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Nucleotide degradation of sodium acetate and potassium sorbate dip treated and vacuum packed Black Pomfret (*Parastromateus niger*) and Pearlspot (*Etroplus suratensis*) during chill storage

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Abstract

Quality changes of vacuum packed Black Pomfret (*Parastromateus niger*) and Pearlspot (*Etroplus suratensis*) treated with sodium acetate and potassium sorbate, respectively, and subsequently stored in vacuum packs in ice during chill storage were evaluated by measuring the volatile base nitrogen values, *K*-values and sensory parameters. The effects of sodium acetate (2% w/v) and potassium sorbate (2% w/v) dip treatments on vacuum packed Black Pomfret and Pearlspot were also examined. Total volatile base nitrogen values were found to increase slowly with time, whereas the *K*-values were found to increase linearly with time in samples of both the species and exceeded the acceptability limit on the day of sensory rejection. The *K*-values, representing the ratio between the sum of inosine and hypoxanthine to the sum of all other ATP breakdown products, was found to be a more reliable method for estimation of the quality of fresh/preserved fish. Sodium acetate/potassium sorbate dip treated and vacuum packed Black Pomfret and Pearlspot exhibited lower *K*-values compared to untreated samples. Treated vacuum packed samples of Black Pomfret were found to be in good and acceptable condition up to 16 days whereas treated vacuum packed samples of pearlspot remained in good condition up to 15 days compared to vacuum packed and air packed samples which were acceptable only up to 10 and 8 days, respectively. The sensory characteristics correlated well with the *K*-values for both the species.

Keywords: Black pomfret; Pearlspot; *K*-values; Total volatile base nitrogen; Sodium acetate; Potassium sorbate

1. Introduction

The consumer's desire to get good quality fresh seafood is on the increase. Fish and fish products are now transported between nations and hence the freshness or quality of these products is becoming more and more important. Fresh fish and other fresh sea products are highly susceptible to spoilage from postmortem microbial growth and enzymatic activity. This spoilage is due to lack of chilling and improper handling during storage, distribution and marketing. The increasing demand for high quality fresh seafood has intensified the search for methods and technologies for better fish preservation. Presently ice and mechanical refrigeration are the most common means of retarding microbial and biochemical spoilage in freshly caught seafood during distribution and marketing.

In fish muscle, autolytic changes take place immediately after death and before microbial spoilage starts. Tests based on nucleotide catabolism have received substantial attention during the past 30 years. Adenine nucleotides are degraded by endogenous enzymes in fish muscle during the early stages of storage of fresh fish, and a series of reactions results in the conversion of ATP through several intermediates to Hypoxanthine (Hx) (Jones & Murray, 1962; Kassemsarn, Perez, Murray, & Jones, 1963). During the later stages of refrigerated shelf life, microbial metabolism also contributes to degradation (Jones, Murray,

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Livingston, & Murray, 1964). Hx formed is thus converted to xanthine, uric acid and ring cleavage products by the developing spoilage microflora (Kassemsarn et al., 1963). In a large variety of fish, nucleotide degradation follows a well-defined process.

$$\text{ATP} \rightarrow \text{ADP} \rightarrow \text{AMP} \rightarrow \text{IMP} \rightarrow \text{Inosine (HxR)} \rightarrow \text{Hypoxanthine (Hx)} \rightarrow \text{Xanthine} \rightarrow \text{Uric acid.}$$

A high level of any of the adenosine related compounds or IMP, in the muscle imparts sweet meaty flavor and is regarded as very fresh fish. Postmortem accumulation of inosine or Hx reflects poor quality. Saito, Arai, and Matsuyoshi (1959) were the first to estimate the freshness of fish muscles from the ratio of the sum of H_xR and H_x to the sum of all other ATP breakdown products. This ratio expressed as % is called the *K*-value, thus

$$K\text{-value}(\%) = \frac{[H_xR] + [H_x]}{[ATP] + [ADP] + [AMP] + [IMP] + [H_xR] + [H_x]} \times 100$$

The larger the *K*-value, the poorer is the eating quality of most species of fish. Ehira (1976) remarked that the *K*-values as proposed by Saito et al. (1959) was one of the most appropriate indicators of freshness. Ehira and Uchiyama (1986) have determined *K*-values in a number of fish species from Japan. Kiesvaara, Hattula, and Karppinen (1990) found that the *K*-values could be applied as a freshness indicator of several finfish fresh water species.

This paper deals with the effectiveness of the *K*-values in assessing the freshness of sodium acetate/potassium sorbate treated and vacuum packed Black Pomfret (*Parastromateus niger*) steaks and whole Pearlspot (*Etroplus suratensis*) in comparison with sensory assessment and total volatile base nitrogen (TVB-N) values.

2. Materials and methods

2.1. Packing and storage of Black Pomfret and Pearlspot

Fresh Black Pomfret and fresh Pearlspot procured from Fortkochi fish landing center were brought to the laboratory in iced condition. The fish were washed in potable water and kept in iced condition during processing. Then, these were beheaded, scaled, gutted and washed in potable water. Dressed fish was used as such in the case of Pearlspot, whereas in the case of Black Pomfret the dressed fish were cut into steaks, which were then washed in chilled potable water.

Fish/steaks were given a dip treatment in 2 ppm chilled chlorine water for 10 min and allowed to drain well. These were then divided into four lots. Lot I was air packed (control air pack – CAP) and lot II vacuum packed (control vacuum pack – CVP) to serve as controls. Lot III was given a dip treatment in 2% (w/v) sodium acetate solution for 30 min, drained well and then vacuum packed (SAVP – sodium acetate vacuum pack). Lot IV was given a dip treatment in 2% (w/v) potassium sorbate solution for 30 min, drained well and then vacuum packed (PSVP – potassium sorbate vacuum pack).

Details regarding the packing of samples, methodology of testing of packaging materials and storage of samples are described in the earlier submitted manuscript (Manju, Srinivasa Gopal, Ravishankar, & Lalitha, 2006). Samples were drawn from each lot at regular intervals and were analyzed for TVB-N, sensory parameters and *K*-value. Sampling was done in triplicate and the mean values were taken.

2.2. Reagents used

Chemicals used for the experiments were of Sigma brand, Analar grade or guaranteed reagent grade. Preservatives, sodium acetate (Merck, Mumbai) and potassium sorbate (s.d. Fine Chemicals Ltd., Mumbai) were used.

2.3. K-values

The nucleotide and related compounds in the muscle (taken from the dorsal region) were determined by the method of Ryder (1985) using HPLC. A Merck system was used, with a Bondapac C18 stainless steel column. Extraction of nucleotide from muscle was done using 0.6 M perchloric acid at 0 °C and neutralized using 1 M KOH. It was then filtered through a Millipore (0.45 μm) syringe filter. Mobile phase comprised of 0.06 M K_2HPO_4 and 0.04 M KH_2PO_4 at pH 6.5–6.8. Buffer solutions were prepared daily in Milli Q water and filtered through a millipore filter (0.45 μm). The flow rate was 1.5 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 onto the column. The *K*-values was computed from the results as defined by Saito et al. (1959). TVB-N was determined by the micro-diffusion method by Conway (1950). Proximate composition was determined as per AOAC (2000).

2.4. Sensory evaluation

Sensory evaluation was based on characterization and differentiation of the various sensory characters such as appearance, texture, odour and flavour. Sensory score was given based on a nine point hedonic scale (Manju et al., 2006). Taste panel scoring of the fish was conducted after boiling the dressed fish in 1.5% brine for 10 min. Sensory score of 4 was taken as the borderline of acceptability.

3. Results and discussion

3.1. Proximate analysis

The proximate composition of fresh Pearlspot analysed had 77.75% moisture, 2.04% crude fat, 19.23% crude pro-

tein and 0.98% ash and the fresh Black Pomfret analysed had 75.92% moisture, 2.48% crude fat, 20.04% crude protein and 1.56% ash. The physical properties of the packaging material used are described in earlier submitted manuscript (Manju et al., 2006). The packaging material used for the study has got enough strength to withstand machine handling. It also exhibited lower water vapour transmission rate and oxygen transmission rate that suits for packing vacuum packed products.

3.2. Total volatile base nitrogen

The changes in the TVB-N values of Pearlspot and Black Pomfret during ice storage are shown in Figs. 1 and 2, respectively. The TVB-N values were found to increase with storage period in control and treated samples. The TVB-N content of Pearlspot increased from an initial value of 5.6–19.45 mg% in CAP and 18.3 mg% in CVP on 10th and 12th day of storage, respectively. In case of SAVP and PSVP samples, the TVB-N content rose to 21.6 and 17.15 mg%, respectively, on 17th day of storage. In Black Pomfret, the TVB-N content increased from an initial value of 4.5–19.6, 18.4, 21.55 and 16.65 mg% in case of CAP, CVP, SAVP and PSVP samples on 10th, 12th and 18th day of storage, respectively.

The TVB-N values of vacuum packed samples were lower than those of the CAP samples in both the species. This is in agreement with Özogul, Polat, and Özogul (2004) who observed lower TVB-N values for vacuum packed samples than air packed samples during storage of vacuum packed sardine at 4 °C. The TVB-N values of treated samples (SAVP and PSVP) were found to be lower than those of the control packs in both the species. Similar results have been reported by Shalini, Jasmine, Shanmugam, and Ramkumar (2000, 2001) and by Rajesh, Ravishankar, Srinivasa Gopal, and Varma (2002). Low levels of TVB-N in treated samples were due to either a reduced bacterial population or a decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds or both (Banks, Nickelson, & Finne, 1980). The TVB-N contents of potassium sorbate treated samples were slightly lower than sodium acetate treated samples for both the species. This might be attributed to the greater inhibition of gram −ve bacteria by potassium sorbate than by sodium acetate. Kim and Hearnsberger (1994) and Kim et al. (1995) observed an inhibition of aerobic gram −ve spoilage bacteria by sodium acetate. 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. A level of 35–40 mg% is usually regarded as the limit of acceptability (Lakshmanan, 2000). However, in the present study TVB-N values of all the samples were well within the suggested limit throughout the storage period and hence do not serve as a good index of spoilage.

3.3. Sensory evaluation

Changes in the overall sensory scores of Pearlspot and Black pomfret during chill storage are presented in Figs. 3 and 4, respectively. There was a significant decline in the sensory score of the control and treated packs with

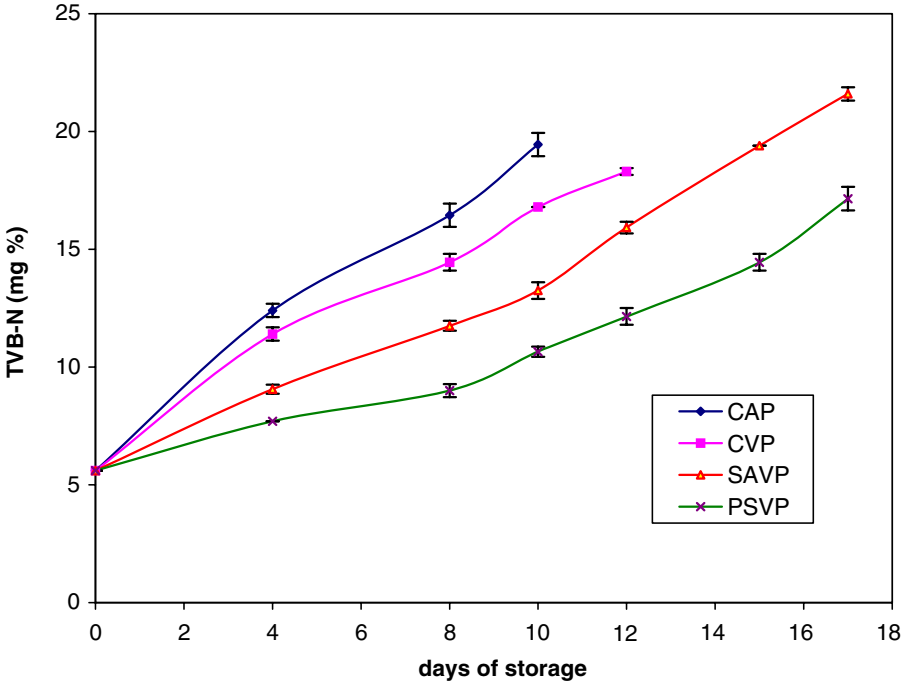

Fig. 1. Changes in TVB-N values of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0–2 °C.

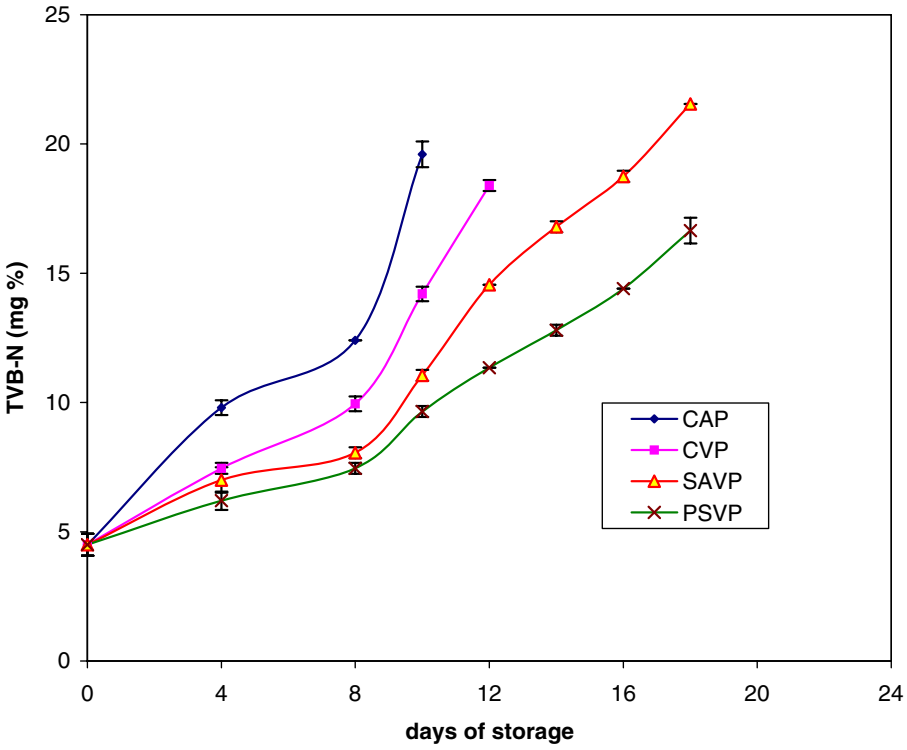

Fig. 2. Changes in TVB-N values of Black Pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0–2 °C.

storage period for both the species. The fish samples were considered to be acceptable for human consumption until the sensory score reached 4. In Pearlspot, the sensory scores declined from an initial score of 8.6–3.4 in CAP and 3.8 in CVP samples on the day of rejection. As the days of storage in ice progressed, the sweet taste of the muscle was lost and the texture became soft and pasty. In SAVP and PSVP samples, the sensory scores on the day of rejection were 3.6 and 3.8, respectively. Thus, CAP and CVP samples were found to be acceptable up to 8 and 10 days, respectively, whereas SAVP and PSVP samples remained in good and acceptable condition upto 15 days.

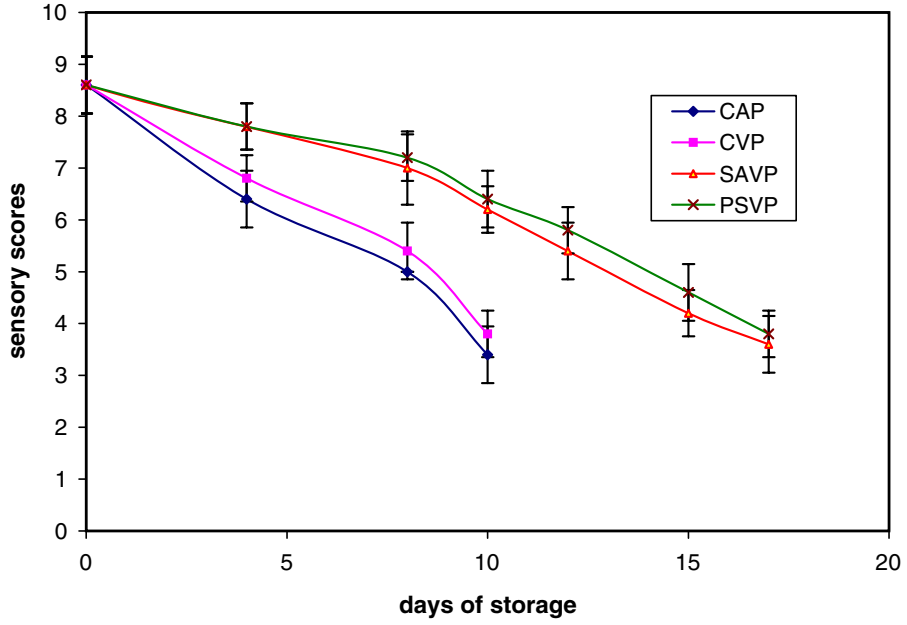

Fig. 3. Changes in overall sensory scores of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0–2 °C.

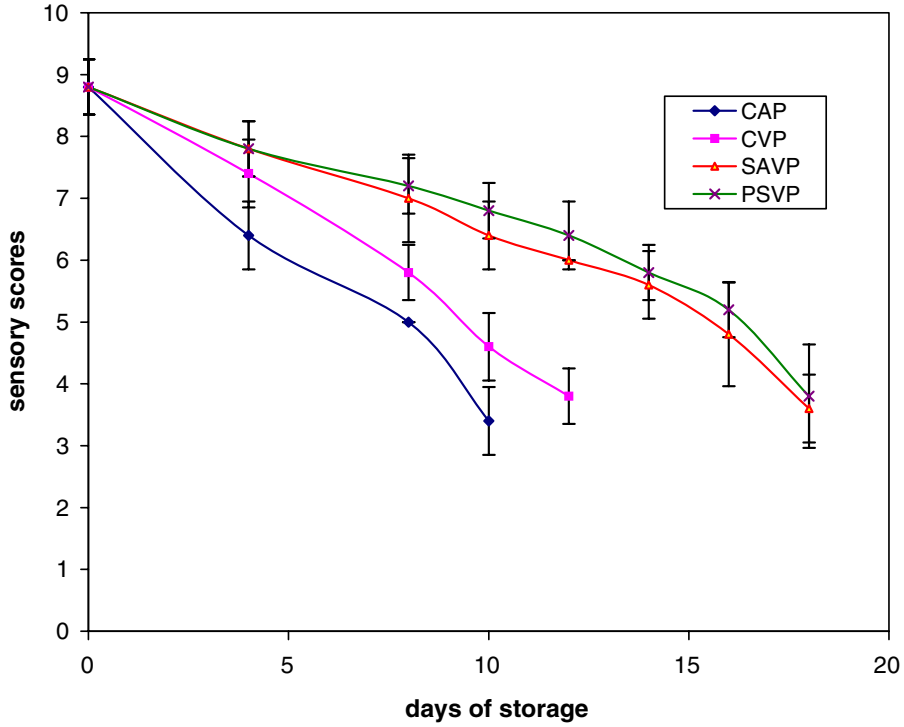

Fig. 4. Changes in overall sensory scores of Black Pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0–2 °C.

In Black Pomfret, the sensory scores declined from an initial 8.8–3.4 in CAP, 3.8 in CVP, 3.6 in SAVP and 3.8 in PSVP samples on 10th, 12th and 18th day of storage, respectively. Thus, air packed samples were acceptable up to 8 days and vacuum packed samples were in good and acceptable condition up to 10 days, whereas SAVP and PSVP samples remained in good and acceptable condition up to 16 days. A significant extension was not noticed due to packing under vacuum in either the species.

Several authors have reported on the shelf life of different fish. Meekin, Hulse, and Bremner (1982) reported that aerobically packed refrigerated (4 °C) sand flat head spoiled in 8–9 days. Reddy, Schreiber, Bazard, Skinner, and Amstrong (1994) reported that tilapia fillets packed under 100% air spoiled after 9 days at 4 °C. Özogul et al. (2004) reported that vacuum packed sardines stored at 4 °C were sensorily acceptable for 8 days. The addition of 2% (dip treatment) sodium acetate has been found to extend the shelf life of refrigerated catfish fillets (Zhuang, Huang, & Beuchat, 1996). Bremner and Statham (1983) achieved suppression of spoilage and a significant extension of shelf life by the addition of potassium sorbate to vacuum packed scallops. Thus, vacuum packaging alone did not extend the shelf life of Pearlspot/Black Pomfret, but vacuum packaging along with preservatives (sodium acetate/potassium sorbate) was found to extend the shelf life by 7 days in Pearlspot and 8 days in Black Pomfret compared to air packed samples which were acceptable only up to 8 days.

3.4. K-value

Changes in the *K*-values of Pearlspot and Black Pomfret are represented in Figs. 4 and 5, respectively. In both the species, the *K*-value was found to increase during storage. In Pearlspot, the *K*-value increased from an initial 4.87–68.7%, 61.0%, 68.4% and 69.4% in CAP, CVP, SAVP and PSVP samples, respectively, on 10th, 12th and 17th days of storage. In Black Pomfret, the value increased from an initial 7.81–67.1%, 63.7%, 63.4% and 62.2% in CAP, CVP, SAVP and PSVP samples, respectively, on 10th, 12th and 18th days of storage (see Fig. 6).

For freshly caught fish, the initial *K*-value reported was around 5% (Aleman, Kakuda, & Uchiyama, 1982). The initial *K*-values obtained for fresh Pearlspot and Black Pomfret in the present study are in agreement with the above observation. In both the species, an increase in the *K*-value was noticed in all the samples with storage time and the values of air packed samples were higher than those of vacuum packed samples. This is in agreement with Özogul et al. (2004) who reported an increase in the *K*-value with time in vacuum packed sardines stored at 4 °C, and the values of air packed samples were higher that those stored under vacuum. Lakshmanan, Brown, and Ames (1993)

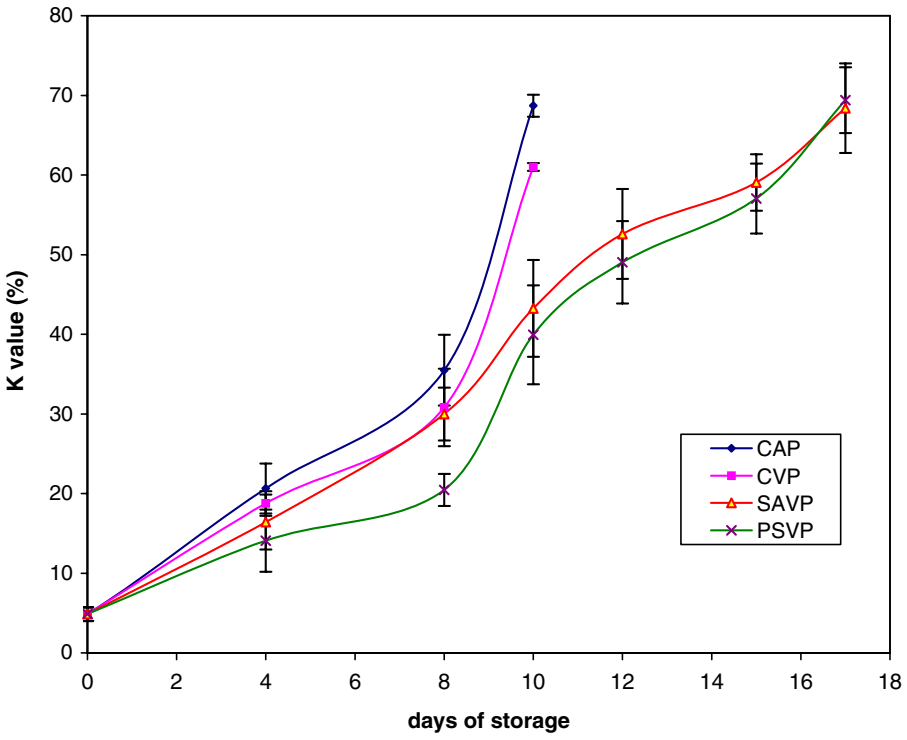

Fig. 5. Changes in *K*-values of pearlspot packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0–2 °C.

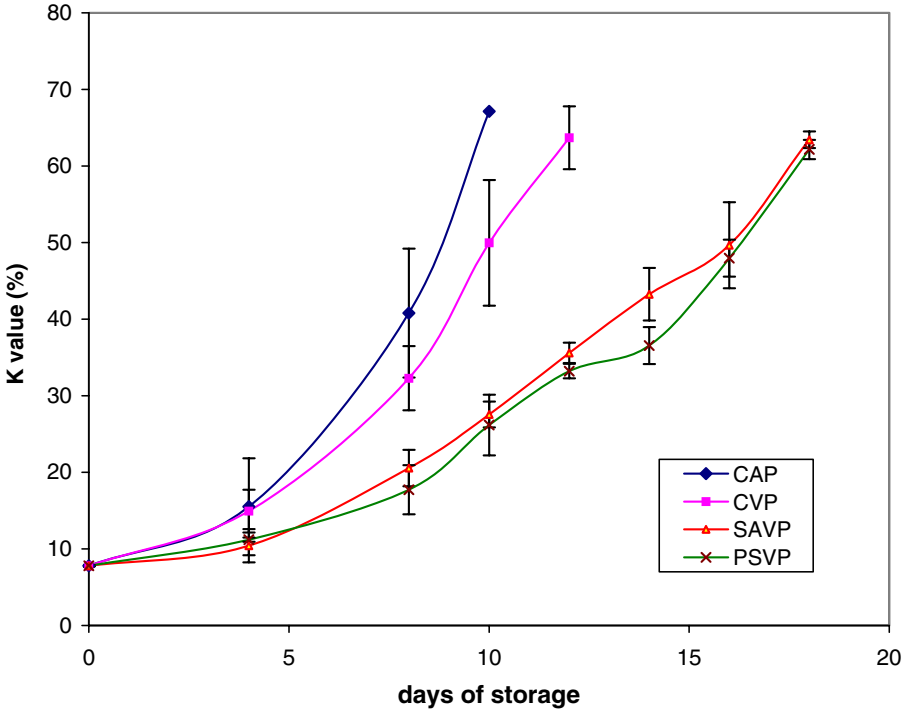

Fig. 6. Changes in *K*-values of Black Pomfret packed under air and vacuum (sodium acetate/potassium sorbate treated and untreated) during storage at 0–2 °C.

observed a constant increase in the *K*-value with time in trout muscle during iced storage. The results of the present study are also in agreement with those of Özogul, Taylor, Quantick, and Özogul (2000) who reported an increase in the *K*-value with storage time in vacuum packed Atlantic herring during chill storage.

An increase in the *K*-value with time was observed by Lakshmanan, Antony, and Gopakumar (1996) in the muscle of Pearlspot and Mullet during ice storage. The rejection levels of the *K*-value observed in the present study are close to the 60% limit set by Ehira (1976) and Ehira and Uchiyama (1974). On the day of sensory rejection, the *K*-values of all the samples exceeded 60%, which indicates good correlation of *K*-values with the sensory scores.

4. Conclusions

In the present study, the TVB-N values increased slowly and never reached the upper limit of 35 mg% in any of the samples during the storage period and hence did not serve as a good index of spoilage. Whereas, the *K*-values increased linearly with storage time in all the samples and crossed the upper limit of 60% on the day of sensory rejection. Thus, good correlation of the *K*-value with sensory scores was observed in the present study and the rate of change of the *K*-values appeared to indicate the real quality of fish. Thus, knowledge of the *K*-values would clearly indicate the state of quality of vacuum packed Pearlspot/Black Pomfret during chill storage. The use of sodium acetate/potassium sorbate and vacuum packaging was found to extend the shelf life of fish samples in the present study also. Vacuum packaging along with 2% sodium acetate/potassium sorbate can be safely used to extend the shelf life of Pearlspot and Black Pomfret samples up to 15 and 16 days, respectively, compared to air and vacuum packed samples which were acceptable only up to 8 and 10 days, respectively.

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