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Nucleotide Degradation of Pearl Spot during Modified Atmosphere Storage

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The effect of modified atmosphere packaging (MAP) at different gas concentration on chill stored pearl spot (*Etroplus suratensis* Bloch) was examined by means of nucleotide degradation and sensory evaluation. K value, a quality index for fish, the ratio of the sum of inosine and hypoxanthine to the sum of ATP and related catabolites expressed as a percentage, was determined until sensory rejection during storage up to 23 days at 0 – 2°C. The CO_2 concentration did not affect the K values of pearl spot. K value increased linearly in samples stored under air and four different modified atmospheres. Identical K values were obtained for Pearl spot packed in either aerobic or carbon dioxide modified atmosphere. K values were independent of sensory spoilage and correlate only with the length of storage of pearl spot packed under MAP.

Keywords : Modified Atmosphere Packaging, K value, Pearl spot, Polyester/Polyethylene laminate

The initial quality loss in fish is primarily caused by autolytic changes and the degradation of nucleotides (ATP- related compounds) is caused by autolytic enzymes. It is widely accepted that the presence of the intermediate nucleotide, inosine monophosphate (IMP), is responsible for the fresh fish flavour. Apart from this, the autolytic changes are also contributing to spoilage by making catabolites available for bacterial growth.

Objective assessment of fish muscle quality is very important, even though ultimate rejection depends on sensory evaluation. Saito *et. al.* (1959) estimated the freshness of fish muscle from the ratios of the sum of the inosine and hypoxanthine to the sum of all other ATP breakdown products. (K value) as an index of estimating fish freshness and is defined as:

$$K\ value = \frac{Hx + HxR}{ATP + ADP + AMP + IMP + Hx + HxR)} x100$$

Uchiyama *et. al.* (1970) suggested that K value could be reliably used as an index of evaluating the freshness of fish. Ehira & Uchiyama (1973) reported that in all the fish species they investigated, K value correlated well with freshness; While hypoxanthine (Hx) could not be used as an index in inosine-forming species, shelf life and overall acceptability were more related to IMP degradation products than bacterial spoilage (Ehira & Uchiyama ,1973). K value served as a freshness indicator for several freshwater Finfish species (Kiesvaara *et al.*1990) brackish water fishes, Pearl spot and Mullet (Lakshmanan *et al.* 1996) and iced stored hoki and rainbow trout (Ryder ,1985). Lakshmanan *et. al.* (1996) also reported a linear increase in K value with storage time. Boyle *et al.* (1991) studied the adenine nucleotide degradation in

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modified atmosphere packed and chill stored whitefish and rainbow trout. The results indicated K values of fish stored in CO_2 atmospheres did not alter from that of aerobically held fish. However CO_2 atmospheres caused decrease in hypoxanthine concentrations compared to aerobic samples. Huynh *et al.* (1992) found similar results with sockeye salmon and herring. Studies on the effect of gas/product ratio and CO_2 concentration on the shelf life of MA packed fish showed that CO_2 concentration did not affect the K values (Randell *et al.*1995 ; Reddy *et al.*, 1997 ; Lopez-Galvez *et al.*1998; Ozogul & Ozogul ,2000), however CO_2 atmosphere affected hypoxanthine concentration. Ozogul *et al.*, (2000) studied the effects of modified atmospheres on K values of herring stored at 2°C and observed that 60% CO_2 atmospheres showed lower K values compared to aerobically and vacuum held fish. Ozogul *et.al.*, (2004) studied the effects of modified atmospheres on K values of Sardines at 4°C and observed the lowest increase in K value in fish stored in MAP, which was possibly influenced by the presence of CO_2.

The objective of the present study was to determine the effect of MAP on degradation of adenine nucleotides in pearl spot under different CO_2 concentrations

Materials and Methods

The fish were collected from Fort Cochin fish landing centre. The species selected was *Etroplus suratensis* Bloch, of average length 22.5 cm and average weight 250g. The fish were stored under ice till packing in different gas mixtures. Prior to packing dip treatment in 2ppm chlorine water was given. See-through pouches (size 22 X 15 cm) having the configuration of 12μm polyester laminated with 75μm polyethylene was used for packing. Physical properties of the pouch such as heat-seal strength (ASTM, 1972), tensile strength and elongation at break were determined in the machine direction and cross direction (BIS, 1984). Oxygen transmission rate (ASTM, 1975) and water vapour transmission rate (BIS, 1960) were determined. Packing was done using Vacuum Sealing Machine with gas flushing facility (Sevana Quick Seal Machine, Kizakambalam, Kerala, India) and the packs were stored at 0-2°C.

K value was determined from perchloric acid extract of fish meat by the method of Ryder (1985). Nucleotide analysis was carried out with a high performance liquid chromatographic method (Ryder,1985). A Merck system was used with a Lichrospher™ C- 18 RP (250X4 mm) stainless steel column. Extraction of the nucleotide from muscle was done using 0.6 M perchloric acid at 0°C and neutralized using 1M KOH. It was then filtered through a Millipore (0.45 μm) syringe filter. Nucleotide standards and Potassium phosphate were obtained from Sigma Chemical Company. The mobile phase was comprised of 0.06 M Di Potassium hydrogen phosphate and 0.04 M Potassium di hydrogen phosphate at pH 6.5-6.8. The buffer solutions were prepared everyday using HPLC grade water (Millipore). The flow rate was 1 ml/ min. and the eluate was monitored at 254 nm. The detector response for each of the six nucleotides found in fish muscle was calibrated daily by injecting 20 μl of 0.166 mM solution of each reference compound. All solutions were passed through a 0.45 μm aqueous filter before injection on to the column. The K value was computed from the results as defined by Saito *et al.* (1959).

From five different packs (I= air pack, II= 40% CO_2 + 60%O_2, III= 50% CO_2 + 50% O_2, IV= 60% CO_2 + 40% O_2 and V= 40% CO_2 + 30% O_2 + 30% N_2) triplicate samples from the anterior dorsal portion of fish was removed and homogenized in 25 ml of 0.6 M perchloric acid (Ryder, 1985). The slurry was centrifuged at 10,000 X g for 30 min. The supernatant was neutralized with 1 M potassium hydroxide. The

extracts were allowed to stand in an ice bath to precipitate potassium perchlorate. Supernatant was stored at –30°C till it was analyzed. Before injecting into the column, each sample was shaken vigorously and filtered through 0.45μm membrane filter.

Sensory evaluation was carried out for the sensory characters such as appearance texture, odour and flavour in cooked fish on a 9-point hedonic scale (Table 1) as described by Amerine *et al.* (1965). A sensory score of 4 was taken as the borderline of acceptability. Additionally, when off odours were detected, panelists were asked to describe them as pungent, sour, marinade, stale, cabbage and putrid.

Results and Discussion

Physical properties of the packaging material used for the study indicated good bond strength and low barrier properties indicating its suitability for MAP (Table 3).

Table 1. Sensory score for taste panel studies

Organoleptic changes (Cooked)	Score
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like or dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

While standardising optimum gas concentration for getting better storage life, the samples were packed using different combinations of CO_2 and O_2 and were stored at 0-2 °C. In order to select the best gas concentration, samples were drawn periodically and subjected to organoleptic evaluation till the end of storage life. The shelf life at different gas concentration was assessed by sensory analysis. The concentration at which the shelf life was maximum was selected as suitable gas concentration.

Changes in the sensory score with various levels of CO_2, O_2 and N_2 are shown in Fig. 1. Based on the sensory score the shelf life is given on Table 4.

Table 2. Sensory characteristics and scores for taste panel studies

Organoleptic changes (Cooked sample)	Score
Sweet and firm muscle	9
Sweet taste and firm muscle	8
Slight loss of sweet taste, slight loss of firmness of muscle	7
More loss of sweet taste and texture soft	6
Blank taste, texture slightly pasty	5
Dull colour, bland taste and pasty consistency	4
Pasty muscle, slight off odor	3

Table 3. Physical properties of the packaging material

Tensile strength	- Machine Direction	363 kg/cm²
	- Cross Direction	349 kg/cm²
Elongation at break (M.D.)		80%
Elongation at break (C.D.)		80%
Heat Seal Strength (M.D.)		249kg/ cm²
Heat Seal Strength (C.D.)		194 kg/ cm²
Water Vapour transmission rate		3.62g/m²/24 hrs at 37°C at 90 ±2 % RH
Oxygen transmission rate (OTR)		65 cc/m²/atmosphere/ 24hrs/temperature (28-32°C)

The odour of fish (evaluated as odour when opening the package) changed as chill storage proceeded from neutral odour upto the appearance of off odours once spoilage had taken place. Air stored fish were sensorially rejected after 11 days of storage. The spoilage pattern of pearl spot packed in CO_2 enriched atmosphere was quite different from that packed under air. No slime was observed and a sour, light marinade odour indicative of spoilage was detected at the time of sensory rejection. Pearl spot packed under MAP showed a much slower development of off-odours than those stored under air. The limit of acceptability reached in samples stored under MAP after 19 days storage except in batch V.Pearl spot stored under 40% CO_2+30%O_2+30%N_2 followed a similar pattern as that of control and rejection occured on 12th day. A shelf life of 19 days were observed for pearl spot stored under MAP

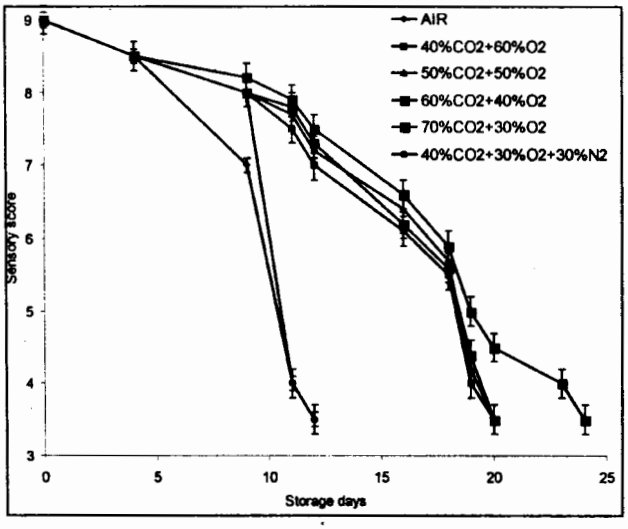

Fig.1 Changes in Sensory score of pearl spot packed under different MAP and Air.

of batches II and III, whereas in Batch IV, appearance of the pearl spot was very good and gave a shelf life of 23 days.

K values determined in samples packed under 5 different gas compositions as described earlier. (Figure-2) showed that Kvalues of pearl spot packed under air and different modified atmospheres differed. Initial K value was 12.5% in pearl spot stored under air and in sample V, K value reached 59% at the time of sensory rejection, whereas in pearl spot stored under II and III, K value reached 68% on rejection day i.e, on 20th day of storage. Pearl spot IV showed a K value of 73.5% at the time of sensory rejection i.e. on 24th day of storage.

Table 4. Shelf life of pearl spot packed under air and MAP based on sesory score

Batch	Type of pack	Shelf life
Batch I	Air pack	11days
Batch II	40% CO_2+60%O_2	19 days
Batch III	50%CO_2+ 50%O_2	19 days
Batch IV	60%CO_2+40%O_2	23 days
Batch V	40%CO_2+30%O_2+30%N_2	11 days

Identical K values were obtained for Pearl spot packed in aerobic or carbon dioxide modified atmosphere. This indicated that presence of CO_2 did not alter the rate of degradation of adenine nucleotides. K value of Pearl spot increased during storage in air and modified atmosphere packed samples, however modified atmosphere packed Pearl spot were still sensorily acceptable even at high K values. K values were independent of sensory spoilage and correlated only with the length of storage of MAP packed Pearl spot. In the present trial, the K value reached 59% at the time of sensory rejection in air stored refrigerated Pearl spot which is in good agreement with the value reported for Pearl spot by Lakshmanan *et. al.*

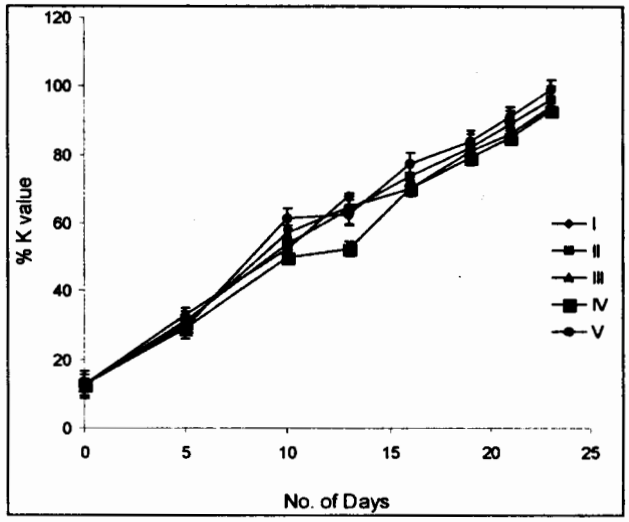

Fig. 2 K values of pearl spot packed under air and different modified atmospheres. (I=air pack, II=40%CO_2 + 60%O_2, III=50% CO_2 + 50%O_2, IV= 60% CO_2 + 40% O_2, V= 40% CO_2 = 30% O_2 = 30% N_2)

(1996) ; Ehira and Uchiyama (1974) and Ehira (1976). Reddy *et.al.*, (1997) found that at 4°C, 8°C and 16°C storage, K value of MAP stored fillets of catfish increased gradually during early and middle storage time and decreased towards the end of storage period with sensory spoilage, indicating no relationship between sensory spoilage and K value.

Fig.3 shows changes in Hypoxanthine values during MAP storage of pearl spot in air and four different gas compositions. Hx content was 0.09 mmol/g initially and increased gradually afterwards. Several fish species are regarded as Inosine formers (Spinelli, 1967; Murata & Sakaguchi, 1986), while others as Hx

formers (Ehira & Uchiyama, 1986). The results suggested that Pearl spot is a Hx former species. Hypoxanthine values of air pack was slightly higher than that of modified atmosphere packed sample. For air pack, hypoxanthine value reached 1.24 μmol/g at the time of rejection (12

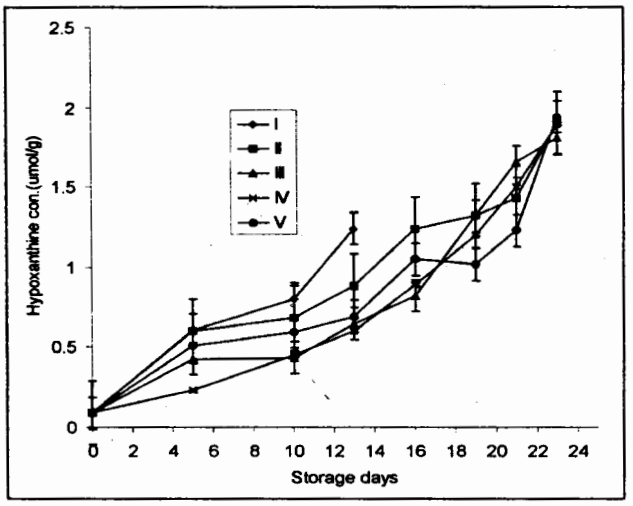

Fig.3 Changes in hypoxanthine values of Pearl spot during MAP storage at 0-2°C (I=air pack, II=40%CO_2 + 60%O_2, III=50% CO_2 + 50%O_2, IV= 60% CO_2 + 40% O_2, V= 40% CO_2 = 30% O_2 = 30% N_2)

days). But in the case of all modified packs hypoxanthine value reached the limit (2 μmol/g) at the time when the samples were rejected organoleptically. This result suggests that CO_2 may have an effect on the hypoxanthine level. In samples III and IV, increase in the hypoxanthine content is similar and lowest compared to all other samples indicating that, most suitable compositions are 50% CO_2 + 50% O_2 and 60% CO_2 + 40% O_2.

Ozogul & Ozogul (2000) got similar results in Rainbow trout stored in ice and in modified atmosphere packaging. Boyle *et. al.* , (1991) found similar effects in chill stored white fish. According to their study, atmosphere altered hypoxanthine production in chill stored white fish.

K value increased linearly in pearl spot stored under air and four different modified atmospheres. K values were independent of sensory spoilage and correlate only with the length of storage of pearl spot packed under MAP. Pearl spot stored under 40%CO_2+30%O_2+30%N_2 followed a similar pattern as that of control and rejection occurred on 12th day. A shelf life of 19 days was observed for pearl spot stored under MAP of batches II and III. Whereas in batch IV, appearance of the pearl spot was very good and it gave a shelf life of 23 days.

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References

Amerine, M.A., Pongborn, R.H, Roescler, E.B. (1965) Principles of Sensory Evaluation

ASTM (1972) *Standard Method of Test for Peel or Stripping Strength of Adhesive Boards*, D 90349, American Society for Testing and Materials, Philadelphia.

ASTM (1975) *Standard Method of Test for Gas Transmission Rate of Plastic Film Sheeting*, D1434. American Society for Testing and Materials, Philadelphia.

BIS (1960) *Methods of sampling and Test for Paper and Allied Products* IS edn, Bureau of Indian Standards. New Delhi.

BIS (1984) *Specification of Low- density Films* IS: 2508 2nd edn. Bureau of Indian Standards. New Delhi.

Boyle, J.L., Lindsay, R.C. and Stuiber, D.A. (1991). Adenosine nucleotide degradation in Modified Atmospheric Chill Storage fresh fish. *J. Food Sci.*, **56**, pp 1265-1270.

Ehira S, and Uchiyama, H. (1974) Freshness lowering rates of cod and seabream viewed from changes in bacterial count total volatile base and trimethyl amine-nitorgen and related compounds, *Bull. Japan. Soc. Sci. Fish.*, **40** , pp 479-487

Ehira, S. (1976). A biochemical study of the freshness of fish. *Bull. Tokai Reg. Fish. Res. Laboratory*, **88**, 1p.

Ehira, S. and Uchiyama, H. (1973), Formation of Inosine and hypoxanthine in fish muscle during storage, *Bull. Tokai Reg. Fish. Res. Laboratory*, **75**, 63p

Ehira, S. and Uchiyama, H. (1986), Determination of fish freshness using the K value and comments on some other biochemical changes in relation to freshness. In: *Proceedings of an International Symposium Coordinated by the University of Alaska Sea Grant Program, Anchorage*, (D.E. Kramer & J. Liston Eds), 185p. *Alaska, U.S.A.*

Huynh, M.D., Mackey, R. and Gawley, R. (1992). Freshness assessment of Pacific fish species using K value. In: *Seafood Sci. Technol.* (Ed. E. G. Bligh), Oxford: Fishing news (books) Limited, pp. 258-268.

Kiesvaara, M, Hattula, T. and Karppinen S (1990) *Development of methods used in the quality classification of fish*, Technical Research Centre of Finland Research Notes, 1193p.

Lakshmanan, P.T., Antony, P.D. and Gopakumar, K. (1996). Nucleotide degradation and quality changes in mullet and perarlspot in ice and at ambient temperatures. *Food Control*, **7**, pp 277-283.

Lopez-Galvez DE Hoz L de la, Blanco, M.and Ordonez Ja (1998) Refrigerated storage (2°C) of sole (*Solea solea*) fillets under CO_2 enriched atmospheres. *J. Ag. Food Chem.*, **46**, pp 1143-1149.

Murata, M. and Sakaguchi, M. (1986). Storage of yellowtail white and dark muscles in ice: Changes in content of adenine nucleotides and related compounds. *J. Food Sci.*, **51**, pp 321-326.

Ozogul, F, Polata A. and Ozogul, Y. (2004) The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardine pitchadus*), *Food Chem.*, **85**, pp 49-57.

Ozogul, F., Taylor, KDA., Quantick, P. and Ozogul Y (2000 a) Arapid HPLC determination of ATP related compounds and its application to herring stored under modified atmosphere, *Int. J. Food Sci.Technol.*, **35**, pp 549-554.

Ozogul, Y.and Ozogul, F. (2000) Adenosine Nucleotide degradation in Rainbow trout stored in ice and in modified atmosphere packaging, *Turkish J. of Zool.* **26**, pp127-103.

Reddy, N. R. Roman MG., Solomon., H.M.., Kauter DA. and Rhodehamel E.J. (1997) Shelf life and *Clostridium botulinum* toxin development during storage of modified atmosphere packaged fresh catfish fillets, *J. Food Sci.*, **62**, pp 878-884.

Randell, K., Ahvenainen Rand Hattula, T. (1995) Effect of the gas/product ratio and CO_2 concentration on the shelf life of MA packed fish, *Packaging Technol. and Sci.*, **8**, pp 205-218.

Ryder, J.M. (1985). Determination of adenine triphosphate and its breakdown products in fish muscle by high performance liquid chromatography. *J. Agric. Food Chem.*, **3**, 673p.

Saito, T, Arai, K. and Matsuyoshi, M. (1959). A new method for estimating the freshness of fish. *Bull. Japan. Soc.Sc.Fish.*, **24**, 749p.

Spinelli, J. (1967). Degradation of nucleotides in ice-stored halibut. *J. Food Sci.* , **32**, pp 38-42.

Uchiyama H., Ehira, S., Kawahara, T. and Shimidu, W. (1970) Significance in measuring volatile base and trimethyl amine nitrogen and nucleotides in fish muscle as indices of freshness, *Bull. Japan. Soc. Scientific Fish.*, **36**, pp 177-181.