.87 on day 16 then significantly ($P<0.05$) decreased to 23.22 meq kg^{-1} at the end of storage period). However, the value of PV was low in treated as compared to untreated sample which shows the antioxidant effect of *A. vera* gel extract.

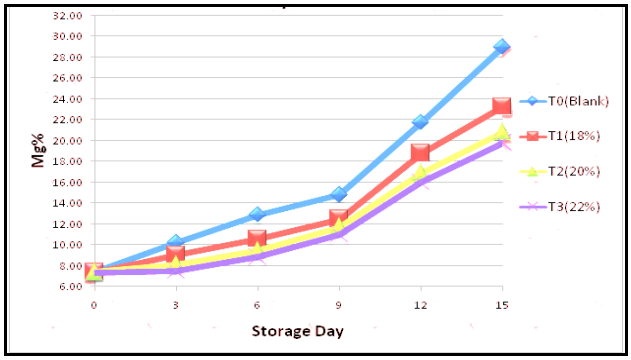

Fig 2: Changes in TVB-N (mg %) during chilled storage study of *T.ilisha*

MICROBIOLOGICAL ANALYSIS

Microbial growth in fresh seafood is the main factor associated with quality deterioration, spoilage and economic loss. Total bacteria on untreated hilsa significantly ($p<0.05$) increased during storage, it reached to 6.753 log cfu g^{-1} at the end of storage. It has reported that microflora on Mediteranian mackerel and blue jack mackerel which were stored in ice at 10 days was < 6 log10 cfu g^{-1} by Tzikas *et al.* (2007).TPC Initial value for T0 was 2.37 ± 0.02 log (cfu/g) which reached to 5.60 ± 0.02 log (cfu/g) on 12th day of chilled storage period which exceeded the limit of acceptability of TPC in fish, whereas samples treated with 18, 20 and 22% *Aloe vera* gel extract, the level of TPC increased from 2.33 ± 0.02 log (cfu/g) to 4.95 ± 0.02 log (cfu/g), from 2.31 ± 0.02 log (cfu/g) to 4.55 ± 0.04 log (cfu/g), from 2.32 ± 0.03 log (cfu/g) to 4.41 ± 0.03 log (cfu/g) respectively (Table 3) after 12th day which is within the limit of acceptability. The samples which were treated with *A. vera* gel extract showed the lowest rate of bacterial growth compare with untreated sample (Fig. 3). The result indicated that microbiological growth was restricted by treatments of *A. vera* gel. Low TPC count in treated sample may be attributed to antimicrobial effect of *A. vera* gel extract.

The microbiological changes of the fish stored at 4 ^{0}C were in good agreement with the observed results of the sensory evaluation. Similar results were found by Winami *et al.* (2012) for gutted sardines stored in boxes at 4^{0}C. However, the interaction effect of *A. vera* gel treatments and chilled storage period (days) were found to be significant ($p<0.05$).

Table 3: Changes in TPC during chilled storage study of *T.ilisha*

Storage day	T0 (Blank)	T1 (18%)	T2 (20%)	T3 (22%)
0	2.37	2.33	2.31	2.32
3	3.81	2.66	2.60	2.56
6	4.40	3.54	3.14	2.98
9	4.91	3.95	3.69	3.61
12	5.60	4.95	4.55	4.41
15	6.66	5.83	4.96	4.87

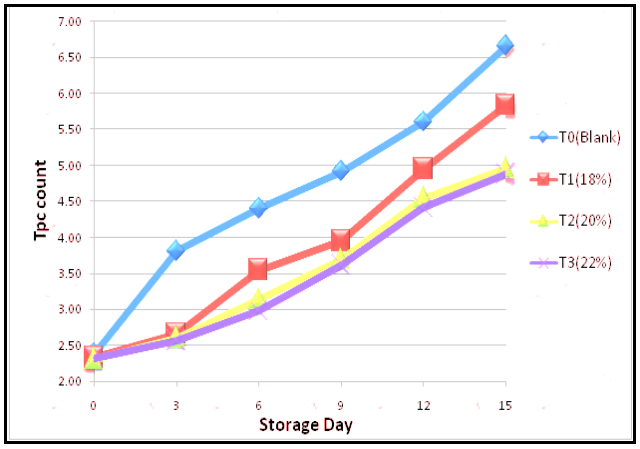

Fig 3: Changes in TPC during chilled storage study of *T. ilisha*

SENSORY CHARACTERISTICS

Sensory evaluation is the most reliable test for raw material and processed fishery products. Sensory analysis of all treatments assessed during 15 days of storage are shown in **(**Table 4**)**. Fresh hilsa exhibited shiny appearance, bright eyes, reddish gill, sea weedy odor and very firm texture. Changes in sensorial properties varied among treatments.

Fastest decreasing rate of sensory attribute was observed in the untreated samples followed by treated samples with 18 and 20 % *A. vera* gel extract. Whilst the least decrease in sensory attribute was observed in the samples treated by 22% *Aloe vera*, its sensory attributes remained at the highest score (Fig. 4). The results of sensory analysis show the preservative effect of *A. vera* sensory analysis also reflect the chemical and microbiological quality of fish described above.

Table 4: Changes in overall acceptability during chilled storage of *T.ilisha*

Storage day	T0 (Blank)	T1 (18%)	T2 (20%)	T3 (22%)
0	9.85	9.87	9.87	9.83
1	8.93	9.33	9.46	9.51
2	8.12	8.85	9.11	9.24
3	7.72	8.27	8.90	8.98
4	7.09	7.85	8.16	8.31
5	6.52	7.32	7.72	7.94
6	6.04	6.75	7.43	7.65
7	5.58	6.51	7.02	7.23
8	5.04	5.99	6.60	6.99
9	4.59	5.79	6.30	6.64
10	4.22	5.39	6.00	6.17
11	3.88	4.53	5.55	5.85
12	3.45	3.92	4.59	5.21
13	2.85	3.88	4.46	4.95
14	2.22	3.47	4.25	4.41
15	2.08	2.97	3.85	3.99

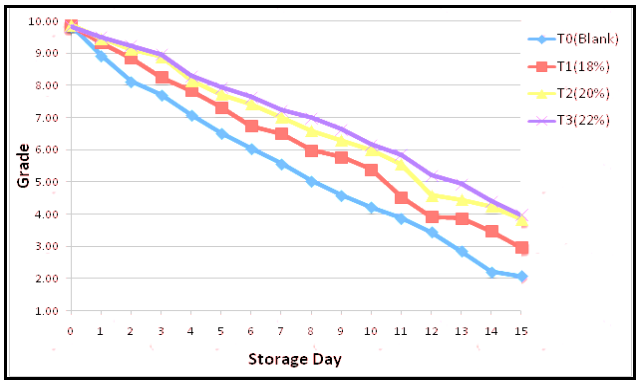

Fig 4: Changes in overall acceptability during chilled storage of *T.ilisha*

Similar result has been found by Winarni *et al.* (2012) where Indian mackerel treated with 20% *Aloe vera* was considered as the most effective treatment. At the end of 15 days storage time, all treated fish except untreated were acceptable by the panelist. Control treatment fish were rejected by the panelists at 11th day of storage. At such concentration fishes were not rejected by the panelist. This effect was possibly due to the complex substances of *Aloe vera* such as aloin which has antibacterial, antifungal and anti-inflammatory activities (Lorenzetti *et al.*, 1964; Das *et al.*, 2011).

These results indicated that treatment with *Aloe vera* gel extract stabilized the sensorial, microbial and chemical properties of hilsa during chilled storage. Moreover, it further extends the chilled storage life for another additional 4 days.

CONCLUSIONS

Changes in the chemical, microbiological and sensorial attributes of treated hilsa varied among concentrations. Higher the concentration less the level of spoilage. 22% *Aloe vera* gel extract treatments were found to be the best among treatments to reduce changes in chemical, microbial and sensorial attributes as well as shelf life than the control during chilled storage. The use of *A. vera* gel extract was found to be effective in minimizing deteriorative changes, both chemically and sensory attribute stored for 15 days at 0^0C. In general, use of *A. vera* gel extract treatments in hilsa holds promise to improve overall quality in terms of chemical, microbiological and sensory characteristics as well as shelf life of *T. ilisha* during chilled storage of 15 days. *A. vera* has also got good keeping quality as a natural preservative for short duration transportation. The findings of the present study, *A. vera* as a whole ignite a keen interest among the researchers, and other entrepreneurs to explore its chemical and biochemical properties. I addition study provides an insight to investigate such natural plant based products with their exhaustive properties as preservatives.

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