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Applications of Potassium Sorbate dip treatment for the extension of shelf life of Indian Mackerel (*Rastrelliger kanagurta*) during chill storage

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Abstract *The aim of the study was to analyze the influence of Potassium sorbate dip treatment on the shelf life of Indian mackerel (Rastrelliger kanagurta) during chill storage. Samples were dip treated in 1, 1.5 and 2% solutions of Potassium Sorbate for 10 minutes and packed in polyethylene pouches. After packing, samples were stored in ice (1:1 fish to ice) and were periodically analyzed for Sensory, chemical (pH, TVB-N, TMA, PV, FFA, and TBA) and microbiological (Total Plate Count) quality changes during the storage period. A significant reduction in the rate of spoilage was observed in the treated samples compared to the control samples. Control air packed samples were found to have a shelf life of fourteen days, whereas potassium sorbate treated samples at 1%, 1.5% and 2 % concentration were acceptable up to 18, 21 and 23 days. The sensory characteristics correlated well with other spoilage parameters and the treatment with Potassium sorbate did not affect the sensory acceptability. Thus, potassium sorbate dip treatment was found to delay rate of spoilage, thereby significantly extending the shelf life of Indian Mackerel during chill storage.*

Key words: *Rastrelliger kanagurta, potassium sorbate, Total volatile base nitrogen,*

Introduction

There is an old Eastern–Finnish proverb that "the fish and unpleasant quests begin to smell after three days." Fish is an extremely perishable food. Seafood remains one of our most perishable food stuffs. The primary restrictive factors in the shelf life of sea foods are the unavoidable contamination by spoilage bacteria throughout processing and by their growth during storage (Ashie et al., 1996).Food production is very important to safety, increasing product shelf life and maintaining the quality provides that customers find appealing.

The method studied in this work seeks to preserve fish and extend its shelf life by the use of a safe chemical preservative such as Potassium Sorbate. It has been shown to inhibit spoilage bacteria which cause deterioration of fish. Potassium sorbate is one of the safest food-grade preservatives commonly used today. The FDA has included potassium sorbate in "generally recognized as safe (GRAS)" list. This food additive is recognized with the E number 202. It is a white crystalline powder with strong antimicrobial properties. It is effective in reducing the risk of food-borne illnesses. It is the inorganic salt of sorbic acid which gets readily dissolved in water and forms sorbic acid. Interestingly, potassium sorbate does not affect the color, taste, or flavor of foods. This food additive is completely metabolized by the human body. It has the lowest allergenic potential amongst different categories of food preservatives as it is effective over a wide range of pH. This food additive prevents the growth of variety of microorganisms including yeast, molds, and fungi that attack foods.

Our study was done to determine the effect of Potassium Sorbate dip treatment on the shelf life extension of Indian Mackerel during chill storage. Secondary goal was to study the biochemical, microbial and sensory changes during the chill storage of Indian mackerel dip treated with different concentrations of Potassium Sorbate. And also we worked out effective utilization mackerel dressing waste by converting it in to fish silage. This procedure simulated processing plant holding of fish over a weekend or for late processing and could also be applicable to certain fishing vessel holding systems.

2. Materials and methods

2.1 Preparation of fish samples:

Commercially important and most commonly available *Rastrelliger kanagurtha* which is popularly known as Indian Mackerel collected from Kalamukku fish landing centre, Cochin. Samples were brought to the laboratory in iced condition (1:1 ratio of fish to ice, 1-2^{0}C). Upon arrival at the laboratory the samples were de-iced and washed with chilled potable water. The average body length of each fish was 20-25 cm. with weight of 250-350 gm. Then these were

scaled, beheaded and gutted, washed with tap water several time to removing the blood and slime. All dressed fishes were cut into steaks. Then these steaks were dipped into 5 ppm chlorine water for 10 minutes and drained it. There after the samples are divided in to four lots. Lot I, II, and lot III are subjected to drip treatment with different concentration of potassium sorbate 1%(w/v),1.5%(w/v), and 2% (w/v) respectively , for 10 minutes, then it was kept it in a slanted position for complete drainage there after these samples are packed in self-sealed polyethen pouches. Lot IV was considered as control and it packed in pouches without dip treatment. All the pouches were packed immediately after packaging all packs were stored in an insulated thermocol box with flake ice, in the ratio 1:1(ice to fish), as layer by layer then kept it in room. Re icing and removal excess water was done in every day. These samples are subjected to chemical, biological and sensory tests, at regular intervals for assessing the changes in the quality.

2.1.2 Chemicals & Preservatives.

The chemicals and preservatives used were of AR grade, from the manufacturers of Merck (Merck and co.Inc. NJ, USA) and Qualigens (Qualigens fine chemicals, Mumbai, India). Only food grade and generally regarded of safe (GRAS) preservatives were used in this study.

2.1.3 Bacteriological media

Dehydrated bacteriological media and supplements from the manufactures of Difco (Voigt global distribution Inc., Lawsence, KS, USA) were used in the study.

2.1.4 Proximate analysis

Moisture Content, Crude fat content, was determined according to (AOAC, 2000). Crude protein content and ash content were determined by using the Kjeldahl method (AOAC, 2000).

2.1.5 Chemical analysis

A total Volatile base in the sample was determined as total volatile base nitrogen (TVB – N) by micro space diffusion method (Conway, 1962). TMA was determined as Trimethyl amine nitrogen (TMA–N) by the micro diffusion method (Conway, 1962). TBA value was determined as described by Tarladgis et al., (1960) and expressed as mg Malonaldehyde kg^{-1} of fish sample. Peroxide value was calculated and expressed as milliequivalent of O_2 kg^{-1} fat and it was determined (AOCS, 1989) method. Free fatty acid value was calculated and expressed as mg % oleic acid (AOCS, 1989).

2.1.5 Physical analysis

pH was measured by homogenizing the fish sample in a distilled water (1:2 w/v) by using a glass electrode digital ph meter (Cybscan 510, Eutech instruments, Singapore) as described in IS: 2168(1971). Drip loss (%) was measured gravimetrically by taking the weight difference of the sample with and without exudates. The exudates were removed by draining and wiping the sample with filter paper.

2.1.6 Texture profile analysis (TPA)

The TPA method of Bourne (1978) based on compression of samples with the universal testing machine (Lloyd Instruments LRX plus, Lloyd Instruments Ltd, Hampshire, UK). The load cell used was a cylindrical probe of 50 mm dia equipped with sensor of 50N. Samples were cut into uniform size of 2 cm^3 from each pack and were used for the analysis. The texture measurement was composed of 2 consecutive compressions (40%) of the sample at a crosshead speed of 12 mm min^{-1}. Force by time data from each test was used to calculate mean values for the TPA parameters.

2.1.7 Microbiological analysis

Ten gram of fish sample was aseptically weighed and homogenized with 90 ml sterile normal saline (0.85%) for one circulator, minute, in a stomacher at 230 rpm (seward stomacher 400 circulator, London, UK). the homogenized sample was serially diluted using sterile 9ml saline for bacteriological analysis. Total mesophilic counts were determined by the spread plate method by using plate count agar (PCA) (Difco, Voigt global distribution inc., Lawsence, KS, USA)) as per Townley and Lanier (1981). 0.5 ml appropriate dilution was spread on the pre-set sterile PCA plates in duplicate. The inoculated plates were incubated at 37^0 c for 48 h for total mesophilic count. Average counts were calculated and expressed as colony forming units per gram (cfu g^{-1}) of the sample

2.1.8 Sensory analysis

Sensory analysis for raw and cooked Mackerel fish steaks was carried out by 10 trained panelists using a 9-pont hedonic scale was prescribed by Meilguard et al., (1999). Samples were cooked in 1.5% salt solution for ten minutes

and provided in a coded plate. The panelists were asked to score for appearance, colour, and odour for the raw sample and for cooked sample. Attributes such as flavor firmness and taste were used. Overall acceptability was calculated by adding the scores for all the attributes and dividing by total number of attributes. A score of above 4.0 was considered as the margin for acceptance.

2.1.9 Statistical analysis

Three packs from each batch were used for each analysis. Results were expressed as mean ± standard deviation. Experimental data were analyzed using the software SPSS version 20.00 (SPSS; 2000). For data analysis, mean, standard deviation and analysis of variance (ANOVA) were used. The level of significance was set at $p \leq 0.05$.

2.1.10 Effective waste utilization by Fish silage preparation.

Fish silage was prepared by using dressing waste of mackerel used for the study (Disney et al., 1997). The raw material was first chopped in to small sizes and added 4% formic acid (98%) into the raw material. The samples were mixed thoroughly by constant stirring so that all the samples come in contact with acid. Constant mixing helps to avoid the pockets of untreated material that may putrefy. During the storage period, utmost care was taken to maintain the pH below 4 to prevent bacterial action.

2.2 Results and Discussion

2.2.1 Proximate Analysis

Proximate analysis conducted at day zero. The results are given in fig.1. It indicated the Indian Mackerel used in the experiments possessed fat content of 5% and the corresponding moisture content was 74%. The protein content of the fish used in the study was 20% and the ash content was 1%.

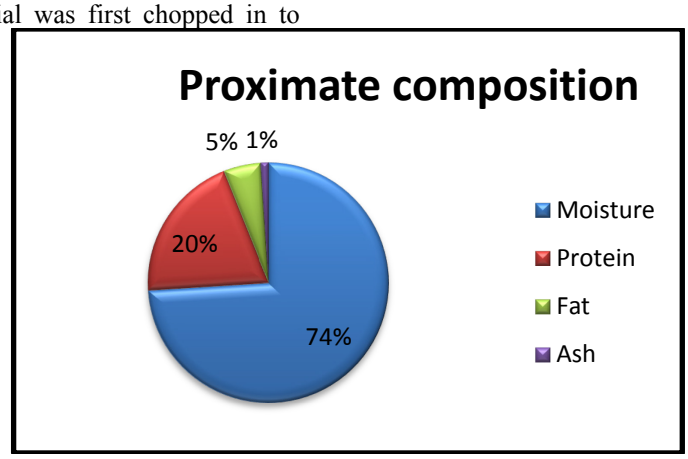

Figure.1. Proximate composition of *Rastreliger kanagurta* used for the study

2.2.2 Physico-Chemical assessments

2.2.2.1 Changes in TBA

The changes in TBA values of Indian Mackerel during ice storage are given in fig.2. An increasing trend was observed to all the samples during chill storage, but the rate of increase was significantly higher ($p \leq 0.05$) for control samples than the treated samples. In all the treated samples,TBA values were within the limit throughout the storage period.TBA value in the range 1-2mg malonaldehyde kg of fish sample is generally taken as the limit of acceptability (Lakshmanan, 2000).The results are in agreement with Rajesh et al (2002) who observed a decrease in TBA values of Potassium Sorbate treated seer fish steaks compared to control samples throughout chill storage. In all the samples, TBA values were within the limit throughout the storage period.

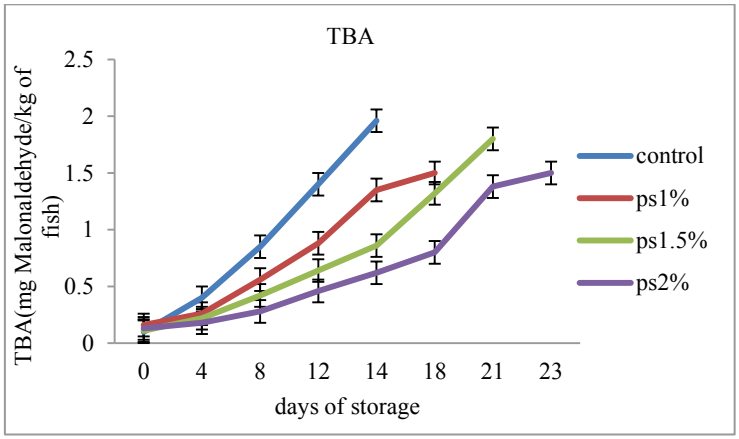

Fig. 2. Changes in TBA values during chill storage

2.2.2.2 Changes in drip loss

The changes in drip loss values of mackerel during chill storage are given in fig. 3 Raw fish was removed from the packages and the volume of drip left in the package (ml) was taken to analyse the drip loss and it was expressed as percentage. An increase in drip loss was observed in case of control and treated packs during chill storage. Drip loss was significantly higher for control samples than the treated samples. Dalgaard *et al.,* (1993) also observed an increase in drip loss throughout storage of vacuum packed and modified atmosphere packed cod fillets.

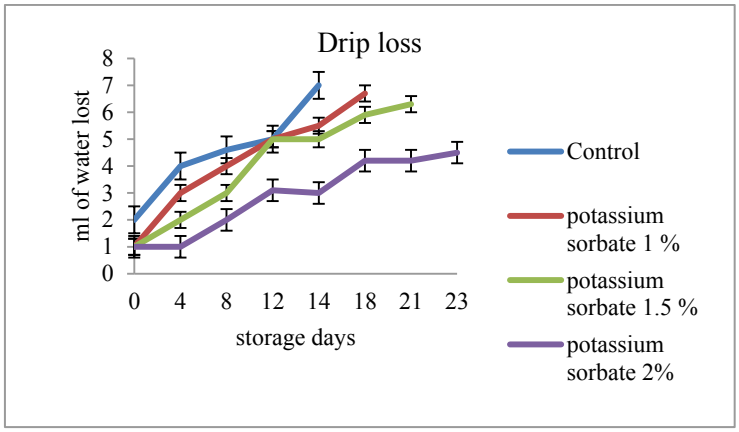

Fig. 3.Drip loss of untreated control sample and treated potassium sorbate sample of *Rastrelliger kanagurta*

2.2.2.3 Changes in pH value

The variations in the pH during chill storage are given in Fig.4. The initial pH of fish sample was found to be 6.72. In potassium sorbate 1%, 1.5% dip treated samples there was a slight reduction in pH values during the initial days of storage and then there was an increasing trend was observed in all treated samples. Slight decrease in pH values may be attributed to the dissolution of CO_2 in the fish muscle. Similar observations were made by Meekin, Hulse, and Bremner (1982). The increase of pH during storage may be recognized to the production of
volatile base compounds by bacterial activity (Cann et al., 1983). Reddy et al., (1997) reported that increase in surface pH of 100% air-packaged tilapia fillets stored at 4^0 C, 8^0C and 16^0C may be fairly due to the production of volatile basic compounds such as ammonia by bacterial action.

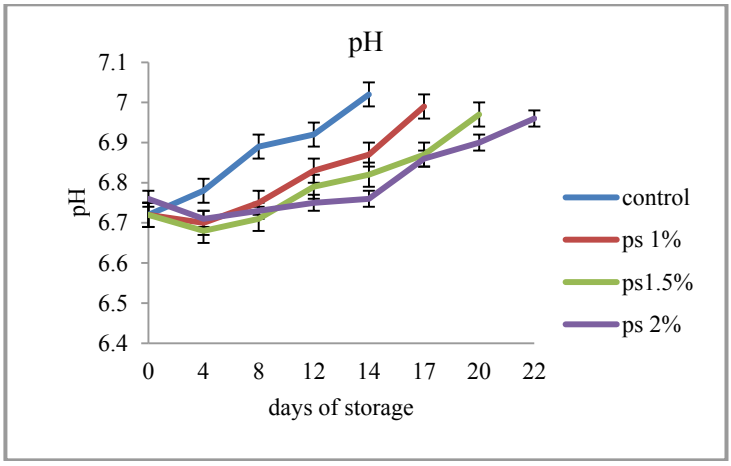

Fig.4. pH changes in mackerel muscle during storage**.**

2.2.2.4 Changes in TVB-N

The changes in TVB-N value of mackerel during ice storage are shown in fig.5. The TVB-N values were found to increase in all samples with storage period. But the rate of increase was significantly lower for treated samples compared to the control samples ($p \leq 0.05$). The low levels of TVB-N in treated samples may be due the reduced bacterial population or decreased capacity of bacteria for oxidative determination of non-protein nitrogen compounds or both. TVB-N levels of the samples increased slowly during storage period, but did not cross the maximum acceptable limit of 35-40 mg% during storage period.

The results of the present study are also in agreement with Debevere and Voets (1972) who observed that Potassium Sorbate inhibited TVB-N formation in prepacked cod fillets stored at 0°C. Botta *et al.,* (1984) reported that there was a definite increase of TVB-N during ice storage of fresh Atlantic cod mainly after 9-11 days. A level of 35-40 mg% is generally regarded as limit of acceptability (Lakshmanan, 2000 and Connell, 1980).

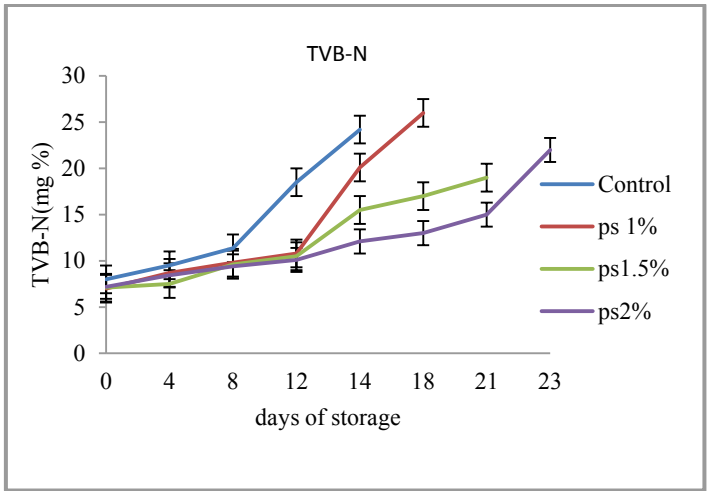

Fig. 5 Changes in TVB-N values during chill storage

2.2.2.5 Changes in TMA-N

The change in TMA-N content of Indian Mackerel during ice storage is shown in fig.6. An increasing trend was noticed in all the samples with storage time. The rate of TMA-N formation was significantly higher ($p \leq 0.05$) for untreated control samples than the treated samples. TMA content of Potassium sorbate 1% treated sample had initial value 2 and increased up to 13.5. Potassium Sorbate 1.5% dip treated sample had initial value of 2.3 and final value 12.3 and also Potassium Sorbate 2% treated

sample TMA value increased from 2.11 to 12.6. TMA-N values of treated samples are found to be comparatively lesser than that of control samples. Similar results were reported by several authors (Parkin and Brown, 1983; Reddy *et al., 1995;*

Jensen *et al.,* 1980). The TMA-N values of treated samples were found to be reasonably lesser than those of control. This might be recognized to the inhibitory effect of potassium sorbate over the growth of bacteria. TMA-N level of 10-15 mg% is typically taken as the limit of acceptability (Lakshmanan, 2000; Sengupta *et al.,* 1972).

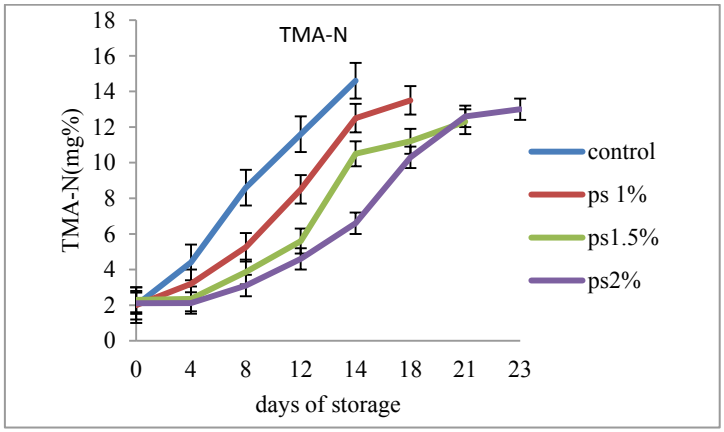

Fig. 6. Changes in TMA-N values during chill storage

2.2.2.6 Changes in peroxide value

The changes in peroxide value content of Indian Mackerel during ice storage are shown in table.1. Initially peroxide value was not observed in all the samples. But during storage the value showed an increasing trend. Peroxide value (PV) shows a general increase during chill storage. In the present study peroxide formation in mackerel after the 0th and 4th days of storage found to be nil. Then the Peroxide values shows significant increase from 8th day in both control and Potassium Sorbate dip treated samples throughout the storage period. The PV value increases from 6.8 -10.1 for control, 6.1-9.9, 5.8-9.1 and 5.1-8.3 for Potassium Sorbate 1%, 1.5% and 2% dip treated samples respectively.

Table. 1. Changes in the Peroxide value during chill storage.

Samples	Storage days							
	0th	4th	8th	12th	14th	18th	21th	23th
Control	Nil	Nil	6.8±0.53	8.5±0.29	10.1±0.17	Nil		
Potassium sorbate1%	Nil	Nil	6.1±0.51	6.5±0.42	8.2±0.31	9.9±0.22	Nil	
Potassium sorbate1.5%	Nil	Nil	5.8±0.54	6.1±0.52	7.5±0.41	8±0.31	9.1±0.21	Nil
Potassium sorbate 2%	Nil	Nil	5.1±0.51	5.3±0.51	6.5±0.51	7±0.42	7.5±0.41	8.3±0.32

4.2.5 Changes in Free Fatty Acid value

The changes in free fatty acid content of Indian Mackerel during ice storage is shown in fig.7.The development of progressive lipid hydrolysis was observed during chill storage of mackerel. FFA (of total lipid as oleic acid) increased significantly from 2 to 14.1 in control pack, 2 to 13.9 in potassium sorbate 1% dip treated samples and 1.5 to 11.5 in potassium sorbate 2% treated samples respectively. Similar findings were obtained. Studies found that Changes in the fatty acid composition and biochemical indices of mackerel (which has a substantial lipid content) and shark (which has negligible lipid content) fillets stored at 18° C for up to six months were calculated. The results showed that, changes of FFA and in shark were significantly ($P< 0.05$) upper than in mackerel. It means that oxidative and hydrolytic deterioration are promoter factors of biochemical changes in mackerel and shark, respectively (Nazemroaya et al., 2011)

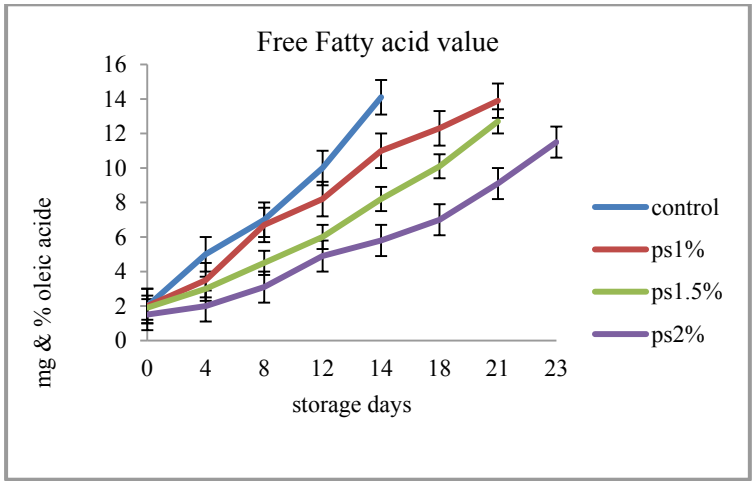

Fig. 7. FFA values of control and potassium sorbate treated samples of *Rastrelliger kanagurta*

4.5 Microbiological changes

The total plate count was found to increase in all the samples during storage period (fig.8.). The lower count in potassium sorbate dip treated samples might be due to the antimicrobial action of potassium sorbate. A similar result was also observed by Zhuang et al., (1996). The total plate count was found to increase in all the samples during storage period**.** Control and 1% potassium dip treated samples reached the microbial acceptable limit on 14th and 18th day respectively. However there was an increase of lag phase on the microbial growth rate was observed for the samples treated with 1.5% and 2% and the samples reached the acceptable limit on 21th and 23nd day respectively. The reduction in microbial growth observed in the treated samples of mackerel can be recognized to the inhibitory effect of potassium sorbate on aerobic spoilage bacteria. The results of the present study are in agreement with result reported in the inhibition of *S. putrefaciens* by sorbate in fresh fish stored under aerobic condition' (Thakur and Patel, 1994). According to Chung and Lee, 1981; Shalini *et al.,* 2000; 2001 and Lalitha *et al., 2003,* prepacking treatments with organic acids and its salts efficiently suppresses growth of aerobic spoilage bacteria on refrigerated fish and shrimp. Gorczyka and Len (1985) reported inhibition of wide range of bacteria in potassium sorbate treated fish.

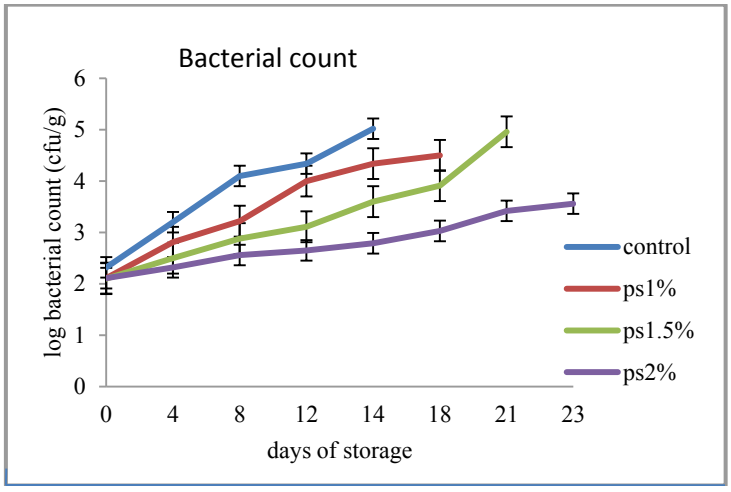

Fig. 8.TPC of control and treated sample of *Rastrelliger kanagurta*

4.4 Texture analysis

Texture profile analysis of control and treated samples are given in the table 2. It is identified as Hardness 1 for the peak during the first compression and Hardness 2 for the peak during the second compression. Decreases in Hardness 1 and Hardness 2 values might be attributed to the weakening of connective tissue of fish muscle during storage. This indicates that there was not much change in the internal bonding of fish muscle during storage. Cohesiveness is the ratio of work done during the second compression divided by the work done during the first compression. The height that the sample springs back between the ends of first compression and the beginning of the second compression is known as springiness. This is sometimes referred to as elasticity. Chewiness refers to the work done. From the study it was observed that there was no significant changes in texture were occurred to the fish samples after dip treatment with potassium sorbate.

Table. 2 Texture profile analysis of Indian mackerel treated with potassium sorbate.

	Control	Potassium sorbate 1%	Potassium Sorbate 1.5%	Potassium Sorbate 2%
Hardness1 (N)	16.06±0.2	19.71±0.1	25.04±0.01	27.99±0.01
Hardness2 (N)	15.20±0.2	16.31±0.21	23.63±0.01	23.96±0.02
Cohesiveness	0.18±0.1	0.20±0.1	0.23±0.11	0.17±0.2
Springiness (mm)	2.09±0.2	1.87±0.2	1.74±0.1	1.46±0.1
Springiness Index	0.432±0.1	0.351±0.1	0.42±0.11	0.351±0.1
Gumminess (kgf)	0.530±0.2	0.326±0.21	0.676±0.01	0.344±0.1
Chewiness (kgf.mm)	1.11±0.1	0.621±0.21	1.17±0.1	0.504±0.3
Fracture Force (kgf)	0.0576±0.01	0.047±0.01	0.047±0.1	0.046±0.21
Adhesive Force (kgf)	0.027±0.1	0.0027±0.1	0.0062±0.01	0.0029±0.1

4.3 Sensory analyses

Changes in overall sensory score of Mackerel during chill storage are presented in fig.9.There was a decline in sensory score of all the control and treated packs with storage period. Fish samples were considered to be acceptable for human consumption until the sensory score reached 4. In Mackerel potassium sorbate 1% treated samples the sensory scores declined from an initial score of 8.5 to 4 , as in the case of potassium sorbate 1.5% treated sample had initial value 8.62 and which declined to 4.2. 2% potassium sorbate treated sample had initial value 8.75 which also declined to 4.3 on the day of rejection. Potassium sorbate treated samples at 1%, 1.5% and 2 % concentration were acceptable up to 18, 21 and 23 days respectively. The control rejected on 14th day of storage. Potassium sorbate 2% treated samples had better sensory scores compared to other packs and remained good and acceptable condition up to 23 days. There was no off flavor was found in potassium sorbate treated samples. As the days of storage in ice progressed, sweet taste of the muscle was lost and the texture became soft.

Result was also reported by Manju et al., (2007) that vacuum packaging along with 2% potassium sorbate samples in Pearl spot and Pomfret samples were good acceptable condition up to 15 and 16 days respectively at 2^{0}C. Huss (1994) has reported 7 days shelf life for excellent quality vacuum packed cod fillets and Ozogul *et al.,* (2000) observed that vacuum packed herring stored at (2±2°C) were sensory acceptable for 8 days. In the present study, 2% potassium sorbate treated samples got an extension of nine days compared to control air packed samples. These results justify the findings of the present study that sodium acetate can increase the shelf life mackerel under chilled condition.

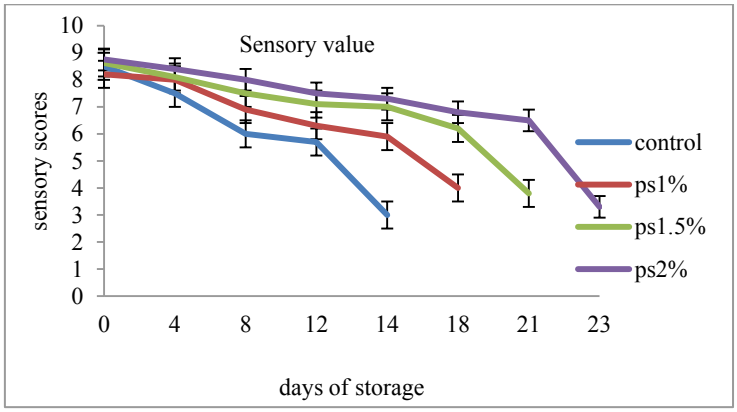

Figure 9. Overall acceptability of the treated and untreated samples of *Rastrelliger kanagurta*

Conclusion

The study was to analyze the effect of Potassium sorbate dip treatment on the shelf life extension of Indian mackerel (*Rastrelliger kanagurta*) during chill storage. A significant reduction in the rate of spoilage was observed in the treated samples compared to the control samples. Control samples were found to have a shelf life of fourteen days, whereas potassium sorbate treated samples at 1%, 1.5% and 2 % concentration were acceptable up to 18, 21 and 23 days. Potassium sorbate is found to be effective in preventing microbial growth and improving shelf life under different storage conditions. The sensory characteristics correlated well with other spoilage parameters and the treatment with Potassium sorbate did not affect the sensory acceptability. The application of potassium sorbate reduces the TMA formation, one of the principle and early indicator of fish spoilage. Thus, potassium sorbate dip treatment was found to delay rate of spoilage, thereby significantly extending the shelf life of Indian Mackerel during chill storage. In summary, this study demonstrated the effectiveness of preserving the nutritional qualities of fish by combining low temperature and preservative treatments during storage over a long period of time, particularly the treatments with 1% and 1.5% and 2% potassium sorbate could aid preservation during storage over a long period of time. Thus this method is therefore recommended for commercial use. Holding 1-5 days old fish in chilled storage containing 1.5% and 2% potassium sorbate was useful in extending shelf life.

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