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Efficacy of ethanolic potato peel extract on the ice storage characteristics of minced lesser sardine (*Sardinella fimbriata*) to prevent lipid and protein oxidation

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Abstract

The present study was carried out to evaluate the effect of ethanolic potato peel extracts (*Solanum tuberosum*) on the ice storage characteristics of mince of a species of lesser sardine (*Sardinella fimbriata*). The concentrations of extract were of 2.5 g/kg of mince and 5 g/kg of mince. The proximate composition of fish mince and also different parameters were analysed like biochemical (TMA-N, TVB-N, PV, FFA, Alpha amino nitrogen and pH), functional (Protein solubility and Water holding capacity) and sensory (Whiteness and other organoleptic characteristics). The biochemical characteristics were increased significantly ($p < 0.05$) except pH ($p > 0.05$) during ice storage. The functional parameters were decreased significantly throughout the storage period ($p < 0.05$). The sensory characteristics of the fish mince were not significantly different ($p > 0.05$). When both the species were compared, TVB-N and AAN showed significant changes between them ($p < 0.05$) but PV did not showed any significant difference ($p > 0.05$). The mince treated with 2.5g/kg of extract was analysed for 8 days in and proved to be the best treatment and the shelf life increased for 3 days as compared to control.

Keywords: Ethanolic potato peel extract, ice storage, minced lesser sardine, lipid oxidation, protein oxidation

1. Introduction

Fish is found to be most perishable as well as difficult to handle among all foods. Shelf life can be defined as the time period, the product takes to reach a point where it becomes unacceptable to consume. Icing is the one of the easiest, cheapest and commonly followed method of preserving fresh fish mince. Lean fishes have longer shelf life than fatty fishes (Gopakumar, 2002) [8]. Potatoes are commonly and highly consumed vegetable throughout and peels are the major wastes of potato processing industries that represent a major disposal, sanitation & environmental problem (Farvin *et al.* 2011) [6]. To increase awareness, sustainability and also upgrading this waste material, it has been used in fish mince, oils to enhance their shelf life. It also acts as a natural antioxidant that prevents protein & lipid oxidation basically rancidity, off odour and off flavor of fish mince. The potato peel extract is helpful in especially fatty fishes like sardines and mackerel which contains higher levels of n-3 PUFA such as EPA and DHA (Farvin *et al.*, 2011) [6]. The study basically aims at:

- Proximate composition of mince of *Sardinella fimbriata* and preparing the mince of it.
- Treatment of mince with different concentrated ethanolic potato peel extract in ice storage.
- To study biochemical, functional and sensory properties of treated mince.

2. Materials and Methods**2.1 Material**

The raw material like the fishes were brought from the landing center and taken to research laboratory in iced condition. The Sava variety of potato (*Solanum tuberosum*), were purchased from local market. Then, potato tubers were initially washed with tap water and then peeled manually using a vegetable peeler.

2.2 Methods

2.2.1 Preparation of potato peel extract

The method of preparation modified from Farvin *et al.*, 2011^[6]. Potatoes were purchased and the tubers were washed and peeled manually. Then the peels were dried at 45 °C for 72 hrs. and powdered by a blender. Then it is sieved through 80 mesh sieve. For preparing ethanolic extract, 5g powdered peel mixed with 50 ml of 96% ethanol and kept overnight at room temperature. Then it was centrifuged at 2800 rpm for 10 min and the supernatant was collected. The filtrate was kept in water bath at 80 °C for 1 hour to evaporate ethanol. Then the liquid part was spread in a petridish and dried in hot air oven at 60 °C. After 2 days, the solid part remaining at the bottom of petridish was scrubbed out as natural extract and kept in airtight bottles.

2.2.2 Standardization of Ethanolic Potato peel extract (PPE) treatment

When the fishes were brought to laboratory and then they were gutted, beheaded and washed properly. The washed fishes were put in a fish deboning machine to separate mince from skin, scales, bones etc. Two concentrations of PPE were made for fish mince i.e. 2.5g/kg & 5g/kg of ethanol extracts of potato peel. The control treatment of sample mince was used without addition of any extract. To ensure even distribution of extracts throughout mince, extracts were dissolved in 10 ml distilled water and then added to mince and properly homogenized (Farvin *et al.* 2011)^[6].

3 samples were prepared as control, 2.5PPE and 5PPE respectively. After addition of extracts, 100g of mince was taken in each plastic bag and packed properly. Then all such bags were stored separately in ice in insulated boxes and from that one bag was taken out every day for analysis till the mince remain in good condition. Mince packs: ice ratio was maintained as 1:1 throughout the ice storage period. At first, control mince without any extract was analyzed, and then the two ethanol extracts treated minced meat were analyzed. The treated minced meat of fishes with the above specified concentrations was analyzed for biochemical, functional and sensory analyses every day.

2.2.3 Biochemical parameters

2.2.3.1 Proximate composition

The proximate composition of minced meat of *S. fimbriata* was analysed. Moisture content, protein content, crude fat and crude ash content were analysed according to AOAC, 2005^[1].

2.2.3.2 pH measurement

5 g of sample was taken and grounded with 45 ml distilled water and then filtered using filter paper. The pH of the filtrate was measured using pH meter (Model EQ 610) according to AOAC, 2005^[1].

2.2.3.3 Determination of TMA-N and TVB-N

The TMA-N content and TVB-N content of fish samples during ice storage were analysed according to Beatty and Gibbons (1937)^[2]. The freshly prepared TCA extract was used in the analysis.

2.2.3.4 Determination of Alpha Amino Nitrogen (AAN)

The AAN content of fish meat was determined by the method given by Pope and Stevens (1939)^[16].

2.2.3.5 Determination of PV and FFA

The PV and FFA of minced meat during chilled storage was analysed according to AOAC (2005)^[1].

2.2.4 Functional parameters

2.2.4.1 Water Holding Capacity (WHC)

The WHC of chilled stored minced meat was determined according to Sultanbawa and Lichan (1998)^[23]. It is expressed in percentage (%).

2.2.4.2 Protein solubility

The protein solubility was analysed according to Choi and Park (2002)^[3].

2.2.5 Sensory parameters

Sensory evaluation of fish mince was done based on the major sensory characters i.e., colour, appearance, odour, texture & overall acceptability. A panel of five experts had conducted analysis of ice stored fish mince. The samples were evaluated on 0-9 point hedonic scale scoring system.

2.2.5.1 Determination of whiteness

The colour of minced meat was measured by colorimeter (Hunter Lab Scan XE USA). The L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) were determined and whiteness was calculated as described by Park *et al.* [1994] as follows:

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

2.2.6 Statistical analysis

The data were analysed to test and check the level of significant difference by applying analysis of variance, (ANOVA) tool available in MS-Excel 2013 and by Student's Newmann Kuel's multiple comparison test. Analysis was mainly performed using a SPSS package (SPSS version 16.0 for Windows SPSS Inc, Chicago, IL, USA). The significant differences were tested by 5% level of significance and mentioned as $p < 0.05$ (Snedecor and Cochran, 1967; Zar, 2014)^[22, 26].

3. Results and Discussion

3.1 Proximate composition of mince of lesser sardine (*S. fimbriata*)

The proximate composition i.e. moisture, protein, fat and ash content of minced meat of *S. fimbriata* in the present study were 73.68±3.94%, 16.78±0.60%, 3.09±0.33%, 1.73±0.50% respectively. Serdaroglu and Felekoglu (2003)^[19] reported that the average proximate composition of the sardine (*Sardina pilchardus*) mince for fat, protein, moisture and ash contents was 5.2, 16.2, 77.2 and 1.2 % respectively. Gokodlu *et al.* (1998) reported that the protein, fat, moisture and ash contents of *Sardina pilchardus* were 20.07±0.02, 14.1±0.09, 69.9±0.7, 1.9±0.009% respectively. There is a slight difference in the proximate composition of *S. fimbriata* in the present study when compared with the studies of above researchers probably due to the seasonal changes of nutrition, living area, fish size, catching season, sexual variation and environmental conditions (Schormuller, 1968; Ludorff and Meyer, 1973)^[18, 12]. The variation also depends upon the species used for the study.

3.2 Biochemical characteristics of Ethanolic potato peel (EPP) extract treated and untreated mince of lesser sardine (*S. fimbriata*)

3.2.1 Trimethylamine nitrogen (TMA-N)

The changes in TMA-N (mg %) content of mince samples of *Sardinella fimbriata* during ice storage at different treatments are shown in table 1. The initial TMA-N value for T1 (PPE of 2.5g/kg of mince) treated sardine species was found to be 3.08±0.28 mg%, 4.66±0.58 mg% on 2nd day, which was steadily increased up to 8th day and proved to be better treatment than control and T2 which lasts for only 5 and 6 days respectively. The TMA-N values for T1 on 7th and 8th day were 14.40±0.75 and 18.20±1.68 mg% respectively. There was significant difference between treatment groups as well as within treated groups in all days ($p<0.05$).

The TMA content is very low in fresh seafood increasing with spoilage. Shinde *et al.* (2012) [21] found there was an increase in TMA-N values of both treated (PPE and GTE) and untreated mackerel minced samples which found to be quite similar with the present study. They stated that production of nonvolatile amines were not significant even though the fish exceeds the limit of acceptability. TMA-N and TVB-N are the most common indices that are used for spoilage of fish and shellfish. The recommended values of TMA-N and TVB-N are 10-15 mg% and 35-40 mg% respectively and considered as the limit of acceptability.

3.2.2 Total Volatile Base – Nitrogen (TVB-N)

The changes in TVB-N (mg %) content of mince samples of *Sardinella fimbriata* during ice storage at different treatments are shown in table 2. The initial values of TVB-N for control were 7.74±0.70 mg % (1st day), 11.29±0.42 mg % (2nd day) and it gave good results upto 5th day (35.60±4.44 mg %). The treatment T1 showed better results than control and T2 and lasts upto 8th day. The initial values for T1 were 5.97±0.42 mg % (1st day), 13.16±1.74 mg % (2nd day) and the value on 8th day was 37.42±3.93 mg %. The initial values for T2 were 6.90±0.58 mg % on 1st day, 9.14±0.42 mg % on 2nd day and the final values on 6th day was 35.84±3.76 mg %. In overall, one way ANNOVA and SNK test showed that there was no significant difference between treatments ($p>0.05$). Gokodlu *et al.* (1998) experimented on physical, chemical and sensory analyses of freshly harvested sardines (*Sardina pilchardus*) stored at 4 °C. The TVB-N values increased significantly ($p<0.05$) throughout the period. It varied from 13.18 to 64.80 mg%. El Marrakchi *et al.* (1990) [5] stated that TVB value was more beneficial in assessing the degree of sardine deterioration. TVB-N value is an important parameter for determining freshness of fish products (Lang, 1979) [10]. They also stated that TVB-N value is affected by species, catching region, season, sex and age of fish.

3.2.2.1 Comparison of changes in TVBN values between two species (*O. ruber* and *S. fimbriata*)

The changes in TVBN values between two species *Otolithes ruber* and *Sardinella fimbriata* are shown in Table 3 and Fig. 1 respectively. In figure, S1 refers to first species i.e. *Otolithes ruber* and S2 refers to second species i.e. *Sardinella fimbriata*. One way ANNOVA and SNK test showed that there was a significant difference between species in different treated groups ($p<0.05$) which might be due to effects of extracts as antimicrobial additives more in *O. ruber* with delayed onset of spoilage that reduces degradation of nitrogen compounds in fish mince. It also might depends upon the

nature of muscle of fish as *O. ruber* is a lean fish.

3.2.3 Peroxide Value (PV)

The changes in PV values of mince samples of *Sardinella fimbriata* during ice storage at different treatments are shown in table 4. The initial values for T0 were 1.39±0.67 meq of O₂/kg of fat on day1, 5.99±1.33 meq of O₂/kg of fat on day 2 and 11.10±2.10 meq of O₂/kg of fat on day 3. T0 was analysed upto 5 days and the value was 21.10±2.34 meq of O₂/kg of fat on day 5. The result for T1 on day 1 was 0.47±0.15 meq of O₂/kg of fat, 5.10±2.69 meq of O₂/kg of fat on day 2. It proved to be better treatment than T0 and T2 that survived upto 8 days and the value was 21.55±1.67 meq of O₂/kg of fat on day 8. The test showed that there was no significance difference between treatments ($p>0.05$). Serdaroglu and Felekoglu (2003) [19] studied on the effects of using rosemary extract and onion juice on oxidative stability of sardine (*Sardina pilchardus*) mince. At 0th and 15th day of storage, there were no significant differences in PV of the treatment groups. Peroxide values of RE, OJ and C treatments were 10.94, 11.96 and 14.24 meq O₂/kg, respectively. They stated that higher PV levels during the initial storage stages might be due to the high degree of unsaturation of fatty acids and the mincing process. It has been already been reported that mincing fish muscle forms a larger surface area and so lipids are easily oxidized (Pastoriza *et al.*, 1994) [15]. The mincing process disturbs the muscle membrane system, thereby exposing the lipid components to oxygen, or causes other reactions (Love and Pearson, 1976) [11]. The PV for all samples increased during frozen storage ($p<0.05$). RE or OJ extracts showed the similar effect on peroxide value at each step which directly relates to the present ice storage study. When both the species were compared, there was no significant difference between treated groups of two species ($p>0.05$). The changes in PV levels did not show a significant effect on the quality of fish mince. *Sardinella* being a fatty fish, had significant changes in the quality of muscle due to PV levels.

3.2.4 Free fatty acid (FFA)

The changes in FFA values of mince samples of *Sardinella fimbriata* during ice storage at different treatments are shown in table 6. The initial values of T0 were 0.46±0.06 % of oleic acid on day 1 and 1.04±0.44 % of oleic acid on day 2. It was finally analysed on day 5 which had value of 2.40±0.42 % of oleic acid. T1 proved to be the better treatment as compared to T0 and T2 as it gave good results upto 8th day (0.31±0.18 % of oleic acid). The initial values for T1 on day 1 and 2 were 0.16±0.10 % of oleic acid and 0.63±0.13 % of oleic acid respectively. The test showed there was no significant difference between treatments but there was significant difference within treatments ($p<0.05$). Sharifian *et al.* (2011) [20] measured the quality changes in tiger tooth croaker (*Otolithes ruber*) during ice storage and stated that oxidation products of FFA have always been a major impact on muscle texture and functionality as they highly interact with myofibrillar proteins and promote aggregation of proteins.

3.2.5 Alpha Amino Nitrogen (AAN)

The changes in AAN values of mince samples of *Sardinella fimbriata* during ice storage at different treatments are shown in table 7. The initial values for T0 was 0.21±0.03 mg % on day 1, 0.46±0.07 mg % on day 2 and the final 5th day got the value of 1.18±0.09 mg %. T1 proved to be the better

treatment as compared to T0 and T2 and the samples were analysed for 8 days. The initial values on day 1 and 2 for T1 and T2 were 0.12 ± 0.01 mg %, 0.17 ± 0.01 mg % and 0.16 ± 0.01 mg %, 0.23 ± 0.01 mg % respectively. The final values for T1 on day 8 was 1.20 ± 0.04 mg % whereas the final value on day 6 for T2 was 1.12 ± 0.09 mg %. The one way ANNOVA and SNK test showed that on day 1, T0 was significantly different from T1 but T2 was not significant with either of them. The test showed that there was significant difference between treatments ($p<0.05$). The treatment showed a significant effect on levels of AAN values in both treated and untreated samples might be due to the effects of phenolic compounds of extracts on the mince.

3.2.5.1 Comparison of changes in AAN values between two species (*O. ruber* and *S. fimbriata*)

The changes in AAN values between two species *Otolithes ruber* and *Sardinella fimbriata* are shown in Table 8 and Fig. 3. When both the species were compared, there was significant difference between species in all treatments ($p<0.05$). The values found to be very less than acceptable limit which might be due to preventing proteolysis and small amount of free amino acids and NPN compounds present in mince.

3.2.6 Changes in pH

The changes in pH values of mince samples of *S. fimbriata* during ice storage at different treatments are shown in table 9 and fig 4 respectively. The initial values for T0 on day 1, 2 and 3 were 6.48, 6.41 and 6.45 respectively. The sample was analysed for 5 days and the value on day 5 was 6.52. For T1, the better treatment as compared to T0 and T2, the initial values on day 1, 2 and 3 were 6.75, 6.68 and 6.71 respectively. The final on day 8 was 6.80. The initial values for T2 on day 1, 2 and 3 were 6.59, 6.55 and 6.58 respectively. The final value on day 6 was 6.64. The two-way annova and SNK test showed that there was no significant difference between treatments ($p>0.05$). Gokodlu *et al.* (1998) analysed physical, chemical and sensory parameters of freshly harvested sardines (*Sardina pilchardus*) stored at 4 °C. The pH values increased with storage period. The range varied from 6.2 to about 7.5 at day 10. Nunes *et al.* (1992) [13] found the pH value of sardine (*Sardina pilchardus*) to gradually increase during storage. Varlyk (1994) [24] found that the pH value of sardine (*Sardina pilchardus*) stored in cold (4°C) increased from 6.35 to 6.71 at day 7 which is in agreement with the present study.

3.3 Functional parameters of EPP extract treated and untreated mince of lesser sardine (*S. fimbriata*)

3.3.1 Protein Solubility

The changes in protein solubility of mince samples of *S. fimbriata* during ice storage at different treatments are shown in table 10. The initial values for T0 were 63.47 ± 1.26 % on day 1, 60.63 ± 0.88 % on day 2 and 56.73 ± 1.31 % on day 3 respectively. T2 was analysed for 6 days and the values were 67.64 ± 1.60 % on day 1, 63.27 ± 0.74 % on day 2, 58.55 ± 1.17 % on day 3 and 37.74 ± 2.41 % on day 6. T1 proved to be better treatment as compared to T0 and T2. There was significant difference between treatments in all days ($p<0.05$). Sarma *et al.* (2000) [17] studied the effect of frozen storage on lipids and functional properties of proteins of dressed Indian oil sardine (*Sardinella longiceps*). They found that protein solubility & emulsifying capacity significantly decreased ($p\leq 0.05$)

throughout storage period. They stated that these changes would might be due to protein aggregation and protein denaturation induced by frozen storage (Grabowska & Sikorski, 1974).

3.3.2 Water Holding Capacity (WHC)

The changes in WHC of mince samples of *S. fimbriata* during ice storage at different treatments are shown in table 11. The initial values for T0 were 62.81 ± 1.70 % on day 1, 57.89 ± 2.96 % on day 2 and the final value on day 5 was 34.28 ± 2.61 %. The treatment T1 was analysed for 8 days and proved to be better treatment as compared to T0 and T2. The initial values were 71.55 ± 4.02 % on day 1, 63.36 ± 1.72 % on day 2 and the final value on day 8 was 31.53 ± 4.24 %. T2 was analysed for 6 days, the initial values were 67.21 ± 2.31 % on day 1, 60.65 ± 1.25 % on day 2 and the value on day 6 was 31.21 ± 2.03 %. The test concluded that there was significant difference between treatments in between days ($p<0.05$). Dhanapal *et al.* (2013) determined quality changes of rohu (*Labeo rohita*) during ice storage. They found that the WHC values significantly decreased at the end of the storage period ($p<0.01$). They stated that the decrease in WHC values leads to loss in texture of fish even though WHC is a good indicator in determining fish quality that relates with the present study directly.

3.4 Sensory characteristics of EPP extract treated and untreated mince of lesser sardine (*S. fimbriata*)

3.4.1 Changes in whiteness

The changes in whiteness values of mince samples of *S. fimbriata* during ice storage at different treatments are shown in table 12. In *S. fimbriata*, both highest whiteness (55.45) and lowest whiteness (30.40) values were observed in T1 samples. The initial values on day 1, 2 and 3 were 52.38, 50.17 and 43.99 for T0, 55.45, 54.24 and 52.76 for T1, 54.50, 50.74, 48.16 for T2. Highest lightness values and lowest lightness values were found to be T1 samples. The two-way annova and SNK test showed that there was no significant difference between treatments in all days ($p>0.05$). Yerlikaya and Gokoglu (2010) [25] observed the effects of green tea (*Camelia sinensis*), grape seed (*Vitis vinifera*) and pomegranate peel (*Punica granatum*) extracts on the sensory and physical properties of frozen bonito (*Sarda sarda*) fillets. They found that L^* , a^* and b^* values were 97.02, 0.08 and 1.75 respectively. There was no difference in the L values in the 1st 2 months. Fillets treated with PPE showed significant decrease in L values for all months ($p<0.05$) while other samples were statistically insignificant. The changes in muscle structure and rigor strengthening occurred with the freezing process. The changes in a values were not significant during the storage period. Redness score found in fillets treated with GSE. PPE and control samples had lower a values. Yellow colour is often associated with oxidation (Ozogul *et al.*, 2006). Oxidation plays the major role in colour deterioration. Plant extracts affect a^* and b^* values and decrease L^* values of control except GTE samples.

3.4.2 Changes in organoleptic characteristics of mince of lesser sardine (*S. fimbriata*)

The five organoleptic parameters were colour, odour, texture, appearance and overall acceptability (Table 13 and Fig 5, 6, 7, 8 and 9). They generally showed a decreasing trend during the storage period. The mince samples became organoleptically unacceptable after 8 days and there was no significant

difference between treatments ($p>0.05$). Gokodlu *et al.* (1998) studied the physical, chemical and sensory analyses of freshly harvested sardines (*Sardina pilchardus*) stored at 4°C. There was shelf life increment of 6 days. The sardines were very

good upto 4th day and of good quality upto 6th days and spoiled nature after 6th days to 10th day. There were significant changes in sensory analyses like taste, texture, odour, appearance ($p<0.05$).

Table 1: Changes in TMA-N content of EPP extract treated and untreated mince of *S. fimbriata* during ice storage between groups

Storage days	Treatments	TMA-N (mg%) of <i>S. fimbriata</i> mince		
		T0	T1	T2
1		4.29±0.42 ²	3.08±0.28 ¹	4.1±0.58 ²
2		7.71±0.88 ³	4.66±0.58 ¹	6.44±0.28 ²
3		11.57±0.42 ³	6.72±0.28 ¹	9.8±0.56 ²
4		15.68±0.28 ³	8.02±0.58 ¹	12.22±0.42 ²
5		17.92±1.22 ³	9.52±0.28 ¹	12.50±0.15 ²
6		NA	12.22±0.42 ¹	16.52±0.56 ²
7		NA	14.40±0.75 ²	NA
8		NA	16.50±1.68 ²	NA
9		NA	NA	NA
10		NA	NA	NA

Values are mean±SD. n=3. Values in the same row with different superscripts are significantly different ($p<0.05$).

Table 2: Changes in TVB-N content of EPP extract treated and untreated mince of *S. fimbriata* during ice storage between groups

Storage days	Treatments	TVB-N (mg %) of <i>S. fimbriata</i> mince		
		T0	T1	T2
1		7.75±0.70 ²	5.97±0.42 ¹	6.90±0.58 ^{1,2}
2		11.29±0.42 ²	13.16±1.74 ²	9.14±0.42 ¹
3		13.72±0.56 ¹	16.33±1.71 ²	12.60±0.56 ¹
4		23.14±2.99 ¹	18.38±0.98 ¹	18.01±2.54 ¹
5		35.60±4.44 ²	21.84±1.00 ¹	27.90±4.09 ¹
6		NA	24.36±1.22 ¹	35.84±3.76 ²
7		NA	28.18±2.80 ²	NA
8		NA	37.42±3.93 ²	NA
9		NA	NA	NA
10		NA	NA	NA

Values are mean±SD. n=3. Values in the same row with different superscripts are significantly different ($p<0.05$).

Table 3: Comparison of changes in TVBN values between two species (*O. ruber* and *S. fimbriata*)

Storage days	Changes in TVBN (mg %) values					
	T0		T1		T2	
	<i>O. ruber</i>	<i>S. fimbriata</i>	<i>O. ruber</i>	<i>S. fimbriata</i>	<i>O. ruber</i>	<i>S. fimbriata</i>
1	6.16±0.28 ¹	7.75±0.70 ²	6.16±0.28 ¹	5.97±0.42 ¹	5.69±0.43 ¹	6.90±0.58 ^{1,2}
2	8.40±0.28 ¹	11.29±0.42 ²	7.75±0.58 ¹	13.16±1.74 ³	7.37±0.43 ¹	9.14±0.42 ¹
3	14.84±0.28 ^{3,4}	13.72±0.56 ^{2,3}	14.56±0.74 ^{3,4}	16.33±1.71 ^{2,3}	9.24±0.28 ¹	12.60±0.56 ²
4	23.71±3.98 ²	23.14±2.99 ²	23.15±3.50 ²	18.38±0.98 ^{1,2}	14.75±1.32 ¹	18.01±2.54 ^{1,2}
5	33.64±3.26 ²	35.60±4.44 ²	26.23±1.97 ¹	21.84±1.00 ¹	21.00±1.56 ¹	27.90±4.09 ¹
6	42.19±0.43 ¹	NA	31.36±0.56 ¹	24.36±1.22 ²	26.04±1.45 ²	35.84±3.76 ¹
7	NA	NA	36.95±2.85 ⁴	28.18±2.80 ²	32.76±2.24 ³	NA
8	NA	NA	42.65±0.56 ³	37.42±3.93 ²	35.00±1.28 ²	NA
9	NA	NA	NA	NA	39.29±1.71 ²	NA
10	NA	NA	NA	NA	44.05±0.98 ²	NA

Values are mean±SD. n=3. Values in the same row with different superscripts are significantly different ($p<0.05$).

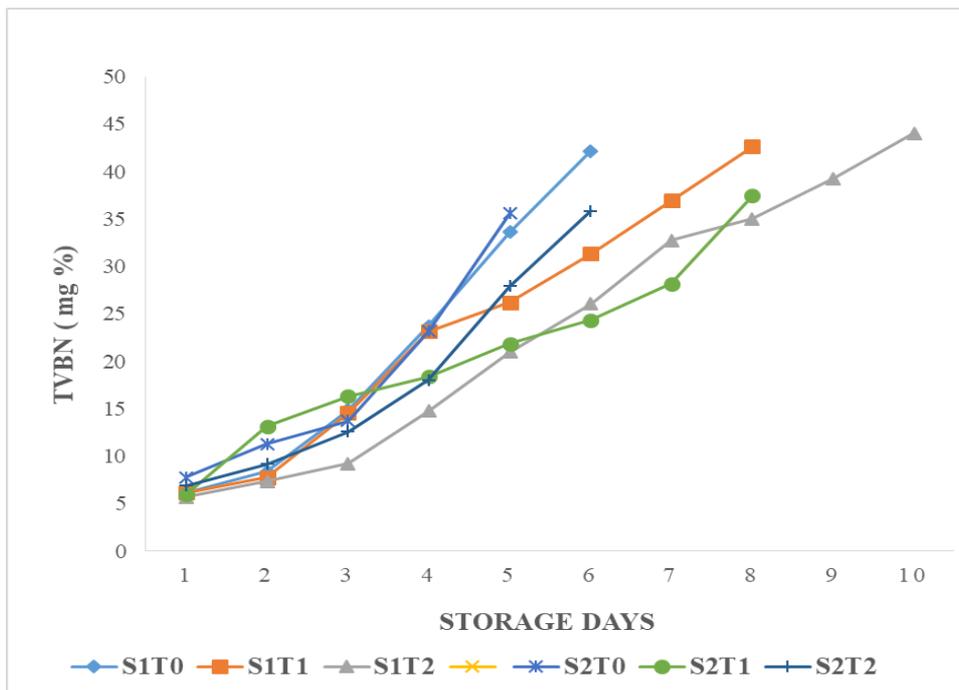


Fig 1: Changes in TVBN values between two species (*O. ruber* and *S. fimbriata*)

Table 4: Changes in PV content of EPP extract treated and untreated mince of *S. fimbriata* during ice storage between groups

Storage days	PV (meq of O ₂ /kg of fat) of <i>S. fimbriata</i> mince		
	T0	T1	T2
1	1.39±0.67 ¹	0.47±0.15 ¹	0.79±0.47 ¹
2	5.99±1.33 ¹	5.10±2.69 ¹	4.22±2.77 ¹
3	11.10±2.14 ¹	10.22±1.01 ¹	12.00±2.00 ¹
4	16.67±1.31 ²	12.88±1.01 ¹	15.99±0.66 ²
5	21.10±2.34 ²	15.55±1.01 ¹	17.99±0.66 ¹
6	NA	18.66±0.66 ²	21.44±0.69 ³
7	NA	21.77±1.01 ²	NA
8	NA	21.55±1.67 ²	NA
9	NA	NA	NA
10	NA	NA	NA

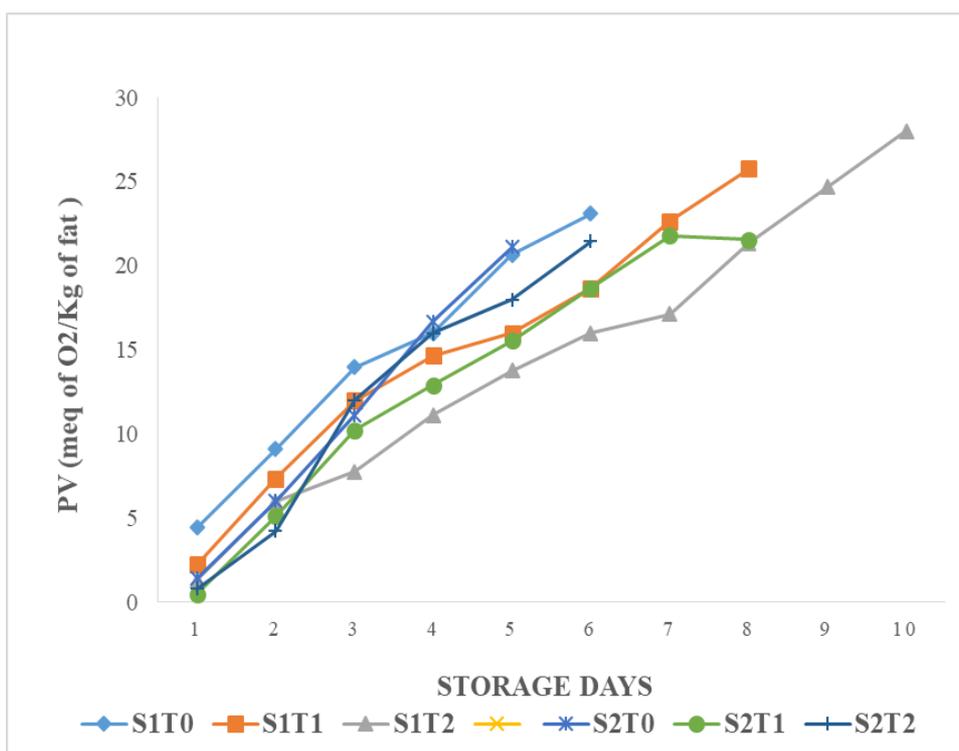


Fig 2: Changes in PV values between two species (*O. ruber* and *S. fimbriata*)

Table 5: Comparison of changes in PV values between two species (*O. ruber* and *S. fimbriata*)

Treatments Storage days	Changes in PV (meq of O ₂ /kg) values					
	T0		T1		T2	
	<i>O. ruber</i>	<i>S. fimbriata</i>	<i>O. ruber</i>	<i>S. fimbriata</i>	<i>O. ruber</i>	<i>S. fimbriata</i>
1	4.44±1.01 ²	1.39±0.67 ¹	2.22±1.01 ¹	0.47±0.15 ¹	1.31±1.31 ¹	0.79±0.47 ¹
2	9.10±1.00 ²	5.99±1.33 ^{1,2}	7.33±0.67 ^{1,2}	5.10±2.69 ^{1,2}	5.98±0.68 ^{1,2}	4.22±2.77 ¹
3	13.98±0.68 ³	11.10±2.14 ^{2,3}	11.99±0.66 ^{2,3}	10.22±1.01 ^{1,2}	7.77±1.01 ¹	12.00±2.00 ^{2,3}
4	15.99±0.66 ³	16.67±1.31 ³	14.66±0.66 ³	12.88±1.01 ²	11.11±1.01 ¹	15.99±0.66 ³
5	20.66±1.76 ³	21.10±2.34 ³	15.99±0.66 ^{1,2}	15.55±1.01 ^{1,2}	13.77±1.01 ¹	17.99±0.66 ²
6	23.11±1.01 ⁵	NA	18.66±0.66 ³	18.66±0.66 ³	15.99±0.66 ²	21.44±0.69 ⁴
7	NA	NA	22.66±1.33 ³	21.77±1.01 ³	17.11±1.01 ²	NA
8	NA	NA	25.77±1.38 ³	21.55±1.67 ²	21.33±0.67 ²	NA
9	NA	NA	NA	NA	24.66±0.66 ²	NA
10	NA	NA	NA	NA	27.99±0.66 ²	NA

Values are mean±SD. n=3. Values in the same row with different superscripts are significantly different ($p<0.05$).

Table 6: Changes in FFA values of EPP extract treated and untreated mince of *S. fimbriata* during ice storage between groups

Treatments Storage days	FFA (% of oleic acid) of <i>S. fimbriata</i> mince		
	T0	T1	T2
1	0.46±0.06 ²	0.16±0.10 ¹	0.28±0.07 ¹
2	1.04±0.44 ¹	0.63±0.13 ¹	0.86±0.20 ¹
3	1.12±0.23 ¹	1.02±0.06 ¹	1.36±0.23 ¹
4	1.86±0.10 ³	1.34±0.09 ¹	1.64±0.07 ²
5	2.40±0.42 ²	1.58±0.29 ¹	1.96±0.13 ^{1,2}
6	NA	1.72±0.17 ¹	2.23±0.30 ²
7	NA	2.11±0.17 ²	NA
8	NA	2.45±0.31 ²	NA
9	NA	NA	NA
10	NA	NA	NA

Values are mean±SD. n=3. Values in the same row with different superscripts are significantly different ($p<0.05$).

Table 7: Changes in AAN content of EPP extract treated and untreated mince of *S. fimbriata* during ice storage between groups

Treatments Storage days	AAN (mg %) of <i>S. fimbriata</i> mince		
	T0	T1	T2
1	0.21±0.03 ²	0.12±0.01 ¹	0.16±0.01 ^{1,2}
2	0.46±0.07 ²	0.17±0.02 ¹	0.23±0.02 ¹
3	0.63±0.03 ³	0.24±0.02 ¹	0.35±0.03 ²
4	0.80±0.05 ³	0.31±0.02 ¹	0.59±0.06 ²
5	1.18±0.09 ³	0.41±0.05 ¹	0.81±0.05 ²
6	NA	0.63±0.06 ²	1.12±0.09 ³
7	NA	0.97±0.11 ²	NA
8	NA	1.20±0.04 ²	NA
9	NA	NA	NA
10	NA	NA	NA

Values are mean±SD. n=3. Values in the same row with different superscripts are significantly different ($p<0.05$).

Table 8: Comparison of changes in AAN values between two species (*O. ruber* and *S. fimbriata*)

Treatments Storage days	Changes in AAN (mg %) values					
	T0		T1		T2	
	<i>O. ruber</i>	<i>S. fimbriata</i>	<i>O. ruber</i>	<i>S. fimbriata</i>	<i>O. ruber</i>	<i>S. fimbriata</i>
1	0.19±0.02 ^{2,3}	0.21±0.03 ³	0.11±0.01 ¹	0.12±0.01 ¹	0.08±0.01 ¹	0.16±0.01 ²
2	0.36±0.03 ³	0.46±0.07 ⁴	0.20±0.01 ^{1,2}	0.17±0.02 ^{1,2}	0.14±0.01 ¹	0.23±0.02 ²
3	0.52±0.05 ³	0.63±0.03 ⁴	0.32±0.05 ²	0.24±0.02 ¹	0.20±0.01 ¹	0.35±0.03 ²
4	0.90±0.05 ⁵	0.80±0.05 ⁴	0.42±0.02 ²	0.31±0.02 ¹	0.33±0.02 ¹	0.59±0.06 ³
5	1.29±0.09 ³	1.18±0.09 ³	0.55±0.03 ¹	0.41±0.05 ¹	0.41±0.03 ¹	0.81±0.05 ²
6	1.40±0.01 ⁵	NA	0.81±0.03 ³	0.63±0.06 ²	0.56±0.02 ²	1.12±0.09 ⁴
7	NA	NA	1.26±0.03 ⁴	0.97±0.11 ³	0.66±0.01 ²	NA
8	NA	NA	1.40±0.07 ⁴	1.20±0.04 ³	0.86±0.03 ²	NA
9	NA	NA	NA	NA	1.29±0.13 ²	NA
10	NA	NA	NA	NA	1.40±0.02 ²	NA

Values are mean±SD. n=3. Values in the same row with different superscripts are significantly different ($p<0.05$).

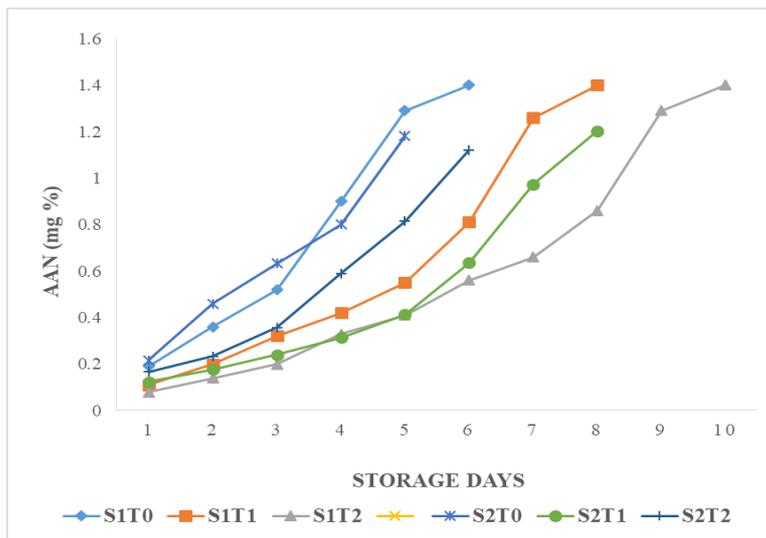


Fig 3: Changes in AAN values between two species (*O. ruber* and *S. fimbriata*)

Table 9: Changes in pH of EPP extract treated and untreated mince of *S. fimbriata* during ice storage

Storage days	Treatments	pH of <i>S. fimbriata</i> mince		
		T0	T1	T2
1		6.48	6.75	6.59
2		6.41	6.68	6.55
3		6.45	6.71	6.58
4		6.49	6.75	6.60
5		6.52	6.69	6.55
6		NA	6.73	6.64
7		NA	6.76	NA
8		NA	6.80	NA
9		NA	NA	NA
10		NA	NA	NA

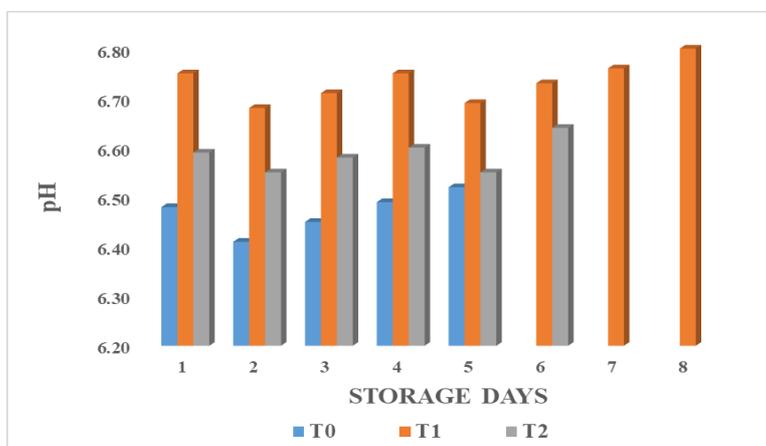


Fig 4: Changes in pH of *S. fimbriata* mince samples during ice storage

Table 10: Changes in Protein Solubility content of EPP extracted and untreated mince of *S. fimbriata* during ice storage between groups

Storage days	Treatments	Protein Solubility (%) of <i>S. fimbriata</i> mince		
		T0	T1	T2
1		63.47±1.26 ¹	70.65±1.80 ²	67.64±1.60 ²
2		60.63±0.88 ¹	65.27±1.40 ²	63.27±0.74 ²
3		56.73±1.31 ¹	60.31±0.70 ²	58.55 ±1.17 ^{1,2}
4		50.48±2.32 ¹	56.40±0.72 ³	51.76± 1.63 ²
5		42.73±5.39 ¹	52.34±1.41 ²	44.17±1.43 ¹
6		NA	46.56±1.94 ³	38.06±2.69 ²
7		NA	42.82±0.23 ²	NA
8		NA	37.74±2.41 ²	NA
9		NA	NA	NA
10		NA	NA	NA

Values are mean±SD. n=3. Values in the same row with different superscripts are significantly different ($p < 0.05$).

Table 11: Changes in WHC content of EPP extract treated and untreated mince of *S. fimbriata* during ice storage between groups

Storage days	Treatments	WHC (%) of <i>S. fimbriata</i> mince		
		T0	T1	T2
1		62.81±1.70 ²	71.55±4.02 ¹	67.21±2.31 ^{1,2}
2		57.89±2.96 ²	63.36±1.72 ¹	60.65±1.29 ^{1,2}
3		45.08±2.80 ²	59.42±4.21 ¹	52.90±3.32 ¹
4		40.35±5.25 ³	57.63±2.29 ¹	47.44±1.26 ²
5		34.28±2.61 ²	56.18±1.83 ¹	38.20±5.64 ²
6		NA	49.80±0.24 ¹	31.21±2.03 ²
7		NA	41.62±1.26 ¹	NA
8		NA	31.53±4.24 ¹	NA
9		NA	NA	NA
10		NA	NA	NA

Table 12: Changes in whiteness of *S. fimbriata* mince samples during ice storage

Storage days	Treatments	L*, a*, b* values of <i>S. fimbriata</i> mince samples									
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
T0	L*	53.97	51.63	44.88	38.56	35.02	NA	NA	NA	NA	NA
	a*	3.45	3.11	2.65	1.89	1.80	NA	NA	NA	NA	NA
	b*	11.70	11.51	10.90	9.40	7.67	NA	NA	NA	NA	NA
whiteness		52.38	50.17	43.99	37.81	34.54	NA	NA	NA	NA	NA
T1	L*	57.19	55.88	54.29	51.72	50.00	45.76	39.15	30.86	NA	NA
	a*	3.60	3.53	3.36	3.23	2.65	2.19	1.56	1.34	NA	NA
	b*	11.76	11.57	11.41	11.28	10.75	10.10	9.13	7.77	NA	NA
whiteness		55.45	54.24	52.76	50.31	48.78	44.78	38.43	30.40	NA	NA
T2	L*	56.18	52.26	49.51	45.72	39.23	31.57	NA	NA	NA	NA
	a*	3.54	3.37	3.17	2.69	1.87	1.37	NA	NA	NA	NA
	b*	11.72	11.63	11.28	10.69	9.19	7.72	NA	NA	NA	NA
whiteness		54.50	50.74	48.16	44.61	38.50	31.12	NA	NA	NA	NA

Where, L*= Lightness, a*= redness to greenness and b*= yellowness to blueness

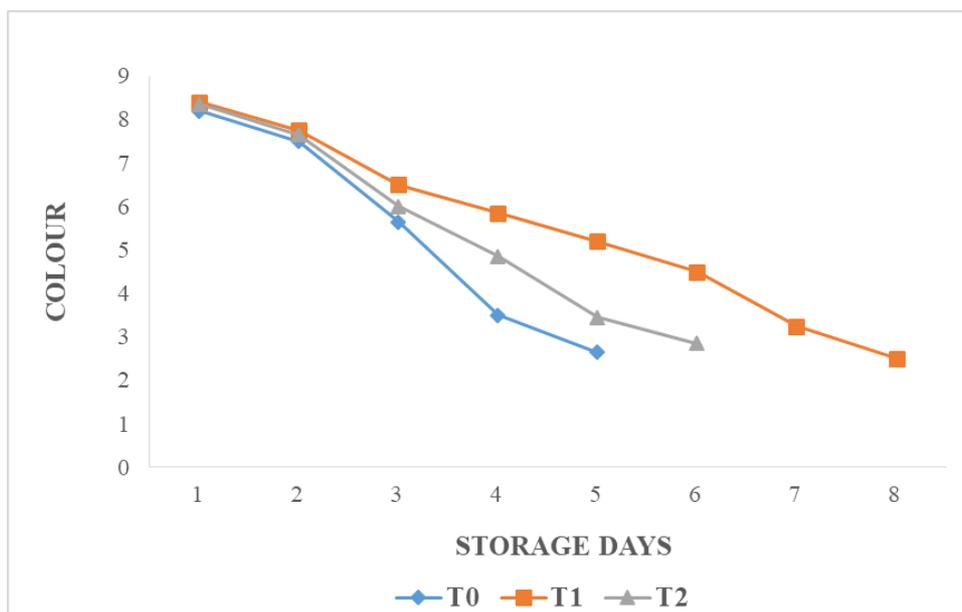


Fig 5: Changes in colour of *S. fimbriata* mince samples during ice storage

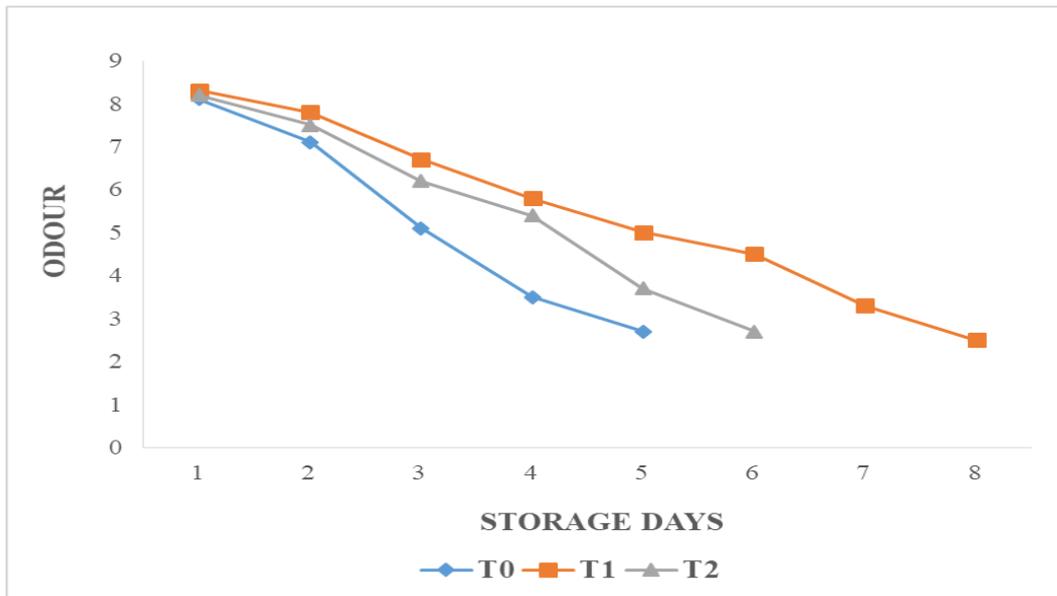


Fig 6: Changes in odour of *S.fimbriata* mince samples during ice storage

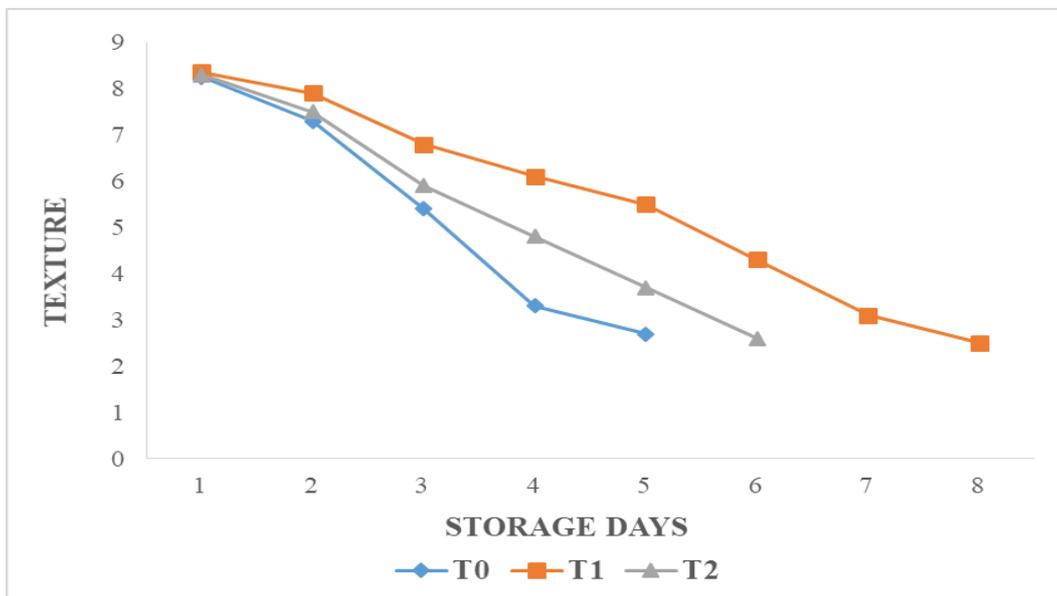


Fig 7: Changes in texture of *S.fimbriata* mince samples during ice storage

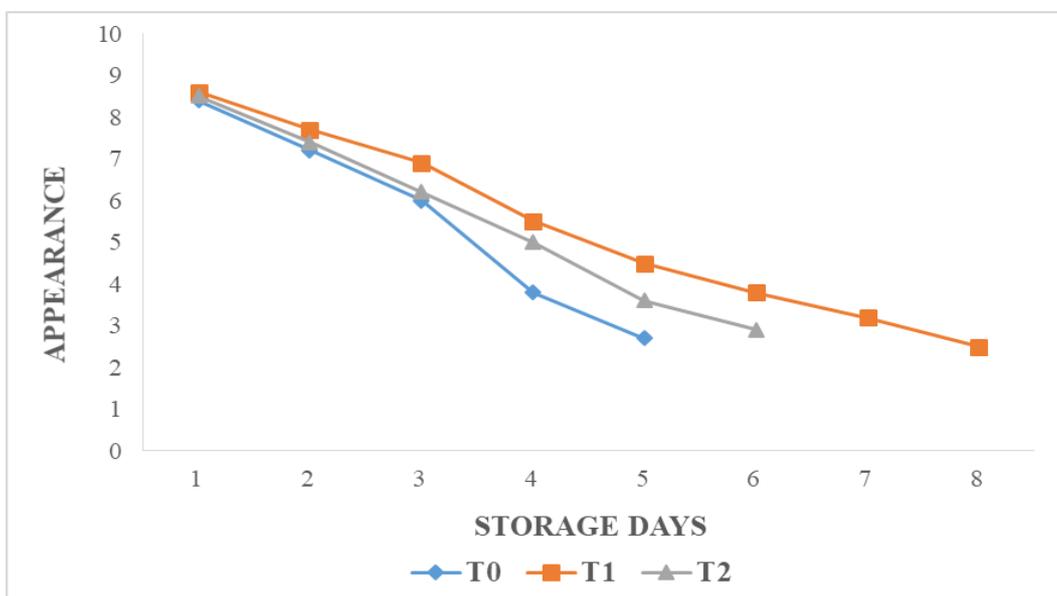
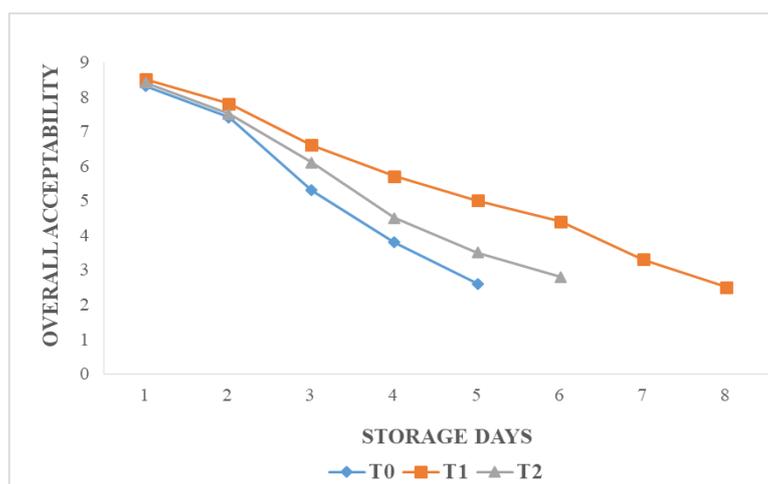


Fig 8: Changes in appearance of *S.fimbriata* mince samples during ice storage

Table 13: Changes in overall acceptability of *S. fimbriata* mince samples during ice storage

Treatments Storage days	Overall acceptability of <i>S. fimbriata</i> mince samples on 0-9 hedonic scale		
	T0	T1	T2
1	8.3	8.5	8.4
2	7.4	7.8	7.5
3	5.3	6.6	6.1
4	3.8	5.7	4.5
5	2.6	5.0	3.5
6	NA	4.4	2.8
7	NA	3.3	NA
8	NA	2.5	NA
9	NA	NA	NA
10	NA	NA	NA

**Fig 9:** Changes in overall acceptability of *S. fimbriata* mince samples during ice storage

4. Conclusion

In the present study, the extracts had a significant effect in biochemical and functional characteristics ($p < 0.05$) except pH ($p > 0.05$). The sensory characteristics of the fish mince were not significantly different ($p > 0.05$). Organoleptically, the control mince samples were acceptable for 5 days in *S. fimbriata*. The mince treated with 2.5 g/kg of extract was analysed for 8 days in *S. fimbriata* and proved to be the best treatment.

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